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REVIEW

Molecular Bases of Immune Complex Pathology

K. V. Shmagel* and V. A. Chereshnev

Institute of Ecology and Genetics of Microorganisms, Ural Branch of Russian Academy of Sciences, ul. Goleva 13, 614081 Perm, Russia; fax: (342) 244-6711; E-mail: shmagel@iegm.ru

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Abstract—The binding of antigens with antibodies forms immune complexes in the body. Usually these complexes are eliminated by the system of mononuclear phagocytes without development of pathological changes. This review highlights principal mechanisms responsible for safe removal of immune complexes in primates and humans. Special attention is given to diseases known as "immune complex diseases", when antigen–antibody complexes induce inflammatory reactions. The review considers key experimental works that significantly contributed to current knowledge of etiology and pathogenesis of type III hypersensitivity. Some factors of the development of immune complex syndrome such as level of humoral immune response to antigen, isotype and affinity of forming antibodies, the amount of immune complexes, and the consequences of their interaction with the complement system and Fc-receptors are analyzed based on the molecular mechanisms involved. The review contains a retrospective analysis of the most significant scientific achievements in immune complex pathology investigation within the last 100 years.

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Immune complex formation *in vivo* is a natural process completing a humoral immune response of the body to an antigen. Generally, resulting antigen–antibody complexes are effectively removed by the system of mononuclear phagocytes. Uptake of the immune aggregates by cells preferentially occurs through complement receptors [1, 2] and Fc-receptors (FcR) [3, 4]. Immune complex formation constantly occurs in healthy organisms. Denatured proteins, antigens of gut bacteria, and dead cells undergo binding to natural antibodies followed by subsequent elimination by macrophages. Although clearance activity is rather high (including removal of about 50 g per day of endogenous material from the blood circulation of a human adult), this natural clearance is characterized by pronounced anti-inflammatory mode and occurs without any uncomfortable symptoms [5, 6].

However, there is a group of diseases known as "immune complex diseases" or "type III hypersensitivity" (according to the Gell–Coombs classification [7]). These diseases are associated with inflammatory effects induced by antigen–antibody complexes. Reasons underlying pathogenicity have been investigated by many researchers [8-17]. However, this problem still requires investigation of many unanswered questions. Why does immune complex pathology appear? Why does it appear in just some individuals even under experimental conditions? Which properties of immune aggregates determine their pathogenicity? Are only properties of immune complexes crucial for the development of these impairments? We consider these problems in the present analytical review.

HISTORICAL BACKGROUND

In 1903, the French scientist M. Arthus described local inflammatory reaction with tissue necrosis appearing after repeated local administration of heterologous (horse) serum to rabbits [18]. Characteristic morphological manifestations of this reaction included hemorrhagic edema and accumulation of neutrophils at the lesion site. During the same time, von Pirquet and Schick described the reaction of the body in response to subcutaneous administration of horse anti-diphtheria serum to children. Within 1-2 weeks after administration of the foreign protein, fever, skin eruption, lymphoadenopathy, arthralgia, leukopenia, and proteinuria appeared in some

Abbreviations: C5aR, receptor for C5a; CIC, circulating immune complexes; CR1, receptor for C3b; DNP, 2,4-dinitrophenol; FcR, Fc receptor; PAF, platelet activating factor.

* To whom correspondence should be addressed.

patients. Since these symptoms were associated with administration of serum, this syndrome was named serum disease. It was postulated [19] that this phenomenon is determined by formation of antibodies against soluble heterogeneous proteins. The authors proposed that antibody–antigen interaction results in formation of "toxic bodies", which are accumulated in tissues and cause destructive changes in them.

The role of antibodies and their immunoprecipitates in the development of experimental Arthus phenomenon (local inflammation) was proved in [20, 21]. The dependence of manifestation of local reaction on the antibody–antigen concentration ratio was investigated by Fischel and Kabat [22]. However, real experimental evidence of the hypothesis proposed by von Pirquet and Schick was obtained 50 years later by the groups of Germuth [23] and Dixon [24]. Independent studies performed by these two groups employed the same experimental model: a single-dose intravenous administration of bovine serum albumin (BSA) to rabbits followed by constant monitoring of blood BSA. One week after the administration, when the immune response resulted in antibody appearance in blood circulation, the rate of antigen elimination from the circulation sharply increased. This was accompanied by simultaneous decrease in serum complement activity followed by the development of proteinuria. Morphologically the kidney glomerular zone was characterized by inflammation and deposit of foreign antigen, immunoglobulins, and complement proteins. These changes temporarily coincided with the presence of high molecular immune complexes in circulation. Disappearance of the immune aggregates from blood was accompanied by normalization of histological characteristics and kidney functioning. The etiological role of immune aggregates in the development of serum disease was finally demonstrated in Benacerraf's laboratory: intravenous administration of antigen–antibody complexes (prepared *in vitro*) to mice and rats was accompanied by vascular lesions and impairments in kidney functioning [25, 26].

