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REVIEW

Extracellular Actin in Health and Disease

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Abstract—This review considers the functions of extracellular actin – cell surface bound, associated with extracellular matrix, or freely circulating. The role of this protein in different pathological processes is analyzed: its toxic effects and involvement in autoimmune diseases as an autoantigen. The extracellular actin clearance system and its role in protection against the negative effects of actin are characterized. Levels of free-circulating actin, anti-actin immunoglobulins, and components of the actin clearance system as prognostic biomarkers for different diseases are reviewed. Experimental approaches to protection against excessive amounts of free-circulating F-actin are discussed.

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Actin is a ubiquitous protein in eukaryotic cells [1]. It exists as a monomer (G) and as a polymer (F) and is a key component of the actin microfilament system – the most dynamic and plastic part of the cytoskeleton [2]. In mammals, several actin isoforms are known: α-actin, including separate forms specific for skeletal and smooth muscle cells and cardiomyocytes, γ-actin of smooth muscle cells, and ubiquitous β- and γ-actin isoforms occurring in the cytosol of all cells [3].

The actin microfilament system is responsible for cell shape [4, 5] and motility [6, 7], and it ensures the interaction of cells with each other [8], with components of the extracellular matrix [9], and with various artificial substrates [10]. The actin cytoskeleton plays a key role in vesicular transport, cell compartmentalization, and distribution of macromolecules within cells [11]. Reorganization of the actin cytoskeleton and expression of different actin isoforms are closely associated with cell differentiation processes [12]. This system is also involved in cell division [11]. The actin skeleton was shown to play an important and rather unambiguous role at different stages of programmed cell death (PCD) [13-17]. In some cases, the actin microfilament system plays the key role in this process. Such type of PCD is classified as actin-mediated apoptosis [18]. It has been shown that actin is also present in the cell nucleus, where it plays a key role in chromatin remodeling, RNA polymerase I, II, and III transcription, and mRNA processing [19].

The actin cytoskeleton, in addition to the above-mentioned fundamental functions in cell vital activity, also plays a key role in the development of different diseases. In particular, the actin microfilament system is involved in oncological processes (cell transformation, invasion, and metastasis) [20-22] and tissue fibrosis [23, 24]. It has been shown that many intracellular parasites (bacteria, viruses) use the actin cytoskeleton in their vital activity [25].

An important feature of actin is that it can be present also in the extracellular environment of an organism. It can be found bound to the outer cell surface [26], in the extracellular matrix [27], or in the systemic circulation – blood, lymph, and liquor [28-30]. The localization of actin in the extracellular space raises many questions whose answers may be of key importance for solving urgent problems of cell biology and medicine. This review is devoted to the analysis of the state-of-the-art in studying the role of extracellular actin in different physiological and pathological processes.

Abbreviations: DAMPs, danger-associated molecular patterns; Ig, immunoglobulins; PCD, programmed cell death.

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ACTIN ON THE CELL SURFACE AND IN EXTRACELLULAR MATRIX

Questions about the mechanisms of the appearance of actin in the extracellular environment have not been sufficiently studied. Most obvious is the release of this protein in case of the impairment of cell wall integrity [31, 32]. In this context, F-actin is rated among the so-called "danger-associated molecular patterns" (DAMPs) – intracellular molecules secreted, released, or exposed on the cell surface during cell death, damage, or exposure to stress [33, 34]. In addition to F-actin, they include Bcl-2, calreticulin, cyclophilin A, heat shock proteins, histones, HMGB1, HMGN1, and mitochondrial DNA. After being released from a cell, these components act as adjuvants or "alarm signals" for the immune system [31].

The possibility of producing G- and F-actin as components of fibroblast exosomes under mechanical stress has also been shown [35]. It can be supposed that subsequent destruction of these vesicles contributes to formation of the pool of extracellular actin. This is especially relevant for connective tissues with extensive and dense extracellular matrix (e.g. cartilage), where there is practically no utilization of exosomes, dead cells, and their fragments because phagocytes have restricted access to them [36, 37].

