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REVIEW

Role of Reactive Oxygen Species in Mast Cell Degranulation

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Abstract—Mast cells are a heterogeneous multifunctional cellular population that promotes connective tissue homeostasis by slow release of biologically active substances, affecting primarily the permeability of vessels and vascular tone, maintenance of electrolyte and water balance, and composition of the extracellular matrix. Along with this, they can rapidly release inflammatory mediators and chemotactic factors that ensure the mobilization of effector innate immune cells to fight against a variety of pathogens. Furthermore, they play a key role in initiation of allergic reactions. Aggregation of high affinity receptors to IgE (FcεRI) results in rapid degranulation and release of inflammatory mediators. It is known that reactive oxygen species (ROS) participate in intracellular signaling and, in particular, stimulate production of several proinflammatory cytokines that regulate the innate immune response. In this review, we focus on known molecular mechanisms of FcεRI-dependent activation of mast cells and discuss the role of ROS in the regulation of this pathway.

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Mast cells (tissue basophils, mastocytes, labrocytes) constitute a multifunctional cell population involved in maintaining local homeostasis of connective tissue and some functional systems (control of blood coagulation, blood–tissue barrier permeability, etc.), as well as in defensive reactions of innate and adaptive immunity (inflammation, defense against microorganisms and parasites, immune response) [1-3]. Their properties are due to the presence of a wide spectrum of biologically active substances that are enclosed in specific granules. Cells of this type are identified in all classes of vertebrates. Some invertebrates, such as ascidia (Urochordates) have primitive leukocyte-like ancestral forms of basophils and mast cells, which along with the protective properties (the primitive local innate immunity reactions) can remodel tissue. They possess phagocytic and bactericidal activity and function as inducers of inflammation. Both types of primitive progenitor cells contain histamine and heparin in the granules. During phylogenesis, functional activity of the mast cells is largely shifted toward the regulation of homeostasis and reparation of connective tissue [4, 5].

Mammalian mast cells belong to the myeloid series and are of medullary origin like the blood basophils [6, 7]. Morphologically and functionally, they are similar to the basophils. However, in contrast to basophils, their final differentiation and maturation is completed in connective

Abbreviations: Akt, protein kinase B; bFGF, basic fibroblast growth factor; BMMCs, bone marrow mast cells; ER, endoplasmic reticulum; Btk, Bruton tyrosine kinase; CTMC, connective tissue mast cells; DAG, diacylglycerol; DNP-BSA, dinitrophenyl conjugated to bovine serum albumin; DNP-HSA, dinitrophenyl conjugated to human serum albumin; ERK1 (ERK2), extracellular signal-regulated kinases; Fyn, tyrosine-protein kinase from Src family; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; InsP3, inositol-1,4,5-trisphosphate; JNK, c-Jun N-terminal kinases; LAT, linker for activation of T cells; LPS, lipopolysaccharide; LTX4, leukotrienes, where X – C, D, E, F; Lyn, tyrosine-protein kinase from Src family; MAPKs, mitogen activated protein kinase; MCP, monocyte chemoattractant protein; MEK, mitogen-activated protein kinase kinase; MIP, macrophage inflammatory protein; MMC, mucosal mast cells; NGF, nerve growth factor; NTAL, non-T-cell activation linker; p38, mitogen-activated protein kinase; PGX, prostaglandins, where X – E2, D2, D4; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PLC (PLD), phospholipase C (D); PtdIns(4,5)P2, phosphatidylinositol-4,5-bisphosphate; RANTES, chemokine, regulated on activation, normal T cell expressed and secreted; RAF, mitogen-activated protein kinase; RAS, small GTPase; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; SCF, stem cell factor; SK, sphingosine kinase; SOD, superoxide dismutase; Syk, spleen tyrosine kinase; TGF-β, transforming growth factor beta; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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tissue. Bone marrow undifferentiated mononuclear precursors of mast cells enter the blood, then migrate into barrier tissues and organs – mucous membranes (especially of the digestive tract), skin derma, serous membranes, spleen, and perivascular areas and become resident connective tissue cells (Fig. 1).

In the tissues, maturation of the specific mast cell granules containing a variety of biologically active substances occurs. The composition of granules defines the functionality of these cells. Basically, they contain mediators by which the mast cells affect their microenvironment – primarily microvascular permeability and function of connective tissue, supporting its normal homeostasis. In 1 g of loose connective tissue, there are 10^4-10^6 mast cells, representing up to 10% of all cells, thus indicating their important role. The regulatory function of mast cells is manifested by the growth of their number in organ stroma with increased functional activity, as well as within and around foci of inflammation, in the healing wounds, and in tumors. Furthermore, they can respond to any harmful tissue effects by participating in initiation and development of inflammation, providing protection against a wide spectrum of microorganisms (bacteria, viruses, fungi, multicellular parasites) by releasing inflammatory mediators and chemotactic factors initiating mobilization of effector cells [9, 10].

One of the most studied and probably the basic mechanism of mast cell activation is IgE binding with the high-affinity receptor FcεRI. It is believed that IgE and IgG derived from a common phylogenetic ancestor – IgY. IgY-like immunoglobulins are found in amphibians, reptiles, and birds. Transition to IgE is observed only in mammals and probably occurred during the separation of the mammalian branch from the branch of reptiles. IgE is primarily associated with development of the Th2-branch of the immune response. IgE binding of antigens (proteins of parasites and allergens) results in initiation of FcεRI signaling. It should be mentioned that only a small part of antigens serves as allergens [11-13]. However, FcεRI signaling can be triggered in the absence of antigen. Thus, binding of IgE stimulates survival of mast cells, exposition of FcεRI receptors, and production and release of histamine and leukotrienes. IgE binding also enhances the response to substance 48/80 and substance P, as well as the adhesion of mast cells to fibronectin and their migration [14].

There are many other receptors of mast cells that play an important role in their activation: receptors coupled with G-protein (including purinergic receptors, receptors to complement component 3a (C3a), chemokines, leukotrienes, prostaglandins, etc.), cytokine receptors, receptors to sphingosine-1-phosphate (S1P), stem cell factor (SCF or c-Kit ligand), and pathogen-associated molecular patterns (PAMPs). These receptors can either modulate FcεRI-mediated activation of mast cells or independently stimulate the release of mediators from mast cells through a variety of mechanisms [15, 16].

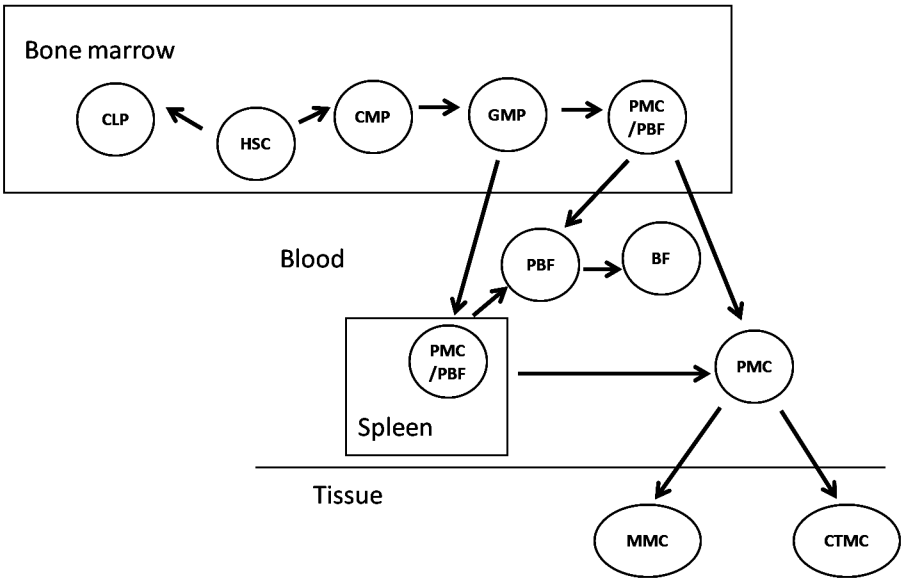

Fig. 1. Schematic diagram of the development, migration, and localization of mast cells and basophils. Mast cells and basophils are derived from hematopoietic stem cells (HSC) giving rise to the common lymphoid progenitors (CLP) and common myeloid precursors (CMP). CMP give rise to granulocyte-monocyte precursors (GMP), which in addition to the common ancestor of mast cells and basophils (PMC/PBF) in the bone marrow and spleen also give rise to neutrophils, eosinophils, and monocytes. Basophils (BF) through basophil precursor (PBF) are differentiated from PMC/PBF and are released into the blood in mature form. In contrast to basophils, mast cells are finally differentiated from the precursors (PMC) in the tissues where maturation of the granules is induced by factors of the microenvironment (the major growth factors for mast cells are cytokines SCF and IL-3) [2, 6, 8]. There are at least two populations of mast cells – mucosal mast cells (MMC) and connective tissue mast cells (CTMC), which differ in composition and arrangement of the granules.