Subsequent studies revealed that antigen–antibody complexes can play an important role in pathogenesis of various human autoimmune [17, 27], infectious [28, 29], and tumor diseases [30-32]. Numerous laboratory methods [14] for detection of immune aggregates in blood (circulating immune complexes, CIC) have been developed for clinical application [14]. However, there is rather a popular viewpoint in the literature indicating lack of any method that would directly evaluate CIC pathogenic properties as well as results of laboratory tests determining severity of a disease and its prognosis [33-35]. Lack of optimism with respect to the diagnostic values of CIC determination can be well illustrated by the title of a paper by Lock and Unsworth: "Measurement of immune complexes is not useful in routine clinical practice" [36]. It should be noted that problems related to methodology of CIC measurement as well as clinical interpretation of laboratory data actually exist. However, it requires serious discussion and detailed analysis of a large body of accumulated information, which is impossible within the framework of this review.

HOW ARE IMMUNE COMPLEXES ELIMINATED?

After antigen binding, many antibodies (human isotypes IgM, IgG3, IgG1, IgG2) acquire ability to activate the complement system [37]. This is accompanied by cleavage of complement component C3 and covalent interaction of C3b with an immune complex. The resulting antigen–antibody complex acquires new properties: high solubility and binding to cell C3b receptor (CR1 receptor). The increase in solubility is determined by two processes: inhibition of immune precipitation (depends on initial steps of the classical pathway of complement activation) and solubilization (realized via the properdin pathway) [38]. This blocks sedimentation of immune aggregates in the capillaries [38, 39]. Among circulating cells in humans and primates, about 90% of CR1 receptors are located on erythrocytes [40]. In the circulation, the major proportion of opsonized C3b complexes is bound to these cells. This phenomenon was discovered by Nelson [41] and named the immune-adherence phenomenon. Aggregate fixation on erythrocytes (in rodents this occurs on platelets [42, 43]) prevents their interaction with other cells [44]. Thus, the activation of complement pathway by CIC can be considered as an adaptive mechanism blocking possible negative reactions.

Subsequently complement component Cb3 providing interaction of immune complexes with erythrocyte CR1 receptors is cleaved by the Cb3 inactivating serum enzyme (factor I) [45] thus undergoing gradual (within several minutes) conversion into iC3b ("i" for inactivated). Although affinity of CR1 for iC3b is lower than to C3b [46], other receptors (CR3 and CR4) exhibit higher affinity towards iC3b than to C3b [47, 48]. CR3 and CR4 receptors are located on macrophages and neutrophils but they are absent in erythrocytes [49]. In liver and spleen where erythrocytes directly contact macrophages, antigen–antibody complexes containing iC3b are transferred from the erythrocyte surface onto the phagocyte membrane (Fig. 1). Erythrocytes are not absorbed by macrophages and are retained in the blood circulation. Experiments on monkeys have shown that within 5 min after intravenous administration of immune complexes their major proportion leaves erythrocytes and is accumulated in liver and spleen [50].

However, it should be noted that binding of objects with CR3 and CR4 is not the only precondition required for their efficient uptake by macrophages [51, 52]. This