It is reliably established that actin can be released from viable, undamaged cells [38]. At the same time, the protein can stay in a complex with the outer surface of the plasma membrane or be excreted into the extracellular environment. The mechanism of this phenomenon remains an enigma [39], because the protein does not contain a signal peptide for transmembrane transfer or a transmembrane domain [40]. Nevertheless, one cannot rule out the existence of other, more complicated ways of appearing on the cell surface. This can be exemplified by the nuclear import and export mechanisms of actin. The actin molecule has no signal sequences for its transport into the nucleus, but it is transferred together with cofilin that does have a signal sequence [41]. Release from the nucleus is determined by two signals (leucine-rich nuclear export signals) for exportin-1-mediated nuclear export. Actin can also be transferred to the cytosol by exportin-6 in complex with profilin [42, 43]. Although it is difficult to compare the transport across the nuclear envelope (through the nuclear pores) with the transport across the plasma membrane, one could assume the existence of mechanisms of actin transport in complex with other proteins acting as carriers. Possible candidates for the role of actin carriers are actin-binding proteins, which are also found on the cell surface [44], e.g. gelsolin, which can penetrate through the membrane due to a special signal peptide [45]. Generally, the question about these mechanisms is still open and clearly needs further investigation.

As mentioned above, extracellular actin molecules can be found on the cell surface [26, 46]. This phenomenon is also typical of other intracellular proteins that are not characteristic of the cell surface [38]. Some of these molecules are also classified as DAMPs. For example, the expression of Hsp70 on the endothelial cell surface was revealed after their incubation with oxidized low-density lipoproteins (LDL) [47]. Such "non-canonical" localization has been established for other components of the actin microfilament system that are actin-binding proteins. For example, filamin A was found on the surface of neuroblastoma cells and other human cell lines [48]. It was assumed that here the filamin A C-terminus is exposed on the cell surface, while the N-terminus is localized in the cytoplasm bound to the actin cytoskeleton. Filamin A is probably involved in the interaction with the extracellular matrix due to the RGD sequence at the C-terminus [48]. Tropomyosin, which was found on the outer surface of the plasma membrane of endotheliocytes activated by fibroblast growth factor-2 (FGF-2), acts as a receptor for antiangiogenic peptides. Like actin, this protein has no transmembrane domain [44]. It has been shown that cell surface localization is also typical of proteins of the microtubule system. There are data on the exposure of tubulin on the surface of lymphoblasts of CCRF-CEM line cells [49]. For the mentioned proteins, it is still unclear what processes of cell vital activity are associated with their noncanonical localization.

Immunofluorescence and immunoelectron microscopy have shown that actin is present on the plasma membrane surface of endotheliocytes having different localization (smooth muscle α-actin on bovine pulmonary artery endothelial cells (BPAEC)) [46], B-lymphocytes and, to a much lesser extent, T-lymphocytes [50], sperm cells [51], and platelets [52]. The data in the cited works [50-52], however, do not clearly show the form (G or F) of actin localized on the surface of these cells. It can be supposed that cell-surface actin is present mainly as the monomeric form. This form does not induce an active immune response since F-actin is a DAMP, against which antibodies are produced in autoimmune processes. If this is true, later we will raise a question about looking for ways to prevent actin polymerization on the cell surface. It is known that spontaneous formation of actin microfilaments in the cytosol is prevented by various proteins binding G-actin [17]. Further studies must elucidate whether it is possible to use these G-actin-binding proteins for preventing actin polymerization on the cell surface. The presence of F-actin has been quite clearly demonstrated for the sperm head surface [51]. At the same time, testicular tissue is protected from contact with components of the immune system by the hematotesticular barrier [53], which eliminates the possibility of immunization against F-actin. Actin was found in the structure of the extracellular matrix of the myometrium and in the glomerular mesangium of the kidney. Immunoelectron microscopy showed the presence of actin in the extracellular matrix of smooth muscle