It is known that reactive oxygen species (ROS) stimulate the production of some proinflammatory cytokines and participate in the regulation of innate immunity. There is increasing evidence to suggest that ROS can cause reversible posttranslational modifications of proteins involved in intracellular signal transduction pathways [17, 18]. Evidently, ROS play an important role in FcεRI-dependent signaling that provides rapid degranulation of mast cells [19-27].

MORPHOLOGY AND CLASSIFICATION

Mast cells vary widely in diameter, i.e. from 4 to 24 μm. Most have round or oval form, and the nucleus follows the shape of the cell. The structures of mitochondria, Golgi apparatus, and endoplasmic reticulum (ER) are typical for these organelles. Microfilaments are present under the plasma membrane in the cytoplasm of mast cells, and under the plasmalemma there are microfilaments, while microtubules are located under the plasma membrane as well as in the perinuclear area near the centrioles. The cytoplasm of mature mast cells is more than 40% filled with secretory granules, surrounded by a single membrane. Among them, there are two main types: lysosome-like azurophilic granules, constituting a small part of the granules, and specific metachromatic – the majority of the granules having a diameter of about 1 μm. These granules contain the whole spectrum of the main active ingredients [28]. Classification of mature mast cells is based on the presence in their specific granules of tryptase and chymase – proteases responsible for cleavage of some neuromediators and cytokines as well as collagen. In humans, as well as in mice, two subpopulations of these cells are identified – mucosal mast cells (MMC), which are characterized by the presence of tryptase without chymase, and mast cells of the connective tissue (CTMC), that contain both enzymes. Some authors also distinguish a third population of mast cells that contain only chymase. However, in humans they are less tissue specific than in mouse. These cells are found mainly in the submucous and/or in the mucous tissue of the stomach, small intestine, and colon. It is worth mentioning that contents of the granules can vary and greatly depend on the extracellular microenvironment. Thus, in the presence of IL-4 the chymase content in human mast cells increases [1, 10, 29]. Differences in morphology of these cells and the response to pharmacological agents reflect differences in their functions *in vivo*. MMC play an important role in parasite invasions and, probably, in allergic reactions. They have smaller size (4-10 μm) and shorter life span than CTMC, and their function depends on T-cells, as they carry more FcεRI receptors, and their cytoplasm contains IgE. MMC contain low levels of histamine, but produce many cysteinyl leukotrienes (LTC4, LTD4, LTE4, LTF4). In turn, CTMC are characterized by higher content of histamine and produce high levels of prostaglandin D2 (Table 1). Drugs have different effects

Table 1. General characteristics of two major subpopulations of mast cells [1, 32, 33]

Main properties	Populations of mast cells	
	MMC	CTMC
Localization	mucous membrane of intestine and respiratory tract	skin and submucous layer of intestine
Histamine content	lower	higher
Peptidoglycans	chondroitin sulfate	heparin
Eicosanoids	LTC4 > PGE2	PGE2 > LTC4
Serine proteases	tryptase	tryptase, chymase
FcεRI expression on mast cell	about 100,000	about 10,000
Nonimmune activation (substance 48/80, substance P, complement component)	absent	present
FcεRI-dependent activation	yes	yes
Growth factors	SCF, IL-3, IL-9, TGF-β	SCF, IL-4
Specific functions	antiparasitic activity, allergic reactions	participation in reparation and remodeling of connective tissue

Table 2. Mediators of mast cells

Groups of substances	Mediators	Effects
	1. Preformed mediators	
Biogenic amines	histamine, serotonin, dopamine, polyamines	regulation of vascular tone, increase in permeability of capillaries, smooth muscle contraction, angiogenesis, excitation of nerve endings
Lysosomal enzymes	β-hexosaminidase, β-glucuronidase, β-D-galactosidase, arylsulfatase A, cathepsins C, B, L, D, and E	hydrolysis of proteins, lipids and, peptidoglycans
Proteases	chymase, tryptase, carboxypeptidase A, cathepsin G, granzyme B, matrix metalloproteinases	collagen degradation; modifications of cyto/chemokines
Other enzymes	kinogenase, heparanase, angiogenin, active caspase 3	synthesis of vasodilator – kinin
Peptidoglycans	heparin, chondroitin sulfate	stimulation of angiogenesis, stabilization of NGF; synthesis of cartilaginous tissue, anti-inflammatory effects
Cytokines	TNF, IL-4, IL-15	stimulation of leukocyte migration
Chemokines	RANTES (CCL5), eotaxin (CCL11), IL-8 (CXCL8), MCP-1 (CCL2), MCP-3 (CCL7), MCP-4 (CCL13)	leukocyte recruitment and migration into inflamed tissues
Growth factors	TGF-β, bFGF, VEGF, NGF, SCF	proliferation and differentiation of various cells
Peptides	corticotropin, endorphin, endothelin-1, cathelicidin LL-37, P substance, vasoactive intestinal peptide, angiogenin, bradykinin, leptin, renin, somatostatin, urocortin	vasodilation, analgesic effect, sepsis, mast cell activation, angiogenesis, synthesis of angiotensin
	2. Newly formed mediators	
Lipid metabolites	prostaglandins D2, E2, leukotrienes B4, C4, PAF	regulation of vascular permeability, smooth muscle contraction, recruitment of immune effector cells, stimulation of mucus secretion
Cytokines	IL-1-6, IL-8-10, IL-12, IL-13, IL-16, IL-17, IL-33, IFN I, II, TNF, MIP-2β	leukocyte migration and activation
Growth factors	SCF, GM-CSF, bFGF, NGF, PDGF, TGF-β, VEGF	regulation of proliferation and differentiation of various cells

on mast cell degranulation depending on the type of mast cells [1, 30, 31].

Functional activity of mast cells depends on the spectrum of the secreted bioactive substances (Table 2). Among the mast cell mediators, it is accepted to distinguish the so-called preformed – synthesized by the resting mast cells and accumulated in their granules, and newly formed – synthesized only by the stimulated mast cells. Preformed mediators include biogenic amines (histamine, serotonin), glycosaminoglycans (heparin, chondroitin sulfate), and enzymes (tryptase, chymase). The newly synthesized include various metabolites of arachidonic acid – prostaglandin D2 (PGD2) and leukotriene D4 (LTD4), platelet-activating factor (PAF), cytokines and chemokines, etc. Preformed mediators are characterized by rapid secretion while the metabolites of arachidonic acid are secreted more slowly [1, 3, 28, 34, 35].

As noted above, several other surface receptors, in addition to FcεRI, play an important role in mast cells activation [9]. Because mast cells play a pivotal role in host defense against bacterial, fungal, and viral infections, Toll-like receptors (TLRs) are the most interesting [10, 36]. Mast cells express multiple receptors to PAMPs (pathogen-associated molecular patterns), including

TLRs. Upon binding of TLR ligands, mast cells secrete cytokines, chemokines, and lipid mediators [10, 37]. Some studies demonstrate that TLR2 activation may also stimulate the degranulation of mast cells [38]. However, in some primary cultures and immortalized mast cells as well as *in vivo* studies, activation of TLR2 does not lead to degranulation of mast cells [39]. Moreover, *in vivo* studies have demonstrated that injection of one of the TLR ligands – peptidoglycan – resulted in mast cell-mediated increase in endothelium permeability, while the other TLR ligand – LPS – had no such effect [39].

There is also information on the effect of TLR ligands on synthetic processes in mast cells. Thus, cultivation of human mast cells with LPS and peptidoglycan causes a change in their cytokine and protease profile. Cultivation of human mast cells LAD2 with LPS leads to increased expression of TLR4 and the further increase in TNF production in response to LPS stimulation [37]. Some controversial data on the effect of TLR2 ligand on mast cell degranulation requires further investigation.

In the context of antigenic stimulation of FcεRI receptor in the presence of agonists of TLR4 – LPS, TLR2/TLR1 – P3C (Pam3CSK4, a synthetic ligand for TLR1/2), and of TLR2/TLR6 – MALP-2 (synthetic ligand for TLR2/TLR6 originally isolated from mycobacteria) and peptidoglycan, cytokines production by the mouse bone-marrow-derived mast cells (BMMC – mast cells isolated from bone marrow) and the MC/9 cells (mouse mast cell line) is enhanced, while degranulation remains at the same level [40]. BMMC and MC/9 cells under the influence of LPS produce more Th2-cytokines, such as IL-5, IL-10, and IL-13, and increase the secretion of these cytokines in response to activation of the FcεRI receptor [41]. Thus, there is mutual enhancement of the activation of TLR and FcεRI.