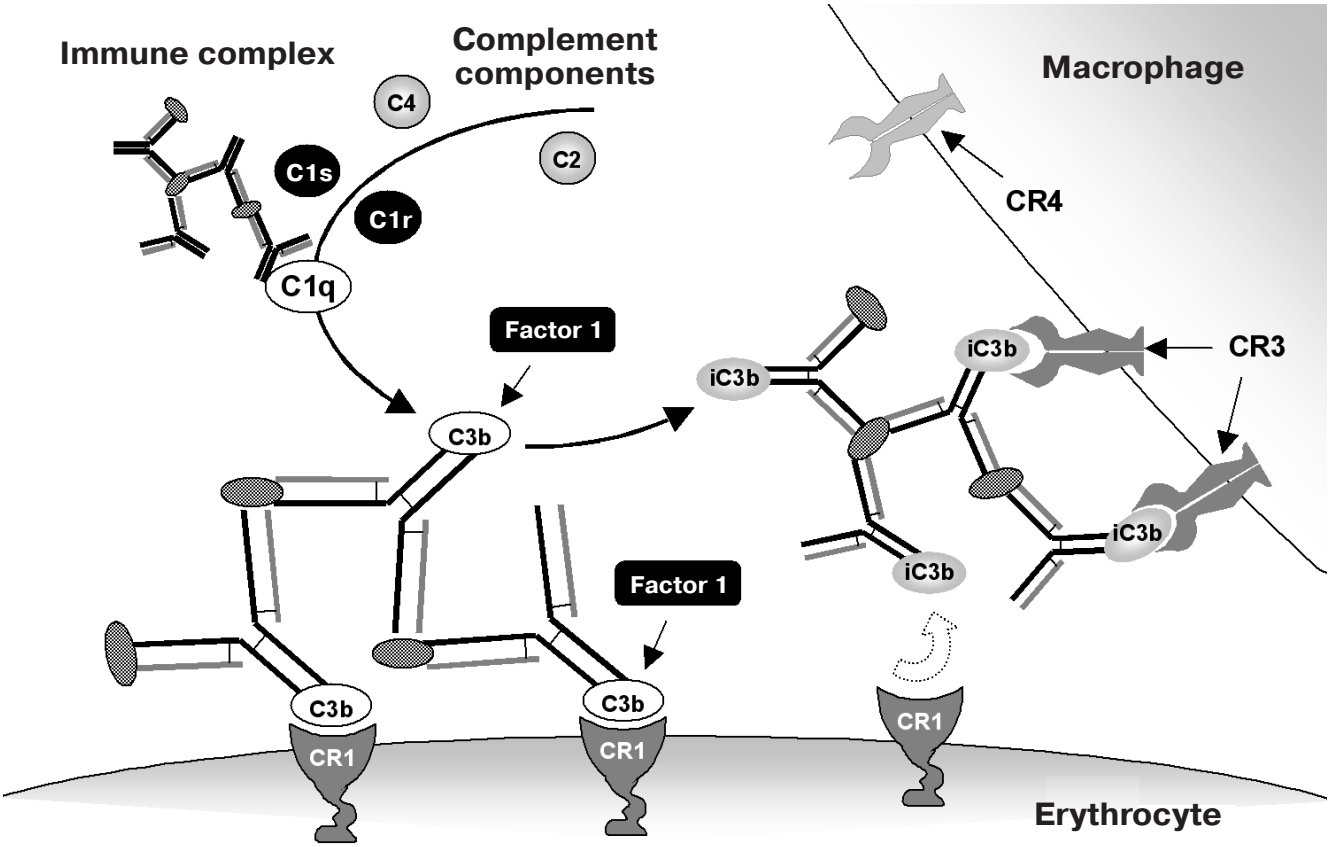

Fig. 1. Molecular mechanisms of fixation of immune complexes on erythrocytes and their subsequent transfer from erythrocytes to macrophages. Immune complexes form C3-convertase and covalently fix its product, C3b; this employs initial steps of activation of the classical complement pathways. CR1 receptors located on erythrocytes bind C3b molecules and C3b bearing immune complexes. Blood plasma factor I causes inactivation of C3b and its conversion into iC3b characterized by low affinity interaction with CR1 and high affinity interaction with CR3 and CR4. During erythrocyte contacts with liver and spleen macrophages, this results in transfer of immune complexes from erythrocytes to phagocytes.

needs involvement of additional factors, for example, Fc receptors or fibronectin [4, 53]. It is possible that elimination of the major proportion of CIC involves Fc receptors. However, this is accompanied by proteolytic cleavage of an external fragment of the CR1 molecule, which undergoes macrophage uptake with immune complexes [54]. Under conditions of excessive and long-term formation of immune complexes, this may have negative consequences leading to the decrease in the transport capacity of erythrocytes. Indeed, the decrease in CR1 content on the surface of erythrocytes has been noted in chronic viral hepatitis [55], systemic lupus erythematosus [56], and malaria [57].