cells, being localized in the inner elastic membrane among fibronectin and elastic fibers, as well as between smooth muscle cells [27]. It was suggested that extracellular actin is involved in interactions of smooth muscle cells – their contacts and mutual sliding during contraction and relaxation of tissues [27]. Actin was shown to be present in the extracellular matrix of the aorta [54] and in the walls of arteries, arterioles, and capillaries [27]. It has been established that complexes consisting mainly of β- and γ-actin and, to a lesser extent, of α-actin and α-actinin, are released from the muscle cell surface (chicken embryo culture) [55, 56].

The functions of cell surface- and extracellular matrix-associated actin are of substantial research and practical interest. It has been demonstrated that actin associated with the outer surface of the plasma membrane can participate in different physiological and pathological processes. Membrane-bound actin was shown to be a binding site for plasminogen [40, 57]. This is of great importance for different processes related to the functions of plasmin. Smooth muscle α-actin bound to endotheliocyte surface is an angiogenin receptor [58, 59] and inducer of angiogenesis [40]. At the same time, the actin/angiogenin complex, similarly to actin, can promote plasmin generation due to tissue plasminogen activator (tPA). This complex, in contrast to actin, does not inhibit plasmin activity [60]. In view of this fact, angiogenin promotes degradation of the extracellular matrix, allowing the penetration of endotheliocytes through the basement membrane and migration during angiogenesis [61]. The mechanisms of plasminogen activation may also be involved in pathological processes. In breast cancer, overexpression of some components of the plasminogen activation cascade (urokinase) results in production of large amounts of plasmin on tumor cell surfaces. This contributes to enhanced metastatic activity of these cells, which is an unfavorable prognosis for breast cancer. The binding of plasminogen to actin is an important event in this process [62]. It has been shown that β-actin expressed on the surface of some tumor cells (PC-3, HT1080 and MDA-MB321) possibly contributes to the formation of angiostatin 4.5 from plasmin [39, 63]. This molecule inhibits the proliferation and migration of endotheliocytes and activates their programmed death [64, 65]. This β-actin-mediated mechanism can constrain neoplastic progression. Accordingly, the expression of β-actin on the surface of tumor cells is considered to be a promising prognostic factor [63]. It has been established that actin expressed on the surface of endothelial cells can interact with lipoprotein(a). At the same time, lipoprotein(a) competes with plasminogen for binding with actin, which may contribute to a decrease in plasmin production and reduction in the intensity of fibrinolysis [40]. On the surface of catecholaminergic cells, γ- and β-actin promote the modulation of neurotransmitter release by interacting with plasminogen and stimulating plasmin production [26].

In view of the functional significance of membrane-bound actin, an interesting fact is the existence of natural anti-actin antibodies. This type of antibodies includes autoreactive antibodies with low affinity for the antigen, which play a regulatory role, in contrast to high-affinity antibodies produced in different pathological processes [66]. Some of the natural antibodies against endotheliocytes of essentially healthy people were shown to have specificity for components of the cytoskeleton – β-actin, vimentin, and α-tubulin. These antibodies are supposed to exert antiinflammatory and antithrombotic effects [67].

It is interesting that the amount of cell-surface actin is related with its functional activity – its content varies under different conditions [68]. Hence, the expression of surface-bound actin by LA350 lymphoblastoid cells increases during DNA synthesis, being maximal within the G_1/early S-phase period of the cell cycle. It was suggested that the extracellular actin exposed on the lymphocyte cell surface can interact with the natural pool of anti-actin antibodies, which seems to be a mechanism of regulation of immune processes [69]. The exposure of F-actin on the sperm head surface (especially at the equatorial segment and the acrosome) is associated with acquiring the ability to penetrate into the oocyte [51, 70]. It is supposed that polymerization of actin occurs at the surface of maturing sperm cells and is critical for this process [71]. Actin appears on the platelet surface after thrombin-induced activation of secretion [72, 73]. Thus, the expression of actin on the cell surface and its activity are regulated by various intracellular and extracellular mechanisms, which emphasizes the importance of its functions in this environment.