Degranulation. Release of bioactive substances from mast cells by exocytosis into surrounding tissue occurs in the process of degranulation, which can be mediated by both immune and non-immune pathways. The immune pathway is usually activated by aggregation of specific surface receptors FcεRI as a result of their binding with several complexes of antigens with IgE (in case of immediate hypersensitivity reaction the antigen is called the allergen) (Fig. 2). The signal transmission via γ-chains of the receptor leads to an increase in calcium concentration in the cytosol, which activates degranulation as well as synthesis of new mediators [1, 15]. The activation of mast cells described in the beginning, soon after the discovery of IgE in 1966, has been presented as the allergic reaction. Currently, it is considered as an important mechanism to protect the organism against penetration of various pathogens, particularly parasites [33].

Non-immune pathways of mast cell activation are triggered by substances such as neuropeptides (substance P, somatostatin, vasoactive intestinal peptide, neuropeptide Y, neurotensin), polycationic mast cell activators (substance 48/80, mastoparan, polymyxin B), cytokines and chemokines (IL-3, IL-4, IL-12, SCF), anaphylatoxins (compliment fragments C3a, C4a, C5a), calcium ionophores (A23187 or ionomycin), eosinophilic granule proteins, components of bacterial cell wall (LPS, peptidoglycan), and some other substances [9, 16, 34].

Two mechanisms of mast cell degranulation have been described: piecemeal degranulation (lasting several days) and immediate (anaphylactic) degranulation (lasting minutes). The structural mechanism of piecemeal degranulation is based on the microvesicular transport of the specific granule content to plasma membrane [42]. In addition to mast cells, this type of degranulation is typical for neutrophils [43], eosinophils [44], basophils [42], and endocrine cells [42]. In the process of gradual degranulation, selective release of mediators contained in the granules occurs. This type of secretion appears to be the main mechanism of excretion of small doses of biologically active substances by mast cells. It is used for regulation of various physiological processes to maintain homeostasis, mainly changing vascular tonus and permeability and, consequently, histotrophic nutrition and electrolyte balance [29, 34, 45]. *In vivo* piecemeal degranulation of mast cells is observed in wound healing, angiogenesis, mechanical and low temperature effects on the skin, as well as in some other pathologies [42]. On the contrary, upon induction of immediate degranulation mediated by exocytosis during the first few minutes there is a fusion between the granules and the cellular membrane, which enables rapid release of the granule content. This type of degranulation is typical for acute processes – acute inflammation, allergic reactions, and anaphylactic shock. This type of degranulation is mediated by IgE-FcεRI signaling (Fig. 2).

FcεRI-mediated activation. One of the best-studied (due to significant clinical importance) scenarios of mast cell activation is mediated by antigen-induced aggregation of the high-affinity receptors to Fc fragment of IgE (FcεRI). FcεRI is a tetrameric receptor composed of a single α-chain, which is responsible for IgE binding, a β-chain, and two γ-chains linked by two disulfide bridges, which are responsible for the initiation of signaling [15] (Fig. 2). FcεRI aggregation leads to phosphorylation of tyrosine residues in ITAM (immunoreceptor tyrosine-based activation motifs) of β- and γ-chains by Lyn kinase, which belongs to the Src family of tyrosine kinases. This leads to the binding of Syc kinase (Src family of tyrosine kinases) to phosphorylated ITAM through SH2-domains. Thus, activated Syc kinase causes phosphorylation of transmembrane adapter molecules LAT (linker for activation of T cells) and NTAL (non-T-cell activation linker) [46]. Phosphorylation of LAT attracts different cytosolic adapter molecules (GRB2, growth-factor-receptor-bound protein 2; GADS, GRB2-related adaptor protein; SHC, SH2-domain-containing transforming protein C; SLP7, SH2-domain-containing leukocyte

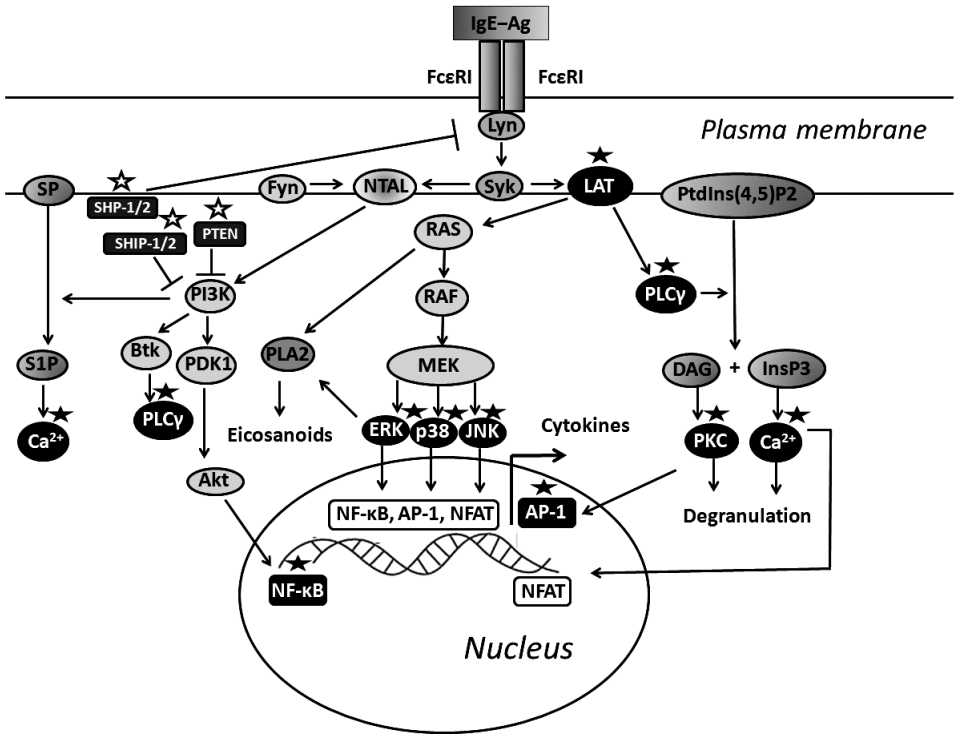

Fig. 2. Scheme of FcεRI-dependent pathways of mast cell activation. Activation of mast cells occurs via the LAT-dependent pathway. The activated state is maintained via the NTAL-dependent pathway. Legend: IgE-Ag –complex of IgE with antigen/allergen. Asterisks denote potential targets of ROS. White stars indicate inhibitory effect and black stars – activating effect.

protein of 65 kDa), guanine-nucleotide-exchange factors SOS and VAV, as well as phospholipase Cγ (PLCγ). This macromolecular complex initiates a further signal transmission [15]. Adapter molecule NTAL is expressed by B cells, mast cells, monocytes, and NK cells. Unlike LAT, NTAL has no PLCγ-binding domains. In the absence of NTAL, phosphorylation of LAT is increased. Conversely, in the absence of LAT an increase in NTAL phosphorylation occurs. However, the lack of both adapter molecules does not lead to full suppression of mast cell activation, indicating that there are additional ways to activate them [47].

An important role in the activation of mast cells is played by PLCγ. As a result of the activation by LAT, PLCγ catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) in the plasma membrane. Inositol 1,4,5-trisphosphate (InsP3) and diacylglycerol (DAG) formed in this reaction induce calcium release from intercellular stores to the cytosol and activation of protein kinase C (PKC). Another signaling pathway is triggered by LAT-dependent activation of the small GTPase RAS, which leads to activation of kinase RAF, further kinase MEK, and finally, the mitogen-activated protein kinases (MAPKs) – ERK1, ERK2, p38, and JNK. MAPKs in turn activate transcription factors such as AP-1, NFAT, and NF-κB, which initiate production of many cytokines. Activation of Fos and Jun proteins (AP-1 components) is regulated by PKC. Activation of NFAT is calcium-dependent [48]. LAT-dependent activation of MAPK-signaling requires the presence of VAV and SOS, which shifts the equilibrium from inactive form of RAS (GDP-bound) to active (GTP-bound). There is also a LAT-independent pathway of mast cell activation that is mediated by Fyn, another kinase from the Src family. This pathway does not lead to activation of PLCγ, but it activates phosphatidylinositol-3-kinase (PI3K) via cytosolic adapter protein GAB2 (GRB2-associated binding protein 2). Results of research on the role of this kinase in activation of mast cells demonstrate that it is not involved in initiation of calcium mobilization but helps to maintain high cytosolic calcium concentration required for successful degranulation of mast cells. PI3K can ensure calcium mobilization in several possible ways: by attracting kinase Btk with further activation of PLCγ or the through sphingosine kinase pathway. Sphingosine kinase (SK) activated through LYN- and FYN-dependent pathways initiates the formation of the sphingolipid mediator sphingosine-1 phosphate (S1P) from sphingosine. S1P participates in calcium mobilization from intracellular stores through an InsP3-independent pathway [47, 49]. SK activity is regulated by phospholipase D (PLD). In turn, PLD is activated by a PI3K-dependent pathway. Currently, NTAL is considered as an adapter molecule that plays an important role in the regulation of

the FYN-GAB2-PI3K-pathway of mast cell activation. Activation of PI3K recruits to the plasma membrane serine/threonine kinase PDK1, which activates another serine/threonine kinase AKT. AKT positively regulates activation of the transcription factor NF-κB due to phosphorylation and further proteolytic degradation of the inhibitor of NF-κB – IκB [47]. It has been shown that inhibition of Fyn did not decrease activation of mast cells. However, inhibition of GAB2 leads to suppression of PI3K activity, thus decreasing MAPK, p38, and JNK phosphorylation, NFAT activation, and TNF production, but has virtually no effect on mast cell degranulation [50]. In summary, it can be assumed that LAT-dependent signaling plays a crucial role in the FcεRI-dependent pathway in the process of degranulation as well as cytokine production by activated mast cells. At the same time, the GAB2-PI3K-pathway is involved in the late response, which consists of maintaining the activation and its strengthening, primarily affecting the secretion of cytokines.