Maximal effectiveness of fixation on the erythrocyte surface was observed in the case of large CIC [58]. This is determined by more effective activation of the complement system by high molecular weight complexes than low molecular weight complexes [59]. In addition, large immune aggregates contain more C3b fragments, and this increases avidity in their interaction with erythrocytes [60].

WHAT DETERMINES PATHOGENIC PROPERTIES OF IMMUNE COMPLEXES?

As mentioned above, immune complexes are constantly formed in the healthy body without hazardous consequences. Taking into consideration this fact, we assume that the development of immune complex syndrome is associated with some of the following reasons: pathogenic immune aggregates have some structural differences compared with non-pathogenic analogs; negative effect of immune complexes is determined by impaired functioning of clearance mechanisms responsible for safe elimination of immune complexes rather than structural features of these complexes; normal functioning of clearance mechanisms cannot provide elimination of large amounts of antigen–antibody complexes exceeding clearance capacity.

Experimental studies on modeling of Arthus phenomenon or serum disease have shown that even under laboratory conditions (when many factors are easily controlled) pathological reactions are reproduced in some

but not all experimental animals [23, 61, 62]. The most reproducible and most frequently employed experimental model of reverse passive Arthus reaction (subcutaneous administration of foreign antibody and intravenous administration of antigen) does not adequately reflect natural development of immunopathology. It should be noted that although there are common features of many pathogenic mechanisms, pathways of realization of Arthus phenomenon and serum disease significantly differ. In the former case, antigen–antibody interaction occurs in tissues where one component is located and the other diffuses from the blood circulation. In the case of serum disease, immune complexes are formed in the circulation and manifestation of the lesion effect requires their penetration through vascular endothelial lining. In this case, one should take into consideration that sizes of even small immune complexes usually (and significantly) exceed sizes of most antigens and free antibodies.

Normally immune complexes do not interact with vascular endothelium and only small quantities of these complexes can leave the vascular bed. In 1963, Cochrane employing experiments on guinea pigs demonstrated that CIC deposits in vascular wall required increased vascular permeability [63]. For this purpose, he induced systemic anaphylaxis in experimental animals or injected intravenously histamine after injection of immune complexes into the blood circulation. Such treatments were accompanied by deposits of immune complexes and complement in walls of small vessels (mainly venules). Injections of antigen–antibody complexes into guinea pigs without increased vascular permeability did not cause formation of these deposits. Later studies by Cochrane's laboratory demonstrated [64] that high CIC concentrations might cause independent system reactions characterized by complex fixation on vascular walls.

Pathogenic properties of CIC depend on the ratio between concentrations of antigen and specific antibodies. This was elegantly demonstrated by Dixon's group [65]. They performed daily intravenous injections of low doses of one protein antigen (BSA, human albumin, bovine γ-globulin, or human γ-globulin) and controlled levels of specific antibodies in blood of experimental rabbits. The animals were divided into three groups according to the extent of the immune response expressiveness. In the first group, they found excessive antibody synthesis versus administered antigen. The foreign protein was rapidly bound and disappeared from circulation and CIC were not found in these rabbits. In the second group, antibody production was low and insufficient for antigen neutralization. CIC were not detected. In the third group, antibody synthesis was higher than in the second group but lower than in the first. It was not sufficient for binding of all foreign proteins, but it was sufficient for formation of soluble immune complexes that remained in the blood circulation for one day (up to injection of the second portion of the antigen). In most rabbits of this group (87.8%) glomerulonephritis subsequently developed. In the first group frequency of its development was less than 3%, and in the second group it was not determined at all.

Results of these experiments have demonstrated that intensity of humoral immune response to antigen rather than properties of antigen itself is the main factor responsible for the development of serum disease. This gives a reasonable explanation why experimental or clinical immunopathological reactions are observed in some but not all individuals. It was also reported [65] that varying blood antigen and antibody concentrations (by intravenous administration of additional quantities of certain component) could increase or decrease activity of pathological process in experimental animals. Several years later Benacerraf [66] discovered immune response genes (*Ir*-genes) determining individual immunologic reactivity to a particular antigen. Thus, the relationship between genetic control of immune response and type III hypersensitivity was established.