The ability of actin to self-assemble into microfilaments in the extracellular medium was confirmed by the results of *in vivo* studies [74]. Laser scanning confocal microscopy of preparations of nucleus pulposus of the intervertebral disk, stained with phalloidin–FITC for actin microfilaments, showed the presence of F-actin aggregates in the extracellular matrix of this tissue in discogenic dorsopathy (L_{IV}-L_V) (N. P. Sudakov, I. V. Klimenkov, V. A. Byvaltsev, S. B. Nikiforov, O. A. Goldberg, A. A. Kalinin, L. A. Bardonova, and E. Belykh, unpublished data). These deposits consisted mainly of radially oriented actin microfilaments and were several times larger than the chondrocytes and the neutrophils infiltrating the necrosis area. Thus, it seems urgent to study the potential involvement of extracellular F-actin in degenerative processes in connective tissues as a DAMP and a potential autoantigen. In this connection, the question of the nature of nucleation centers for actin aggregates in the extracellular matrix is of interest – is this process always spontaneous, or can G-actin also be polymerized by interacting with components of extracellular substance? It has been shown that actin *in vitro* can be bound with fibronectin [75], which can be considered as

a potential nucleation center in self-assembly of extracellular F-actin.

Thus, the mechanisms of the release of actin into the extracellular environment have not yet been sufficiently characterized. However, the available data demonstrate that different actin isoforms localized on the outer cell surface and in the extracellular matrix are involved in various physiological and pathological processes. Hence, it is important to thoroughly investigate the functions of this protein and the mechanisms of their regulation.

ACTIN IN SYSTEMIC CIRCULATION AND OTHER BIOLOGICAL FLUIDS

It has been established that actin can enter the systemic circulation (blood [76], liquor [30] and lymph [29]), as well as into urine [77] and bronchoalveolar lavage [78]. The mechanism of penetration of actin into these extracellular environments is probably the destruction of endotheliocytes or the production of active actin by the latter [31, 32, 46]. The possibility of penetration of this protein across the endothelial barrier from surrounding tissues (via endothelial fenestrae, transcytosis, and tight junction opening) should not be excluded [79]. It was shown that α-actin was present in the blood of patients with myocardial infarction, the maximum amount of this protein being found in case of acute anterior wall myocardial infarction [80]. In addition, the release of α-actin into systemic circulation in angina pectoris (stenocardia), when high concentrations of circulating actin were revealed in patients with Class III B disease (according to Braunwald's classification) [28]. High concentrations of α-actin in the serum of patients with non-insulin-dependent diabetes and neuropathy can be a marker of high risk of developing acute myocardial infarction or stenocardia [76]. It has been shown that the actin α-isoform typical of smooth muscle cells is released into blood plasma after extensive tissue damage to the small intestine in rats with ischemia–reperfusion injury of the small intestine and in patients with necrotizing enterocolitis. It can be used for diagnosing intestinal muscle damage [81]. A relationship between release of actin into blood and lethal effect in the case of hepatic necrosis and septic shock has been shown in clinical practice [82-85].