Protein phosphatases such as SHP-1, SHP2, SHIP1, SHIP2, and PTEN play an important role in the regulation of FcεRI-mediated activation of mast cells [47]. The β-subunit of FcεRI is constitutively associated with SHP-1, while SHP-2 recruitment occurs only after aggregation of FcεRI. SHP-1 and SHP-2 can directly dephosphorylate ITAM in FcεRI. However, there are some indications that SHP-1 can have either inhibitory or activating effect on cytokine production**,** while providing no effect on degranulation. The same applies to the phosphatase SHP-2: SHP-2-deficient mast cells demonstrate decreased activation of Fyn and Erk as well as the secretion of TNF. Such a complex effect of these phosphatases in the FcεRI-dependent activation of mast cells can be explained by the participation of these phosphatases in the signaling pathways that regulate the survival and differentiation of mast cells [27].

Phosphatases PTEN, SHIP-1, and SHIP-2 are negative regulators of PI3K-signaling; however, their inhibition leads to different consequences. Thus, inhibition of PTEN using RNA-interference enhances mast cell degranulation. In SHIP-1-deficient mice, there is only a slight increase in degranulation. At the same time, it has been shown that at supra-optimal antigen doses Lyn-dependent binding of SHIP-1 with β-subunits of FcεRI occurs, leading to inhibition of degranulation as well as production of proinflammatory cytokines [27, 51, 52].

Based on these data, we propose the following scheme of FcεRI-dependent pathways of mast cell activation (Fig. 2).

Role of calcium in mast cell activation. The degranulation process depends on increase in cytosolic Ca^{2+} concentration. Degranulation of mast cells includes translocation of granules, their docking to the plasma membrane, and finally fusion with the membrane and release the contents into the extracellular environment. The first stage – the translocation of the granules – is Ca^{2+}-independent, whereas the next stages of degranulation are Ca^{2+}-dependent [53]. In addition, there are indications of involvement of Ca^{2+} in other aspects of mast cell activation: synthesis of leukotriene, prostaglandin, and cytokine [54]. There are two sources of calcium ions for degranulation – intracellular stores and influx of extracellular Ca^{2+} through plasma membrane channels. Activation of PLCγ results in formation of InsP3, which binds to its receptor (InsP3R) at the ER membrane causing Ca^{2+} release through ryanodine-sensitive channels [55]. Emptying of the Ca^{2+} depot in ER is followed by rapid Ca^{2+} entry from extracellular medium controlled by the STIM1–ORAI1 system consisting of molecular sensor STIM1 at the ER and Ca^{2+} channel CRAC at the plasma membrane [54]. There is also another way of Ca^{2+} influx from extracellular medium, which involves Fyn kinase and Ca^{2+}-channels TRPC1 [56]. Recently, one more type of Ca^{2+}-channels – Cav1.2 (belonging to the family of LTCCs channels), which plays an important role in regulation of mast cell activation and survival was described [54].

There are some indications that successful degranulation of mast cells requires calcium transport across the mitochondrial membrane. It has been shown that decreased expression of MCU (mitochondrial calcium uniporter) – a protein responsible for calcium transport across the inner mitochondrial membrane – resulted in FcεRI-mediated degranulation. However, it remains unclear how these processes are related [57].

Role of ROS in mast cell activation. Reactive oxygen species (ROS) are free radicals (molecules or fragments of molecules having one or more unpaired electrons on the atomic or molecular orbitals) that are derived from oxygen molecules. ROS also include hydrogen peroxide, hypochlorous acid, and singlet oxygen, which can form radicals in the extracellular and intracellular environment. There are several sources of ROS in a cell. These include the electron transport chain of mitochondria, matrix dehydrogenases, the p66shc protein in the intermembrane space and monoamine oxidase in the outer mitochondria membrane, xanthine oxidase, cyclooxygenase, myeloperoxidase, NADPH-oxidase (NOX-enzymes), cytochrome P450, and lipoxygenase. Most of these enzymes form superoxide (O_2^-), which is then converted to peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) formed in the Fenton reaction. NOX-enzymes and lipoxygenase generate ROS in response to hormones, growth factors, and cytokines. It is worth noting that the bulk of ROS produced in a cell are unstable, and therefore their signaling function can be limited. Only hydrogen peroxide is a relatively stable substance, but it is rapidly decomposed in a cell due to high content of peroxiredoxins and other components of the internal antioxidant system [58]. In high concentrations, ROS exert damaging effects on DNA, lipids, and proteins. However, at low

concentrations ROS are important mediators involved in regulation of cell growth, adhesion, differentiation, cell death, etc. [25, 59].

Numerous studies have shown that degranulation of mast cells induced by chemical agents (salts of mercury and gold, substance 48/80, Ca^{2+} ionophores, etc.) as well as by physiological stimuli (antigens, neurotrophic growth factor, substance P, etc.) is accompanied by generation of ROS [21]. However, there are conflicting data on the sources of ROS in mast cells as well as their role in the activation of mast cells. According to some reports, a major source of ROS in mast cells is NADPH-oxidase 2 (NOX2) [60]. Inhibition of NADPH-oxidase attenuates phosphorylation of Lyn and the related signaling [61].

It has been established that a powerful source of ROS in humans and mice BMMCs is lipoxygenase-5, and to lesser extent cyclooxygenase-1. However, inhibition of ROS and ROS production by these enzymes has no effect on secretion of cytokines and degranulation of mast cells [62].

The use of inhibitors of various components of FcεRI-signaling showed that ROS generation depended on the kinases of Src family (Lyn and Syk) and on PI3K. Activation of PI3K stimulates translocation of Btk kinase to lipid rafts and its following phosphorylation by members of the Src kinase family. This can be a key step in signal transduction leading to ROS production. Activation of Syk and/or Btk can stimulate ROS production by NOX2. The produced ROS participates in the formation and/or maintenance of the macromolecular complex that regulates Ca^{2+} mobilization [21]. In the model of ovalbumin-induced food allergy, data were obtained that indicate the participation of ROS (stimulated by PI3K pathway) in enhancing SOC-dependent Ca^{2+} mobilization due to increased expression of components of this system [26].

Activation of mast cells by the factors causing phagocytosis can also be accompanied by ROS production. Thus, BMMCs stimulated with silicone dioxide produce TNF, IL-13, and MCP-1 in ROS-dependent manner. At the same time, silicon dioxide does not affect degranulation of mast cells [63].

ANTIOXIDANTS, INHIBITORS OF ROS PRODUCTION, AND ACTIVATION OF MAST CELLS

By now, many experimental data, often contradictory, have been accumulated about a significant role of ROS in activation and degranulation of mast cells *in vitro* and *in vivo*. According to different authors, inhibition of ROS production in mast cells leads to ambiguous results due to various characteristics of different mast cell lines, to different modes of their activation, to different methods of ROS modulation, and to complexity of data interpretation in animal models.

Thus, it was shown that inhibition of ROS accumulation using MnTBAP, exhibiting the both superoxide dismutase (SOD) and peroxidase activities, prevents FcεRI-dependent degranulation and secretion of leukotrienes in mouse BMMCs. Similar results were obtained for mast cells of RBL-2H3 line (cells derived from rat basophilic leukemia) [21]. At the same time, the glutathione peroxidase mimetic ebselen that selectively decreases the level of H_2O_2 inhibits the cytokine production, but it has no effect on degranulation of mast cells. This indicates a possible difference in the role of O_2^- and H_2O_2 in activation of mast cells [21, 64].

Several studies have shown that the use of antioxidants (SOD-mimetics, resveratrol) *in vitro* and *in vivo* inhibits degranulation of mast cells as well as production of cytokines. Endogenic antioxidants such as thioredoxin-1 and heme oxygenase-1 also affect the activation of mast cells [65]. BMMCs of transgenic mice with overexpression of thioredoxin-1 differ from BMMCs of wild-type mouse by reduced ability to secret histamine, but they do not differ in the ability to secrete cytokines [66].