At the same time, structural features of immune complexes promoting manifestation of their pathogenic properties have also been found. In this case, isotypes of immunoglobulins constituting immune aggregates play a crucial role. For example, apoptotic cells are preferentially opsonized by class M natural antibodies [67]. Antibodies of this isotype form large complexes and activate the complement system. Antibodies of class G usually elaborated in response to foreign antigens frequently form complexes of smaller sizes. Complement activating ability of human IgG subclasses decreases in the following order [68]: IgG3 > IgG1 > IgG2; aggregates containing IgG4 do not cause activation of complement. Antigen bound and aggregated IgA cannot cause complement activation via the classical pathway [69], but they can initiate complement activation via the alternative pathway [37]. It was also demonstrated that the rate of solubilization of complexes formed by IgA is significantly lower than the rate of solubilization of complexes formed by antibodies of the other isotypes [70], and effectiveness of their binding to erythrocytes is significantly lower than corresponding activity of IgG immune aggregates of the same size [71]. Liver and spleen macrophage uptake of IgA-CIC administered into baboon blood circulation was less effective than that of IgG-CIC, and IgA-CIC deposits were found in lungs and kidneys (glomeruli) of these monkeys. The appearance of circulating IgA-complexes and their presence in kidney glomeruli is typical for acute vascular purpura (known as the Schoenlein–Henoch syndrome) and IgA nephropathy [72, 73].

The role of immunoglobulin isotypes in pathogenicity of their immune aggregates has also been demonstrated in rodents. Model immune complexes based on 2,4-dinitrophenyl-labeled BSA (DNP-BSA) and specific to DNP monoclonal antibodies of subclasses IgG1, IgG2a, and IgG3 were tested in mice [74]. Studies have shown that intravenously administered IgG1-CIC as well as

IgG3-CIC were effectively eliminated from circulation (90% disappeared within 8 min after injection). Clearance of IgG2a-CIC was more than 2 times lower, and significantly higher amounts of IgG2a-complexes were deposited in kidney glomeruli compared with complexes formed by the other antibody isotypes. The other study was performed using (NZB×NZW) F1 mice predisposed to spontaneous development of lupus nephritis: animals received daily injections of anti-DNA monoclonal IgM antibodies, and this resulted in the decrease of renal lesions and lethality [75]. The injections did not attenuate synthesis of their own anti-DNA IgG antibodies, which were spontaneously formed and exhibited pathogenic properties and determining the development of this disease. These results convincingly prove the important role of particular isotype antibodies in realization of pathological effects of immune complexes. Clinical observations are consistent with these experimental data. For example, in patients with serum disease developed after administration of horse γ-globulin, 80% of antibodies against the foreign protein belonged to IgG1 subclass [76].

Affinity of antigen–antibody interaction is another factor influencing pathogenicity of immune complexes. There are demonstrative experiments performed on mice of various strains differing by ability to synthesize high and low affinity antibodies to the same antigen (BSA). Daily administration of BSA to animals of both strains for more than 40 days resulted in CIC formation and their depositions in kidneys [77]. However, blood concentrations of immune complexes in mice synthesizing low affinity antibodies significantly exceeded those detected in animals producing high affinity antibodies. In addition, accumulation of immune deposits in kidneys was higher in the first group than in the second. At the same time, impairments in kidney functioning were found only in the mice producing low affinity antibodies. Subsequent studies have shown that in some animals producing low affinity antibodies the development of immune reaction to antigen was accompanied by an increase in binding constant for specific antibodies. Subsequent selection resulted in formation of a strain of animals in which immune response was not accompanied by the increase in antibody affinity. This strain, especially males, was even more susceptible to the development of immune complex pathology. Chronic administration of antigen to these males cased 52% lethality, and 81% of these animals suffered from glomerulonephritis [78] (these parameters in male mice producing high affinity antibodies and treated with the same dose of the antigen were 6 and 19%, respectively). The authors also found that lack of affinity maturation was associated with low level of specific antibody production. These results are consistent with the above-mentioned results of Dixon [65], who determined a particular role of the ratio between antigen and antibody concentrations in the development of type III hypersensitivity.