Data of *in vitro* and *in vivo* studies characterize free-circulating actin not only as a marker of cell damage. The release of F-actin into the systemic circulation may have a direct lethal effect. Intravenous injection of G-actin into rats resulted in intravascular self-assembly of actin microfilaments leading to the formation of microthrombi and endothelial damage [74]. Data from various studies demonstrate the mechanisms of events described in this experiment. It has been shown that thrombosis is activated during the formation of actin microfilaments in the bloodstream [84]. In contrast to G-actin, F-actin can activate platelet aggregation due to the bound ADP [86, 87]. The possibility of direct interaction between F-actin and fibrin (but not fibrinogen) has been demonstrated [88]. At the same time, the inclusion of actin microfilaments into a fibrin clot impedes its lysis due to the binding of plasmin and inhibition of its activity [89]. It is also supposed that actin microfilaments can directly change the characteristics of blood flow in vessels and even lead to obstruction of small vessels [85]. The addition of G-actin or actin-containing serum of patients with respiratory distress syndrome to a culture medium had a toxic effect on endotheliocytes from sheep pulmonary artery – the cells die by the mechanism of necrosis. However, the addition of gelsolin to the culture had an opposite effect [90]. The intravenous administration of G-actin to Wistar rats with the model of mesangial proliferative glomerulonephritis induces a great number of microaneurysms and persistent lysis of the mesangium. It was supposed that such effect of exogenous actin is associated with the competition of free-circulating actin for the binding of angiogenin to actin on the endotheliocyte surface and, thereby, the inhibition of restoration of capillary structure [91].

The negative effects of actin in systemic circulation determine the necessity of its elimination from this environment. It is known that in blood plasma there is a system for extracellular actin sequestration and clearance (elimination from the systemic circulation), which involves gelsolin and Gc-globulin (Gc-globulin: group-specific component), also called vitamin D-binding protein [92, 93]. At the same time, gelsolin depolymerizes actin, while Gc-globulin captures actin monomers and accelerates the elimination of actin from the systemic circulation [82]. The kinetics of elimination of circulating G- and F-actin from an organism was investigated. It was found that nephrectomy had no substantial effect on the clearance of Gc-globulin or actin [94, 95]. Liver cells play the key role in this process: G-actin, mainly in complex with Gc-globulin, is captured by Kupffer cells, while F-actin is captured by the endothelial cells of liver sinusoids [96]. At the same time, the clearance of actin/Gc-globulin complexes proceeds much more rapidly compared to the native Gc-globulin [94, 95]. Inadequate functioning of the actin utilization system leads to excessive production of F-actin, which exerts the previously characterized negative effects on cells and components of the extracellular environment [85].

The mechanism of decrease in gelsolin level in the systemic circulation is an interesting issue. This process is supposedly caused both by increase in extracellular actin level [29] and by modulation of gelsolin level by different inflammatory mediators [97].

The effects of intravenous injections of G-actin into mice with Gc-globulin gene knockout (*DBP null*$^{(-/-)}$) and in wild-type mice (*DBP*$^{+/+}$) were investigated [98]. The DBP$^{+/+}$ mice had a more severe form of acute pneu-

monia. Under conditions *in vitro*, the purified actin/Gc-globulin complexes induced damage and death of cultivated endothelial cells from human lung microvessels and umbilical vein. It is interesting that cells incubated with actin/Gc-globulin complexes demonstrated a significant decrease in viability already after 4 h, but this effect was reversible if the cells were further cultivated in fresh medium for 24 h [98].

Studies show that considerable amounts of actin are released into the extracellular space under acute lung damage, and circulating actin/gelsolin complexes can be found in peripheral blood [99]. Patients of intensive care units with decreased plasma gelsolin levels are characterized by greater probability of fatality, longer hospital stay, and longer period of artificial pulmonary ventilation. The level of this protein increased after the state of the patients improved [100]. It has been shown that gelsolin levels in patients undergoing hemodialysis are associated with the development of systemic inflammation [101], as well as with higher mortality risk in the first year of observation [102]. The level of gelsolin is reduced after stroke. Also, its concentration decreases prior to the development of multiple organ failure [97]. It has been shown that the decrease in gelsolin level in blood within a short time after hematopoietic stem cell transplantation can be a predictor of the development of idiopathic pneumonia [103].