It was shown that rutin and chlorogenic acid, polyphenol antioxidants from tobacco leaves, reduce histamine secretion by IgE-activated mast cells but at the same time increase production of IL-6, IL-10, IL-13, TNF, IFNγ [67]. Another study demonstrated that epigallocatechin-3-gallate, a polyphenol from green tea, inhibits secretion of leukotriene C4 and degranulation of mast cells [68].

Studies in mice deficient for antioxidant protein DJ-1 showed that their BMMCs have reduced ability for degranulation and production of TNF and IL-4 [69].

It was found that UCP2 (uncoupling protein 2), a protein of the inner mitochondrial membrane capable of regulating the production of ROS, suppresses activation of mast cells [70]. UCP2 belongs to the family of uncoupling proteins whose title member UCP1 causes thermoregulatory uncoupling of oxidative phosphorylation in the mitochondria of brown fat [71]. By analogy with UCP1, it was suggested that the other proteins of this family possess the same activity in mitochondria of other tissues. However, recent studies have shown that UCP2 is not capable of uncoupling, but catalyzes the transport of malate, oxaloacetate, and aspartate in exchange for phosphate across the inner mitochondrial membrane [72]. Export of C4 substrates from mitochondria causes inhibition of the Krebs cycle and increases glutaminolysis, thus considerably altering mitochondrial metabolism and, in particular, decreases the production of ROS. BMMCs of *Ucp2*-deficient mice contain increased amounts of histamine, and this shift is inhibited by SOD-mimetic MnTBAP that is mostly accumulated in the mitochondria [70]. These cells produce more IL-6 and prostaglandin D2 (PGD2) and contain elevated levels of the phosphorylated (activated) form of ERK, which regulates prostaglandin synthesis. It has also been demon-

Table 3. Effects of antioxidants and inhibitors of ROS production on mast cells

No.	Model used in study	Modulator(s) of ROS production	Activator of degranulation	Effect	References
1	2	3	4	5	6
1	Peritoneal mast cells of Brown Norway (BN) rats	deferoxamine (forms a stable complex with iron, inhibiting hydrogen peroxide decomposition to form hydroxyl radical)	mercury chloride (allergen)	prevention of serotonin release	[82]
2	MC-9 cell line (mice mast cells)	rutin and chlorogenic acid (polyphenol antioxidants)	sensitization of anti-DNP-IgE and stimulation of DNP-BSA	decrease in histamine secretion, increase in production of IL-10, IL-13, IFN-γ, IL-6 and TNF	[67]
3	RBL-2H3 cells (cells derived from rat basophilic leukemia)	diphenyl iodonium – inhibitor of flavoprotein oxidoreductases; inhibits ROS production by NADPH-oxidases and by mitochondria [83]	sensitization of anti-DNP-IgE and stimulation of DNP-BSA; A23187 (calcium ionophore)	decrease in histamine, β-hexosaminidase and LTC4 secretion; inhibition of Ca^{2+} influx	[64, 84]
	BMMCs generated from BDF1 mice	ebselen (antioxidant that mimics activity of glutathione-peroxidase)		decrease in LTC4 secretion; no effect on secretion of histamine and β-hexosaminidase; inhibition of Ca^{2+} influx with its partial retention	
4	RBL-2H3 cells	overexpression of hemoxygenase-1 (an enzyme with antioxidant properties)	A23187 (calcium ionophore)	decrease in β-hexosaminidase secretion	[85]
5	RBL-2H3 cells; leukocytes (including basophils) isolated from human blood	diphenyl iodonium	antibodies to FcεRI (high-affinity receptor for IgE)	inhibition of histamine and LTC4 secretion	[86]
6	Guinea pig model of bronchial asthma	SOD mimetic M40403 (antioxidant)	albumin (used to induce allergic reactions in the model)	reduced mast cells degranulation, and other parameters of inflammation in the airways	[87]
7	BMMCs from BDF1 mice; RBL-2H3 cells	MnTBAP – agent exhibiting activity of superoxide dismutase (SOD) and peroxidase	sensitization of anti-DNP-IgE and stimulation of DNP-BSA	reduction of degranulation and secretion of leukotrienes	[21]
8	BMMCs from transgenic C57BL/6 mice with overexpression of thioredoxin-1	overexpression of thioredoxin-1 (enzyme with antioxidant properties that play important role in protection of cells from oxidative stress, together with the glutathione system)	sensitization of anti-DNP-IgE and stimulation of DNP-BSA	reduced secretion of histamine, no effect on the secretion of TNF and IL-6	[66]
9	RBL-2H3 cells	liposomal hydrogel with povidone-iodine (antioxidant)	sensitization of anti-DNP-IgE and stimulation of DNP-HSA	reduced secretion of β-hexosaminidase	[88]
10	Ucp2$^{-/-}$ C57BL/6 mice	lack of Ucp2 – mitochondrial protein that regulates the production of ROS in mitochondria	substance P (biologically active peptide, facilitates mast cells degranulation)	increased vascular permeability	[70]

Table 3. (Contd.)

1	2	3	4	5	6
	BMMCs from Ucp2$^{-/-}$ C57BL/6 mice	lack of Ucp2	sensitization of anti-DNP-IgE and stimulation of DNP-HSA	increased secretion of histamine and IL-6 production, PGD2	
			substance 48/80 (polyamine, which activates receptor for substance P and induces histamine secretion in mast cells)	increased secretion of histamine and PGD2 production	
			substance P; A23187	increased secretion of histamine	
	LAD2 human mast cells line	increased expression of Ucp2	substance P; IgE sensitization and stimulation by anti-IgE antibodies	reduced histamine secretion	
11	RBL-2H3 cells	resveratrol (phytoalexin – a steroid metabolite with antioxidant properties)	sensitization of anti-DNP-IgG and stimulation of DNP-HSA	reduced secretion of histamine and β-hexosaminidase	[89]
	BALB/c mice			inhibition of passive cutaneous anaphylaxis reaction	
12	DJ-1 knockout C57BL/6 mice	lack of DJ-1 (protein with antioxidant properties)	sensitization of anti-DNP-IgG and stimulation of DNP-HSA	inhibition of passive cutaneous anaphylaxis reaction	[69]
	BMMCs from DJ-1 knockout C57BL/6 mice			reduced secretion of β-hexosaminidase, TNF and IL-4	

strated *in vivo* that vascular permeability induced by substance P, in *Ucp2*-deficient mice, is higher than in wild-type animals. Furthermore, UCP-2 inhibits exocytosis of insulin granules of pancreatic β-cells [73] and dopamine-containing vesicles of pheochromocytoma PC12 [74].

UCP2 is a unique protein that not only neutralizes ROS but also prevents their formation. UCP2 can also affect the kinetics of calcium influx required for degranulation of mast cells [75]. UCP2 is important not only for the functioning of mast cells, but also for other cells of the immune system. It is expressed in mitochondria of lymphocytes, dendritic cells, neutrophils, and macrophages. *Ucp2*-deficient mice develop more severe forms of autoimmune encephalomyelitis and diabetes induced experimentally. Macrophages of these mice are characterized by higher sensitivity to LPS, while macrophages with increased expression of UCP2 produce less ROS in response to LPS and have a reduced ability for transendothelial migration [70].

Degranulation of stimulated mast cells is also affected by agents that uncouple oxidative phosphorylation. The uncoupler carbonyl cyanide *m*-chlorophenylhydrazone inhibits antigen-stimulated secretion of β-hexosaminidase of rat mast cells RBL-2H3 [76]. The commonly used antibacterial and antimycotic agent triclosan, which also has uncoupling properties, inhibits degranulation of some types of mast cells stimulated by IgE–antigen complex as well as by thapsigargin (inhibitor of Ca^{2+}-ATPase of ER inducing passive release of Ca^{2+} from intercellular stores into cytoplasm) [77]. Mechanisms of the inhibitory action of mitochondrial uncouplers on degranulation of mast cells have not been investigated. In addition to inhibition of ATP production, uncouplers can reduce the excessive generation of ROS by mitochondrial respiratory chain due to inhibition of Ca^{2+} accumulation in mitochondria [78].

It should be mentioned that degranulation is accompanied by Drp-1-mediated fragmentation of mitochon-

dria and their translocation to the plasma membrane [79]. Inhibition of Drp1 activity or its expression inhibits mitochondria fragmentation and translocation to the plasma membrane, thus decreasing degranulation of mast cells (human mast cell line LAD2 and mast cells derived from human umbilical blood) and secretion of TNF [79]. It is known that fragmentation of mitochondria in cells can be induced by mitochondrial ROS [80, 81], which may contribute to the regulation of mast cell activation.

Table 3 summarizes data on the effects of antioxidants and inhibitors of ROS production on the activation of mast cells. In practically all cases, inhibition of degranulation as well as cytokine production decline was observed.