Formation of deposits of immune complexes in kidneys might be determined by cationic properties of protein aggregates. Identification of negatively charged sites on basal membrane of kidney glomeruli [79] initiated studies on the effects of molecular charges on CIC fixation in kidneys. Natural BSA was chemically modified with formation of cationic and anionic BSA. These cationic and anionic proteins were intravenously injected into rabbits using the following scheme: first injection of 1 mg and then after 1 week interval daily injections (25 mg) for 4 weeks and finally daily injections (50 mg) for an additional 2 weeks [80]. In animals treated with cationic BSA (pI > 9.5) there were diffuse granular deposits containing IgG, C3, and BSA in capillary walls. In animals treated with natural (pI 4.5-5.1) and anionic (pI 3.5-4.6) proteins, immune complexes were preferentially localized in mesangium. Ultrastructural studies showed that aggregates containing cationic BSA were mainly located along *lamina rara externa* of glomerular basal membrane. Such localization was not typical for complexes formed by native and anionic BSA. Average values of proteinuria in animals treated with cationic BSA were 5 times higher than in rabbits treated with natural or anionic BSA. The importance of these findings is determined by the fact that subepithelial deposits of positively charged immune complexes correspond to the morphological pattern of membranous glomerulonephritis, the most common form of immune damage of kidneys in humans. The effect of charge of immune aggregates on the development of kidney immunopathology was also demonstrated using the model of passive serum disease in mice [81, 82].

The other important aspect related to pathogenicity of immune complexes is their size. The size is mainly determined by the ratio of molar concentrations of antigen and antibody [83, 84]. The largest aggregates were formed in the equivalence zone [85]. Small complexes were formed on significant excess of antigen. Structures of intermediate size were formed when the antigen/antibody ratio was moderately shifted from the equivalence zone. Low molecular weight complexes poorly activated complement [86, 87] and weakly interacted with leukocyte Fc-receptors [88]; this significantly attenuated their pathogenic properties. In contrast, large aggregates readily bind complement and easily adhere to erythrocytes, but are effectively eliminated from circulation by mononuclear phagocytes [89]. It is believed that most frequently complexes of intermediate size are responsible for tissue damage [90]. They are eliminated from circulation less effectively than large aggregates, but they can activate the complement system.

However, the latter is rather arbitrary because the situation is often determined by CIC concentration, activity of the complement system, erythrocyte ability to bind antigen–antibody complex, and functioning of the mononuclear phagocyte system (Fig. 2). Impairments in