The level of Gc-globulin was shown to be reduced in the blood of patients with fulminant hepatic necrosis, especially in non-survivors [104]. At the same time, the concentration of Gc-globulin/actin complexes in blood considerably increases [94, 95, 105]. Decrease in Gc-globulin level in serum is also associated with unfavorable outcome under acute hepatic failure [106]. The serum level of this protein decreases in hamsters with the model of acetaminophen-induced liver damage [107]. Exhaustion of the pool of Gc-globulin under acute hepatic damage is most probably because hepatocytes are the major producers of this protein in an organism [74].

The level of circulating Gc-globulin immediately decreases after severe injuries [108]. The reduced gelsolin concentration is associated with high mortality risk in patients remaining in a critical state after injuries and burns [29].

Hypogelsolinemia is observed in patients with different activities of acute or chronic inflammatory processes – sepsis, atrophic arthritis, and multiple sclerosis [109]. The concentration of gelsolin in blood was also shown to decrease in a model of multiple sclerosis – experimental autoimmune encephalomyelitis in mice [110].

Decrease in the plasma level of gelsolin was shown in human sepsis and in animal models. The extent of decrease in its concentration correlates with the mortality risk [83]. The level of Gc-globulin is considerably reduced in the serum of patients with septic shock. The percentage of circulating Gc-globulin in complex with actin substantially increases, and the concentration of these complexes is closely associated with disease severity and mortality [111]. The release of actin into the systemic circulation and insufficiency of the system of its removal is associated with the development of complications in case of sepsis and organ failure, in particular, respiratory distress and thrombocytopenia [108].

It is noteworthy that components of the actin clearance system interact with various lipids that are mediators of inflammation. It has been shown that Gc-globulin can bind arachidonic acid, which is a substrate for eicosanoid generation under septic shock. At the same time, the formation of a complex with actin disturbs the binding of arachidonic acid [111]. Gelsolin can also be involved in the inflammatory response, exerting an immunomodulatory effect due to binding proinflammatory lipids – lysophosphatidic acid, sphingosine 1-phosphate, and phosphoinositides [29, 109, 112, 113]. In addition, this protein can bind platelet activation factor [114] and bacterial lipopolysaccharides [115]. Nevertheless, there is also a reverse side of this phenomenon – the reduced ability of gelsolin to depolymerize F-actin when binding sphingosine 1-phosphate [109] and lipoteichoic acid (a lipopolysaccharide of Gram-negative bacteria) [116]. Generally, the ability of components of the actin clearance system to interact with lipid mediators of inflammation is another facet of the pathological effects of actin in the systemic circulation. The formation of complexes of these proteins with actin most probably disturbs the immunomodulatory effects of this system due to reduction of its ability to sequester these biologically active lipids [29].

Actin and gelsolin were shown to be present in lymph from mesentery vessels of Sprague Dawley rats at concentrations comparable with the values in blood plasma [29]. The level of gelsolin considerably decreases in the lymph of mesentery vessels under hemorrhagic shock. Its decrease supposedly leads to the enhancement of biological activity of proinflammatory lipids involved in triggering the mechanisms of capillary vessel damage in lungs and other organs.

The free-circulating actin and components of its clearance system have been found in cerebrospinal fluid. Patients with neurodegenerative processes (multiple sclerosis [30], Alzheimer's disease [117]) demonstrate high level of actin in the liquor. The dynamics of its level correlates with the disease progression [118]. In turn, gelsolin concentration in cerebrospinal fluid is substantially reduced in case of various neurological diseases including multiple sclerosis [119, 120]. In Japanese encephalitis, there was a significant increase in the levels of cytoplasmic actin and Gc-globulin in the liquor. It was suggested that this is a consequence of impaired integrity of the blood–brain barrier [121]. The concentration of gelsolin in the liquor is substantially reduced in epilepsy [122]. It has been shown that gelsolin can bind β-amyloid protein in the liquor of patients with Alzheimer's disease [123]. It

is important to mention that gelsolin is able not only to inhibit the formation of fibrils from this protein, but also has a lysing activity against β-amyloid [124]. It may be supposed that the previously discussed actin increase in the liquor in case of Alzheimer's disease [117] will result in the weakening of this protective effect of gelsolin and, therefore, will contribute to the progression of the neurodegenerative disease.