ROS can cause reversible posttranslational modifications of proteins involved in intracellular signaling. Thus, some of them have functionally important cysteine residues that can be oxidized. For example, H_2O_2 can oxidize sulfhydryl groups (-SH) to form sulfenic acid (-SOH), which can react with glutathione to form disulfide bond or with the amides to form sulfonamides. The sulfenic acid residue (-SOH) can be oxidized to sulfinic acid ($-SO_2H$) and further to sulfonic acid ($Cys-SO_3H$). Each of these modifications can change protein activity, thus altering its function in signal transduction pathways [17, 18].

Now we review the potential protein targets of ROS involved in the signaling pathways mediating mast cells activation. One of the main events modulated by ROS and/or by changes in redox status of cells is elevation of cytosolic Ca^{2+}, which plays an important role in degranulation of mast cells. This is achieved by redox-sensitivity of the Ca^{2+} channels as well as proteins involved in the regulation of their activity [22, 90]. In particular, an important role in Ca^{2+} mobilization is played by mitochondrial ROS [22, 91], but these mechanisms are not yet fully understood. It is worth mentioning that the changes in intracellular concentration of Ca^{2+}, in turn, also affect the generation of ROS [22].

Phosphatases SHP1, SHP2, and PTEN participating in mast cell activation have in their catalytic center cysteine residues that serve as one of the possible targets of ROS [23, 24]. It has been shown that inhibition of phosphatases with H_2O_2 and/or with pervanadate promotes phosphorylation of tyrosine residues of β- and γ-subunits of FcεRI as well as calcium influx and mast cell degranulation [27].

All PKC isoforms contain zinc finger domains and high concentrations of cysteine residues located in the regulatory area, as well as free sulfhydryl groups in the catalytic site. Additionally, the redox dependence of PKC can be associated with oxidative activation of PLCγ [92, 93] and Ca^{2+} mobilization, as well as with phosphorylation of tyrosine residues by redox-sensitive kinases of the Src family [94]. Consequently, one of the mechanisms regulating mast cell activation may be mediated by ROS-dependent activation of PKC.

Another possible target for ROS is an adapter protein LAT [19], which facilitates the induction of FcεRI-mediated pathway of mast cell activation.

Regarding the secretion of proinflammatory cytokines and eicosanoids, it is known that MAPK-dependent signaling is ROS-dependent [95] and at least two transcription factors that are activated following FcεRI stimulation are redox-sensitive – NF-κB [96, 97] and AP-1 [20, 96].

Based on the currently available data, it is possible to make a well-founded suggestion that ROS play an important role in the regulation of the best studied and, apparently, the most important for mast cell degranulation FcεRI-signaling, affecting the activity of most of the known participating proteins. Potential targets for ROS established to date are presented in the diagram (Fig. 2).

Studies on the impact of ROS on various aspects of mast cell activation, especially on the FcεRI-dependent pathway, open perspectives for the development and introduction into clinical practice of drugs based on antioxidants and inhibitors of ROS production. These drugs may be used for treatment of pathologies associated with hyperactivation of mast cells, first of all allergic reactions.

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REFERENCES

1. Omelyanenko, N. P., and Slutsky, L. I. (2009) *Connective Tissue (Histophysiology and Biochemistry)* [in Russian], Vol. 1, Izvestiya, Moscow, p. 378.
2. Yarilin, A. A. (2010) *Immunology* [in Russian], GEOTAR-Media, Moscow, p. 752.
3. Da Silva, E., Jamur, M., and Oliver, C. (2014) Mast cell function: a new vision of an old cell, *J. Histochem. Cytochem.*, **62**, 698-738.
4. Mulero, I., Sepulcre, M. P., Meseguer, J., Garcia-Ayala, A., and Mulero, V. (2007) Histamine is stored in mast cells of most evolutionarily advanced fish and regulates the fish inflammatory response, *Proc. Natl. Acad. Sci. USA*, **104**, 19434-19439.
5. Crivellato, E., Travan, L., and Ribatti, D. (2015) The phylogenetic profile of mast cells, *Methods Mol. Biol.*, **1220**, 11-27.
6. Voehringer, D. (2013) Protective and pathological roles of mast cells and basophils, *Nat. Rev. Immunol.*, **13**, 362-375.

7. Dahlin, J. S., and Hallgren, J. (2015) Mast cell progenitors: origin, development and migration to tissues, *Mol. Immunol.*, **63**, 9-17.
8. Sasaki, H., Kurotaki, D., and Tamura, T. (2016) Regulation of basophil and mast cell development by transcription factors, *Allergol. Int.*, **65**, 127-134.
9. Molderings, G. J. (2010) Mast cell function in physiology and pathophysiology, *Biotrend Chem. AG*, **5**, 1-11.
10. Weller, C. L., Collington, S. J., Williams, T., and Lamb, J. R. (2011) Mast cells in health and disease, *Clin. Sci. (London)*, **120**, 473-484.
11. Du Pasquier, L., and Litman, G. W. (2012) *Origin and Evolution of the Vertebrate Immune System*, Springer Science & Business Media, New York, p. 326.
12. Brinkworth, J. F., and Pechenkina, K. (2013) *Primates, Pathogens, and Evolution*, Springer Science & Business Media, New York, p. 428.
13. Fitzsimmons, C. M., Falcone, F. H., and Dunne, D. W. (2014) Helminth allergens, parasite-specific IgE, and its protective role in human immunity, *Front. Immunol.*, **5**, 1-12.
14. Kawakami, T., and Kitaura, J. (2005) Mast cell survival and activation by IgE in the absence of antigen: a consideration of the biologic mechanisms and relevance, *J. Immunol.*, **175**, 4167-4173.
15. Gilfillan, A. M., and Tkaczyk, C. (2006) Integrated signaling pathways for mast cell activation, *Nat. Rev. Immunol.*, **6**, 218-230.
16. Yu, Y., Blokhuis, B. R., Garssen, J., and Redegeld, F. A. (2016) Non-IgE mediated mast cell activation, *Eur. J. Pharmacol.*, **778**, 33-43.
17. Sena, L. A., and Chandel, N. S. (2012) Physiological roles of mitochondrial reactive oxygen species, *Mol. Cell*, **48**, 158-167.
18. Kalinina, E. V., Chernov, N. N., and Novichkova, M. D. (2014) The role of glutathione, glutathione transferase, and glutaredoxin in regulation of redox-dependent processes, *Biochemistry (Moscow)*, **79**, 1562-1583.
19. Gringhuis, S. I., Papendrecht-van der Voort, E. A., Leow, A., Levarht, E. W., and Verweij, F. C. (2002) Effect of redox balance alterations on cellular localization of LAT and downstream T-cell receptor signaling pathways, *Mol. Cell. Biol.*, **22**, 400-411.
20. Gius, D. (2004) Redox-sensitive signaling factors and antioxidants: how tumor cells respond to ionizing radiation, *J. Nutr.*, **134**, 3213S-3214S.
21. Suzuki, Y., Yoshimaru, T., Inoue, T., Niide, O., and Ra, C. (2005) Role of oxidants in mast cell activation, *Chem. Immunol. Allergy*, **87**, 32-42.
22. Csordas, G., and Hajnoczky, G. (2009) SR/ER-mitochondrial local communication: calcium and ROS, *Biochim. Biophys. Acta*, **1787**, 1352-1362.
23. Hamanaka, R. B., and Chandel, N. S. (2010) Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes, *Trends Biochem. Sci.*, **35**, 505-513.
24. Ostman, A., Frijhoff, J., Sandin, A., and Bohmer, F. D. (2011) Regulation of protein tyrosine phosphatases by reversible oxidation, *J. Biochem.*, **150**, 345-356.
25. Cosentino-Gomes, D., Rocco-Machado, N., and Meyer-Fernandes, J. R. (2012) Cell signaling through protein kinase C oxidation and activation, *Int. J. Mol. Sci.*, **13**, 10697-10721.
26. Yang, B., Yang, C., Wang, P., Li, J., Huang, H., Ji, Q., Liu, J., and Liu, Z. (2013) Food allergen-induced mast cell degranulation is dependent on PI3K-mediated reactive oxygen species production and upregulation of store-operated calcium channel subunits, *Scand. J. Immunol.*, **78**, 35-43.
27. Hughes, M. R., and McNagny, K. M. (2015) *Mast Cells: Methods and Protocols, Methods in Molecular Biology*, Springer Science & Business Media, New York, p. 540.
28. Wernersson, S., and Pejler, G. (2014) Mast cell secretory granules: armed for battle, *Nat. Rev. Immunol.*, **14**, 478-494.
29. Wenk, P. A. (2012) Mast cells, *Mikro Graf*, **41**, 4-6.
30. Collington, S. J., Williams, T. J., and Weller, C. L. (2011) Mechanisms underlying the localisation of mast cells in tissues, *Trends Immunol.*, **32**, 478-485.
31. Blank, U., Falcone, F. H., and Nilsson, G. (2013) The history of mast cell and basophil research – some lessons learnt from the last century, *Allergy*, **68**, 1093-1101.
32. Westerberg, C. M., Ulleras, E., and Nilsson, G. (2012) Differentiation of mast cell subpopulations from mouse embryonic stem cells, *J. Immunol. Methods*, **382**, 160-166.
33. Saito, H., Ishizaka, T., and Ishizaka, K. (2013) Mast cells and IgE: from history to today, *Allergol. Int.*, **62**, 3-12.
34. Kondashevskaya, M. V. (2011) *Heparin – a New Paradigm of Effects* [in Russian], MDV Studio, Moscow, p. 274.
35. Theoharides, T. C., Alysandratos, K. D., Angelidou, A., Delivanis, D. A., Sismanopoulos, N., Zhang, B., Asadi, S., Vasiadi, M., Weng, Z., Miniati, A., and Kalogeromitros, D. (2012) Mast cells and inflammation, *Biochim. Biophys. Acta*, **1822**, 21-33.
36. Pawankar, R., Holgate, S. T., and Rosenwasser, L. J. (2009) *Allergy Frontier. Classification and Pathomechanisms*, Springer Science & Business Media, New York, p. 606.
37. Sandig, H., and Bulfone-Paus, S. (2012) TLR signaling in mast cells: common and unique features, *Front. Immunol.*, **3**, 1-13.
38. Supajatura, V., Ushio, H., Nakao, A., Akira, S., Okumura, K., Ra, C., and Ogawa, H. (2002) Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity, *J. Clin. Invest.*, **109**, 1351-1359.
39. Yu, Y., Yip, K. H., Tam, I. Y. S., Sam, S. W., Ng, C. W., Zhang, W., and Lau, H. Y. A. (2014) Differential effects of the Toll-like receptor 2 agonists, PGN and Pam3CSK4 on anti-IgE induced human mast cell activation, *PLoS One*, **9**, 1-9.
40. Qiao, H., Andrade, M. V., Lisboa, F. A., Morgan, K., and Beaven, M. A. (2006) FcεR1 and Toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells, *Blood*, **107**, 610-618.
41. Masuda, A., Yoshikai, Y., Aiba, K., and Matsuguchi, T. (2002) Th2 cytokine production from mast cells is directly induced by lipopolysaccharide and distinctly regulated by c-Jun N-terminal kinase and p38 pathways, *J. Immunol.*, **169**, 3801-3810.
42. Crivellato, E., Nico, B., Mallardi, F., Beltrami, C. A., and Ribatti, D. (2003) Piecemeal degranulation as a general secretory mechanism? *Anat. Rec. A Discov. Mol. Cell Evol. Biol.*, **274**, 778-784.
43. Mahmudi-Azer, S., Weller, P. F., Dvorak, A. M., Moqbel, R., and Pare, P. D. (2010) Eosinophil granules function