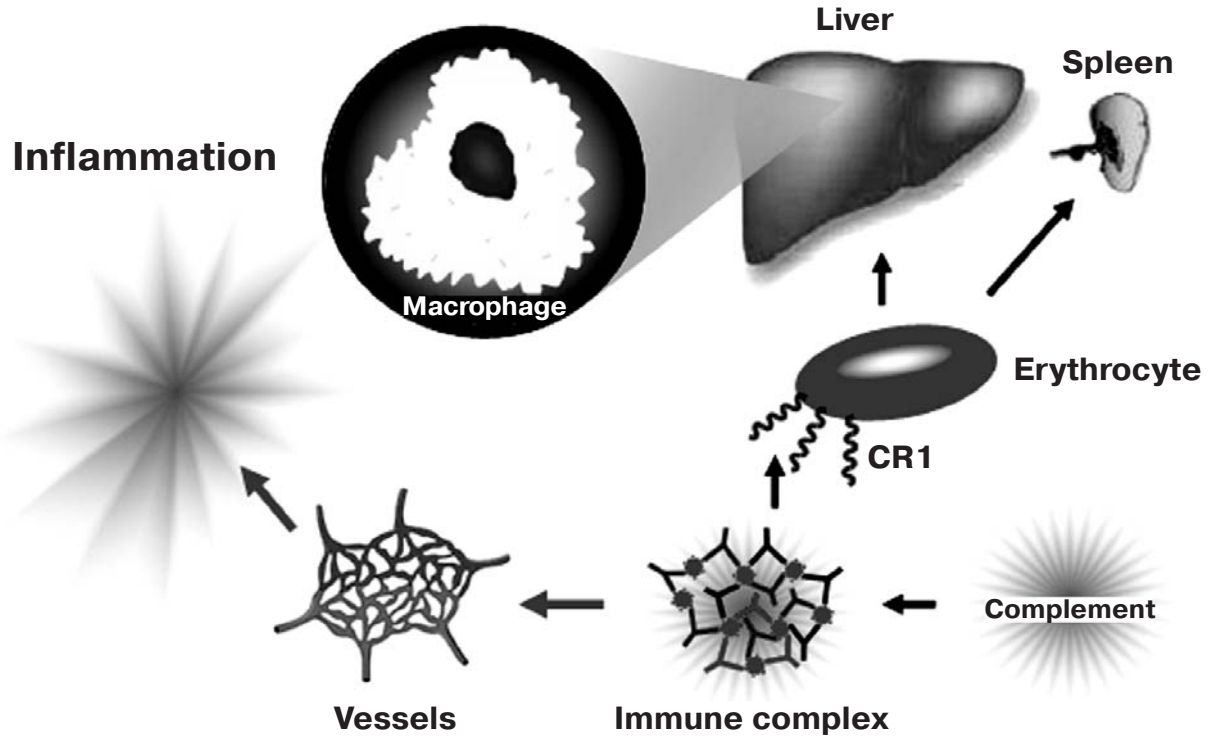

Fig. 2. System of safe removal of CIC from the circulation in humans and primates and its impairments. Effective removal of CIC depends on their ability to activate the complement system, which subsequently determines their binding to erythrocyte CR1 receptors. In liver and spleen, these complexes are removed from erythrocyte surface by macrophages and then undergo uptake and proteolysis. Impairments in the complement system, decrease in fixating capacity of erythrocytes, decrease in uptake by mononuclear phagocytes, as well as intensive and long-term formation of CIC can result in increase in their concentration in the circulation and the development of inflammation.

any link of this system significantly increase risk of the development of type III hypersensitivity [15]. Large immune aggregates not bound to erythrocytes exhibit marked proinflammatory properties [44, 91]. At high concentrations, they can increase vascular permeability [64] and be deposited in vascular walls. At the same time, low molecular weight CIC formed with antigen excess easily penetrate into tissues where they bind newly synthesized free antibodies arriving from the circulation. In this case, sizes of immune aggregates will gradually increase and they finally acquire ability to fix complement. This will inevitably result in formation of pathogenic deposits *in situ* (a variant of the Arthus phenomenon) [10].

PATHOGENIC EFFECTS OF IMMUNE COMPLEXES

Inflammatory reactions induced by immune complexes are mainly determined by the ability of the complexes to activate the complement system and interact with various cells via Fc receptors. It should be noted that these receptors play a decisive role in the realization of type III hypersensitivity syndrome. This was demonstrated using mice with genetic defect of γ-chain determining signal transduction inside cells from FcγRI, FcγRIII, and FcεRI [92]. Animals with intact complement system but with abnormal FcγR were characterized by weak Arthus reaction. Later studies performed in the same laboratory on genetically modified mice deficient in C3 and C4 complement components demonstrated that the complement system does not determine the reverse passive Arthus phenomenon [93]. These data underline the priority role of Fcγ-signals in tissue damage rather than "reject" complement involvement in multistage and polyetiologic immune complex syndrome. This is especially important in the case of systemic diseases when a pathological process requires CIC penetration out of the blood circulation.

We have already mentioned above that antigen–antibody interaction in blood is accompanied by complement utilization [24]. Opsonization of immune complexes results in formation of anaphylatoxins C3a and C5a [94]. In lupus erythematosus, their content can be increased several-fold [95, 96]. Acting on perivascular mast cells, C3a and C5a initiate release of histamine, proteases, and TNF-α and this results in an increase in vascular permeability [97, 98]. Tissue macrophages exposed to anaphylatoxins produce TNF-α and IL-1β [99, 100], which in

turn activate endothelial cells [101]. These processes can obviously provide a reasonable level of vascular permeability required for penetration of CIC into perivascular space. However, anaphylatoxin effects do not last long as they are rapidly inactivated by plasma carboxypeptidases [102]. In addition, the half-life of C5-convertase (source of C5a) is 2-3 min at 37°C, and maximal enzyme activity is achieved only during effective formation of C3b [103].