The effects of proinflammatory cytokines on the proteome composition of bronchial epithelial secretions *in vitro* were investigated. The control and the experiment demonstrated the presence of β- and γ-isoforms of actin, actin-like protein 3 (Arp3), and gelsolin. It has been shown that interleukin 4 induces an increase in the secretion of gelsolin precursor into the extracellular environment, which may be of great importance for utilization of actin filaments formed in bronchoalveolar lavage from the products of disintegration of epithelial cells in inflammatory processes [78]. Enhanced gelsolin concentration in bronchoalveolar lavage was also found in asthma patients [125]. The presence of actin in urine was shown after ischemic effect during kidney transplantation [77].

The protective effect of the F-actin depolymerization system and the clearance of F-actin monomers predetermine the need for restoration of the level of its components for therapeutic purposes [83, 97, 101, 126]. Restoration of the plasma level of gelsolin due to the introduction of recombinant gelsolin was shown to reduce mortality in experimental animals with models of sepsis [84, 102, 109], autoimmune encephalomyelitis [110], and stroke [100].

Thus, free-circulating actin can be a biomarker of damage in different tissues. Its polymerized form has multiple negative effects on an organism, acting both on cells and on components of the extracellular environment. Increase in actin level in the systemic circulation can also weaken the additional protective effects of its clearance system not related to actin utilization. Hence, the extracellular actin clearance system is a promising object for the development of technologies for protecting an organism from the lethal effects of circulating actin microfilaments.

EXTRACELLULAR ACTIN AND AUTOIMMUNE PROCESSES

It has been shown that extracellular actin can be involved in the development of various pathologies as an inducer of autoimmunity [127]. As already mentioned, F-actin is one of DAMPs [33] – the intracellular macromolecules and their complexes that can induce inflammatory response when released into the extracellular environment [31, 32]. An important fact confirming the involvement of extracellular F-actin in autoimmunity is the detection of DNGR-1 (CLEC9A) receptors specific for F-actin on the surface of cytotoxic T-lymphocytes and dendritic cells [33, 128, 129].

It has been shown that actin can interact with the component C1q of the complement system (complement component 1, q subcomponent). It was supposed that actin microfilaments can activate the complement cascade due to C1q activation that is not mediated by antibodies [130].

It has been established that Gc-globulin is involved in the activation of macrophages and stimulates chemotaxis in monocytes and neutrophils [131, 132]. However, actin/Gc-globulin complexes *in vitro* cannot activate complement or neutrophils [97].

Data on anti-actin antibodies and their involvement in many pathological processes (hepatitis, celiac disease, atherosclerosis, acute transplant rejection, acute coronary syndrome, autoimmune kidney diseases) are the subject of wide speculation in the literature [133-137].

Most of the research in this field is devoted to type I autoimmune hepatitis [138, 139]. Immunoglobulins specific for F-actin found in blood are an important diagnostic criterion of this disease, on one hand [140, 141], and a predictor of unfavorable outcome on the other [142]. It has been shown that high titers of anti-actin antibodies are associated with the hepatitis activity index [143]. The presence of anti-actin antibodies in the case of autoimmune hepatitis is associated with lower efficacy of corticosteroid therapy in patients [144]. High titers of antibodies against F-actin in serum were revealed in patients with hepatitis C, reflecting more active autoimmunity in liver tissue [145]. The production of anti-actin antibodies was also shown in the case of clometacin-induced hepatitis [146].