extracellularly as receptor-mediated secretory organelles, *Allergy Asthma Clin. Immunol.*, **6**, 1-20.
44. Melo, R. C., and Weller, P. F. (2010) Piecemeal degranulation in human eosinophils: a distinct secretion mechanism underlying inflammatory responses, *Histol. Histopathol.*, **25**, 1341-1354.
45. Theoharides, T. C., and Cochrane, D. E. (2004) Critical role of mast cells in inflammatory diseases and the effect of acute stress, *J. Neuroimmunol.*, **146**, 1-12.
46. Zhu, M., Zou, J., Li, T., O'Brien, S. A., Zhang, Y., Ogden, S., and Zhang, W. (2015) Differential roles of phospholipase D proteins in FcεRI-mediated signaling and mast cell function, *J. Immunol.*, **195**, 4492-4502.
47. Rivera, J. (2005) NTAL/LAB and LAT: a balancing act in mast-cell activation and function, *Trends Immunol.*, **26**, 119-122.
48. Ma, J. (2006) *Gene Expression and Regulation*, Springer, New York.
49. Jolly, P. S., Bektas, M., Olivera, A., Gonzalez-Espinosa, C., Proia, R. L., Rivera, J., Milstien, S., and Spiegel, S. (2004) Transactivation of sphingosine-1-phosphate receptors by FcεRI triggering is required for normal mast cell degranulation and chemotaxis, *J. Exp. Med.*, **199**, 959-970.
50. Siraganian, R. P., De Castro, R. O., Barbu, E. A., and Zhang, J. (2010) Mast cell signaling: the role of protein tyrosine kinase Syk, its activation and screening methods for new pathway participants, *FEBS Lett.*, **584**, 4933-4940.
51. Xiao, W., Nishimoto, H., Hong, H., Kitaura, J., Nunomura, S., Maeda-Yamamoto, M., Kawakami, Y., Lowell, C. A., Ra, C., and Kawakami, T. (2005) Positive and negative regulation of mast cell activation by Lyn via the FcεRI, *J. Immunol.*, **175**, 6885-6892.
52. Huber, M. (2013) Activation/inhibition of mast cells by supra-optimal antigen concentrations, *Cell Commun. Signal.*, **11**, 1-11.
53. Nishida, K., Yamasaki, S., Ito, Y., Kabu, K., Hattori, K., Tezuka, T., Nishizumi, H., Kitamura, D., Goitsuka, R., Geha, R. S., Yamamoto, T., Yagi, T., and Hirano, T. (2005) FcεRI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane, *J. Cell Biol.*, **170**, 115-126.
54. Islam, M. S. (2012) *Calcium Signaling*, Springer Science & Business Media, Dordrecht-New York.
55. Tshori, S., and Razin, E. (2010) Editorial: mast cell degranulation and calcium entry – the Fyn-calcium store connection, *J. Leukoc. Biol.*, **88**, 837-838.
56. Sanchez-Miranda, E., Ibarra-Sanchez, A., and Gonzalez-Espinosa, C. (2010) Fyn kinase controls FcεRI receptor-operated calcium entry necessary for full degranulation in mast cells, *Biochem. Biophys. Res. Commun.*, **391**, 1714-1720.
57. Furuno, T., Shinkai, N., Inoh, Y., and Nakanishi, M. (2015) Impaired expression of the mitochondrial calcium uniporter suppresses mast cell degranulation, *Mol. Cell Biochem.*, **410**, 215-221.
58. Bystrova, M. F., and Budanova, E. N. (2007) Hydrogen peroxide and peroxiredoxins in redox regulation of intracellular signaling, *Biol. Membr.*, **24**, 115-125.
59. Holmstrom, K. M., and Finkel, T. (2014) Cellular mechanisms and physiological consequences of redox-dependent signaling, *Nat. Rev. Mol. Cell Biol.*, **15**, 411-421.
60. Li, Z. Y., Jiang, W. Y., and Cui, Z. J. (2015) An essential role of NAD(P)H oxidase 2 in UVA-induced calcium oscillations in mast cells, *Photochem. Photobiol. Sci.*, **14**, 414-428.
61. Itoh, T., Fujita, Y., Ito, M., Masuda, A., Ohno, K., Ichihara, M., Kojima, T., Nozawa, Y., and Ito, M. (2009) Molecular hydrogen suppresses FcεRI-mediated signal transduction and prevents degranulation of mast cells, *Biochem. Biophys. Res. Commun.*, **389**, 651-656.
62. Swindle, E. J., Coleman, J. W., DeLeo, F. R., and Metcalfe, D. D. (2007) FcεRI- and Fcγ-receptor-mediated production of reactive oxygen species by mast cells is lipoxygenase- and cyclooxygenase-dependent and NADPH oxidase-independent, *J. Immunol.*, **179**, 7059-7071.
63. Brown, J. M., Swindle, E. J., Kushnir-Sukhov, N. M., Holian, A., and Metcalfe, D. D. (2007) Silica-directed mast cell activation is enhanced by scavenger receptors, *Am. J. Respir. Cell Mol. Biol.*, **36**, 43-52.
64. Suzuki, Y., Yoshimaru, T., Matsui, T., Inoue, T., Niide, O., Nunomura, S., and Ra, C. (2003) FcεRI signaling of mast cells activates intracellular production of hydrogen peroxide: role in the regulation of calcium signals, *J. Immunol.*, **171**, 6119-6127.
65. Catalli, A., Karpov, V., Pundir, P., Dimitrijevic, A., and Kulka, M. (2010) Comparison of the inhibitory effects of resveratrol and tranilast on IgE, 48/80 and substance P-dependent mast cell activation, *Allergy Asthma Clin. Immunol.*, **6**, 1-14.
66. Son, A., Nakamura, H., Kondo, N., Matsuo, Y., Liu, W., Oka, S., Ishii, Y., and Yodoi, J. (2006) Redox regulation of mast cell histamine release in thioredoxin-1 (TRX) transgenic mice, *Cell Res.*, **16**, 230-239.
67. Chen, S., Gong, J., Liu, F., and Mohammed, U. (2000) Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation, *Immunology*, **100**, 471-480.
68. Inoue, T., Suzuki, Y., and Ra, C. (2010) Epigallocatechin-3-gallate inhibits mast cell degranulation, leukotriene C4 secretion, and calcium influx via mitochondrial calcium dysfunction, *Free Radic. Biol. Med.*, **49**, 632-640.
69. Kim, D. K., Kim, H. S., Kim, A. R., Kim, J. H., Kim, B., Noh, G., Kim, H. S., Beaven, M. A., Kim, Y. M., and Choi, W. S. (2013) DJ-1 regulates mast cell activation and IgE-mediated allergic responses, *J. Allergy Clin. Immunol.*, **131**, 1653-1662.
70. Tagen, M., Elorza, A., Kempuraj, D., Boucher, W., Kepley, C. L., Shirihai, O. S., and Theoharides, T. C. (2009) Mitochondrial uncoupling protein 2 inhibits mast cell activation and reduces histamine content, *J. Immunol.*, **183**, 6313-6319.
71. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997) Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia, *Nat. Genet.*, **15**, 269-272.
72. Vozza, A., Parisi, G., De Leonardis, F., Lasorsa, F. M., Castegna, A., Amorese, D., Marmo, R., Calcagnile, V. M., Palmieri, L., Ricquier D., Paradies, E., Scarcia, P., Palmieri, F., Bouillaud, F., and Fiermonte, G. (2014) UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation, *Proc. Natl. Acad. Sci. USA*, **111**, 960-965.
73. Li, Y., Maedler, K., Shu, L., and Haataja, L. (2008) UCP-2 and UCP-3 proteins are differentially regulated in pancreatic beta-cells, *PLoS One*, **3**, e1397.