It has been shown that actin as an autoantigen can induce Th1 cellular immune response in patients with atherosclerosis and thereby participate in the development of systemic inflammation in this disease [147]. The level of anti-actin antibodies positively correlates with the intima-media thickness and adventitial diameter of carotid arteries in atherosclerosis [148].

Anti-actin and anti-myosin IgGs were found in acute coronary syndrome. The degree of their production was associated with the severity of damage to the heart muscle [137]. Autoimmune responses to troponin I, tropomyosin, and actin were revealed in case of dilated cardiomyopathy – one of the major complications of Emery–Dreifuss muscular dystrophy [149].

The production of IgG against some components of the endothelial cytoskeleton (actin, vimentin, tubulin, and keratin) in the blood of patients is associated with acute heart transplant rejection [135]. However, antibodies against HLA antigens of the transplant, which are the major and most widespread factor in the mechanisms of its humoral rejection [150] and are clearly diagnosed at the pretransplant stage [151], were not found in the examined patients. If the antibodies against the endothe-

lial cytoskeleton were present in the recipient's blood before the transplantation, the transplant was rejected in the early post-transplantation period [135].

The presence of anti-actin IgA in case of celiac disease is closely associated with small intestinal villous atrophy [152]. High levels of these antibodies are a marker of severity of damage to the mucous coat of the small intestine and villous atrophy [153, 154].

There is evidence of generation of an immune response to actin in other diseases. In the case of IgA nephropathy, actin-binding antibodies were found among the IgA of blood serum of patients [155]. Anti-actin IgMs were found in blood also in case of idiopathic nephrotic syndrome [136]. Antibodies against β-actin were found in the serum of patients with autoimmune inner ear disease (Meniere's disease, sudden deafness, rapidly progressing sensorineural hearing loss, otosclerosis) [156]. In autoimmune neutropenia, Wegener's granulomatosis and microscopic polyangiitis are accompanied by the production of anti-neutrophil antibodies (actin being one of their autoantigens) [139, 157]. The possibility of production of anti-actin antibodies has been shown for amyotrophic lateral sclerosis [158].

Altogether, the currently available data provide convincing evidence of the concept that extracellular F-actin not only exerts a direct toxic effect on cells, has a negative influence on the system of hemostasis, and weakens the antiinflammatory properties of components of its clearance system, but it also actively participates in the development of autoimmunity as an autoantigen. The anti-actin antibodies thus produced can be used as prognostic biomarkers of different pathological processes.

The analysis of vast research into the biological role of actin in the life of cells and whole organisms shows that the functions of actin are not confined to its fundamental role as a component of the cytoskeleton. This protein can be released into the extracellular environment through different mechanisms (some of them still need to be determined); there, it (i) remains bound to the cell surface, (ii) binds to the extracellular matrix, or (iii) enters the systemic circulation. Being in complex with the cell surface, actin is a plasminogen-binding site, which determines its important and rather unambiguous role in regulation of angiogenesis and modulation of neurotransmitter release. We suppose that the impairment in these functions of extracellular actin as a result of changes in the level of its expression at the cell surface may be a cause of development and/or complication of certain pathologies. F-actin, being released directly into the systemic circulation or produced there from G-actin due to self-assembly, plays a role in the development of a rather broad range of disorders. Actin microfilaments can exert negative effects both on tissue and organ cells and on components of the extracellular environment (induction of death of endotheliocytes, activation of thrombogenesis and the inhibition of fibrinolysis, the altered rheological properties of blood, reduced ability of the actin clearance system to sequester inflammatory mediators), which may result in fatal outcome. Being a representative of DAMPs, F-actin can induce an autoimmune response, another mechanism of its involvement in various pathological processes. Further studies on the pattern of appearance of extracellular actin and its role in physiological and pathological processes will undoubtedly contribute, in addition to fundamental significance, the development of new technologies for diagnostics, prevention, and therapy for many socially significant diseases.

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