74. Yamada, S., Isojima, Y., Yamatodani, A., and Nagai, K. (2003) Uncoupling protein 2 influences dopamine secretion in PC12h cells, *J. Neurochem.*, **87**, 461-469.
75. Trenker, M., Malli, R., Fertschai, I., Levak-Frank, S., and Graier, W. F. (2007) Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport, *Nat. Cell. Biol.*, **9**, 445-452.
76. Mohr, F. C., and Fewtrell, C. (1987) The relative contributions of extracellular and intracellular calcium to secretion from tumor mast cells. Multiple effects of the proton ionophore carbonyl cyanide *m*-chlorophenylhydrazone, *J. Biol. Chem.*, **262**, 10638-10643.
77. Weatherly, L. M., Shim, J., Hashmi, H. N., Kennedy, R. H., Hess, S. T., and Gosse, J. A. (2016) Antimicrobial agent triclosan is a proton ionophore uncoupler of mitochondria in living rat and human mast cells and in primary human keratinocytes, *J. Appl. Toxicol.*, **36**, 777-789.
78. Korshunov, S. S., Skulachev, V. P., and Starkov, A. A. (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria, *FEBS Lett.*, **416**, 15-18.
79. Zhang, B., Alysandratos, K. D., Angelidou, A., Asadi, S., Sismanopoulos, N., Delivanis, D. A., Weng, Z., Miniati, A., Vasiadi, M., Katsarou-Katsari, A., Miao, B., Leeman, S. E., Kalogeromitros, D., and Theoharides, T. C. (2011) Human mast cell degranulation and preformed TNF secretion require mitochondrial translocation to exocytosis sites: relevance to atopic dermatitis, *J. Allergy Clin. Immunol.*, **127**, 1522-1531.
80. Pletjushkina, O. Y., Lyamzaev, K. G., Popova, E. N., Nepryakhina, O. K., Ivanova, O. Y., Domnina, L. V., Chernyak, B. V., and Skulachev, V. P. (2006) Effect of oxidative stress on dynamics of mitochondrial reticulum, *Biochim. Biophys. Acta*, **1757**, 518-524.
81. Wu, S., Zhou, F., Zhang, Z., and Xing, D. (2011) Mitochondrial oxidative stress causes mitochondrial fragmentation via differential modulation of mitochondrial fission-fusion proteins, *FEBS J.*, **278**, 941-954.
82. Wolfreys, K., and Oliveira, D. B. (1997) Alterations in intracellular reactive oxygen species generation and redox potential modulate mast cell function, *Eur. J. Immunol.*, **27**, 297-306.
83. Li, Y., and Trush, M. A. (1998) Diphenyleneiodonium, an NAD(P)H oxidase inhibitor, also potently inhibits mitochondrial reactive oxygen species production, *Biochem. Biophys. Res. Commun.*, **253**, 295-299.
84. Matsui, T., Suzuki, Y., Yamashita, K., Yoshimaru, T., Suzuki-Karasaki, M., Hayakawa, S., Yamaki, M., and Shimizu, K. (2000) Diphenyleneiodonium prevents reactive oxygen species generation, tyrosine phosphorylation, and histamine release in RBL-2H3 mast cells, *Biochem. Biophys. Res. Commun.*, **276**, 742-748.
85. Takamiya, R., Murakami, M., Kajimura, M., Goda, N., Makino, N., Takamiya, Y., Yamaguchi, T., Ishimura, Y., Hozumi, N., and Suematsu, M. (2002) Stabilization of mast cells by heme oxygenase-1: an anti-inflammatory role, *Am. J. Physiol. Heart Circ. Physiol.*, **283**, 861-870.
86. Yoshimaru, T., Suzuki, Y., Matsui, T., Yamashita, K., Ochiai, T., Yamaki, M., and Shimizu, K. (2002) Blockade of superoxide generation prevents high-affinity immunoglobulin E receptor-mediated release of allergic mediators by rat mast cell line and human basophils, *Clin. Exp. Allergy*, **32**, 612-618.
87. Masinia, E., Banib, D., Vannaccia, A., Pierpaolia, S., Mannaionia, P. F., Comhairc, S. A. A., Xuc, W., Muscolid, C., Erzurumc, S. C., and Salveminie, D. (2005) Reduction of antigen-induced respiratory abnormalities and airway inflammation in sensitized guinea pigs by a superoxide dismutase mimetic, *Free Radic. Biol. Med.*, **39**, 520-531.
88. Beukelman, C. J., Van den Berg, A. J., Hoekstra, M. J., Uhl, R., Reimer, K., and Mueller, S. (2008) Anti-inflammatory properties of a liposomal hydrogel with povidone-iodine (Repithel) for wound healing *in vitro*, *Burns*, **34**, 845-855.
89. Han, S. Y., Bae, J. Y., Park, S. H., Kim, Y. H., Park, J. H. Y., and Kang, Y. H. (2013) Resveratrol inhibits IgE-mediated basophilic mast cell degranulation and passive cutaneous anaphylaxis in mice, *J. Nutr.*, **143**, 632-639.
90. Song, M. Y., Makino, A., and Yuan, J. X. (2011) Role of reactive oxygen species and redox in regulating the function of transient receptor potential channels, *Antioxid. Redox Signal.*, **15**, 1549-1565.
91. Waypa, G. B., Marks, J. D., Mack, M. M., Boriboun, C., Mungai, P. T., and Schumacker, P. T. (2002) Mitochondrial reactive oxygen species trigger calcium increases during hypoxia in pulmonary arterial myocytes, *Circ. Res.*, **91**, 719-726.
92. Gonzalez-Pacheco, F. R., Caramelo, C., Castilla, M. A., Deudero, J. J., Arias, J., Yague, S., Jimenez, S., Bragado, R., and Alvarez-Arroyo, M. V. (2002) Mechanism of vascular smooth muscle cells activation by hydrogen peroxide: role of phospholipase C gamma, *Nephrol. Dial. Transplant.*, **17**, 392-398.
93. Jang, J. Y., Min, J. H., Chae, Y. H., Baek, J. Y., Wang, S. B., Park, S. J., Oh, G. T., Lee, S.-H., Ho, Y.-S., and Chang, T.-S. (2014) Reactive oxygen species play a critical role in collagen-induced platelet activation via SHP-2 oxidation, *Antioxid. Redox Signal.*, **20**, 2528-2540.
94. Steinberg, S. F. (2015) Mechanisms for redox-regulation of protein kinase C, *Front. Pharmacol.*, **6**, 128.
95. Son, Y., Cheong, Y. K., Kim, N. H., Chung, H. T., Kang, D. G., and Pae, H. O. (2011) Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? *J. Signal. Transduct.*, **2011**, 792639-792644.
96. Karin, M., Takahashi, T., Kapahi, P., Delhase, M., Chen, Y., Makris, C., Rothwarf, D., Baud, V., Natoli, G., Guido, F., and Li, N. (2001) Oxidative stress and gene expression: the AP-1 and NF-kappaB connections, *Biofactors*, **15**, 87-89.
97. Mohora, M., Greabu, M., Totan, A., Mitrea, N., and Battino, M. (2009) Redox-sensitive signaling factors and antioxidants, *Farmacia*, **57**, 399-411.