Indeed, marked inflammatory manifestations are preferentially determined by binding of immune complexes to cell Fc receptors. This was first found during administration of soluble human recombinant FcγRIIA to the zone of induction of reverse passive skin Arthus reaction in rats [104]. Free receptors specifically and in a dose-dependent manner suppressed local inflammatory reaction induced by antigen–antibody complex. At the same time, it was reported [104] that neutrophil infiltration typical for Arthus phenomenon was not abolished by receptor administration (this may be attributed to effects of complement products), whereas the size of a pathological lesion and inflammation severity evaluated by edema and hyperemia significantly decreased. Later the role of Fc receptors in type III hypersensitivity was confirmed in experiments on mice with defects in signal γ-chain [92] (this has already been mentioned above). Subsequently, the role of low affinity FcγRIII in Arthus phenomenon has been recognized [105]. Genetically modified mice with deficit of ligand binding α-chain of FcγRIII were unable to demonstrate antigen-dependent NK-determined cytotoxicity and phagocytosis of IgG1-opsonized particles, and the Arthus reaction was sharply attenuated in these animals. In addition, in these animals (in contrast to animals with normal FcγRIII) it was impossible to induce mast cell degranulation by adding immune complexes.

In our view, elucidation of the regulatory role of FcγRIIB receptor carrying the inhibitory sequence known as ITIM (immunoreceptor tyrosine-based inhibitory motifs) in the development of immune complex pathology represented an important achievement in this field [106, 107]. All other Fc-receptors contain activation ITAM motifs (immunoreceptor tyrosine-based activation motifs) responsible for transmission of proinflammatory signals into cells. The importance of FcγRIIB was demonstrated in experiments employing FcγRIIB-knockout mice with initiated immune complex alveolitis [106]. Administration of sub-threshold doses of immune complexes (which did not cause the development of pathological syndrome in wild type mice) was accompanied by marked manifestation of inflammatory reaction. Functional analysis of macrophages derived from FcγRIIB-deficient mice has shown that in contrast to corresponding cells derived from wild type mice, these cells respond to stimulation by more potent calcium signal and exhibit increased phagocyte activity.

Although there is certain progress in elucidation of the role of Fc-receptors in the immune complex syndrome, data characterizing importance of C5a receptor (C5aR) in this pathology should not be ignored. Knockout of *C5aR* gene in mice almost totally blocked the development of Arthus reaction in lungs and significantly attenuated its skin and peritoneal manifestations [108]. There was significant decrease in neutrophil infiltration and vascular permeability in the inflammation zone. Recent studies provided convincing evidence for the interaction of signals initiated by complement component C5a and Fc fragments of IgG antibodies of immune complexes in type III hypersensitivity [109, 110]. Usually C5aR (CD88) and FcγRIII (CD16) are simultaneously present on macrophages [111]. It was shown [110] that during antigen–antibody attack C5a significantly increased FcγRIII-mediated activation of alveolar macrophages. The cited authors also found the effect of C5a–C5aR interaction on expression of mRNA responsible for synthesis of FcγRIII and FcγRIIB. In C57BL/6 mouse macrophages, C5a increased the content of mRNA encoding the activatory Fc-receptors and decreased the content of mRNA encoding the inhibitory Fc-receptors. In $C5aR^{-/-}$ animals derived from the same strain, these effects were not observed. Subsequent studies have shown that the effect of C5a is also realized in the number of corresponding Fc-receptors on the cell surface [112]: on macrophage membrane, FcγRIII density increased, whereas FcγRIIB decreased (Fig. 3).

Studies of the influence of various factors on neutrophil infiltration into the zone of the development of Arthus phenomenon suggest a dominating role of C5a and C5aR. Blockade of C5aR by Fab fragments of anti-C5aR antibodies, employment of a specific antagonist of this receptor, and finally experiments with $C5aR^{-/-}$ mice have demonstrated almost total blockade of polymorphonuclear leukocyte migration to the inflammation zone [112]. It is known that anaphylatoxins significantly increase granulocyte adhesion to endothelial cells. This is achieved by increased expression of CD11b/CD18 integrins on the surface of neutrophils (C5a) and eosinophils (C3a and C5a) [113-115] and increased expression of the adhesion molecules ICAM-1 and VCAM-1 on membranes of the endothelial cells [115, 116]. In the inflammation zone, neutrophils are controlled by platelet-activating factor (PAF). The latter is synthesized by macrophages [117] and mast cells [118]. This lipid mediator increases vascular permeability [119] and thus provides CIC migration or immune aggregate formation *in situ*, stimulates synthesis of TNF-α [120], and increases free radical production by neutrophils during interaction with immune complexes [121]; it also leads to aggregation and deposition of platelets in the inflammation zone [122].

At the same time, these pathological changes can be maintained only under conditions of constant formation of immune complexes, and they also require complement activation via the alternative pathway. This pathway is the

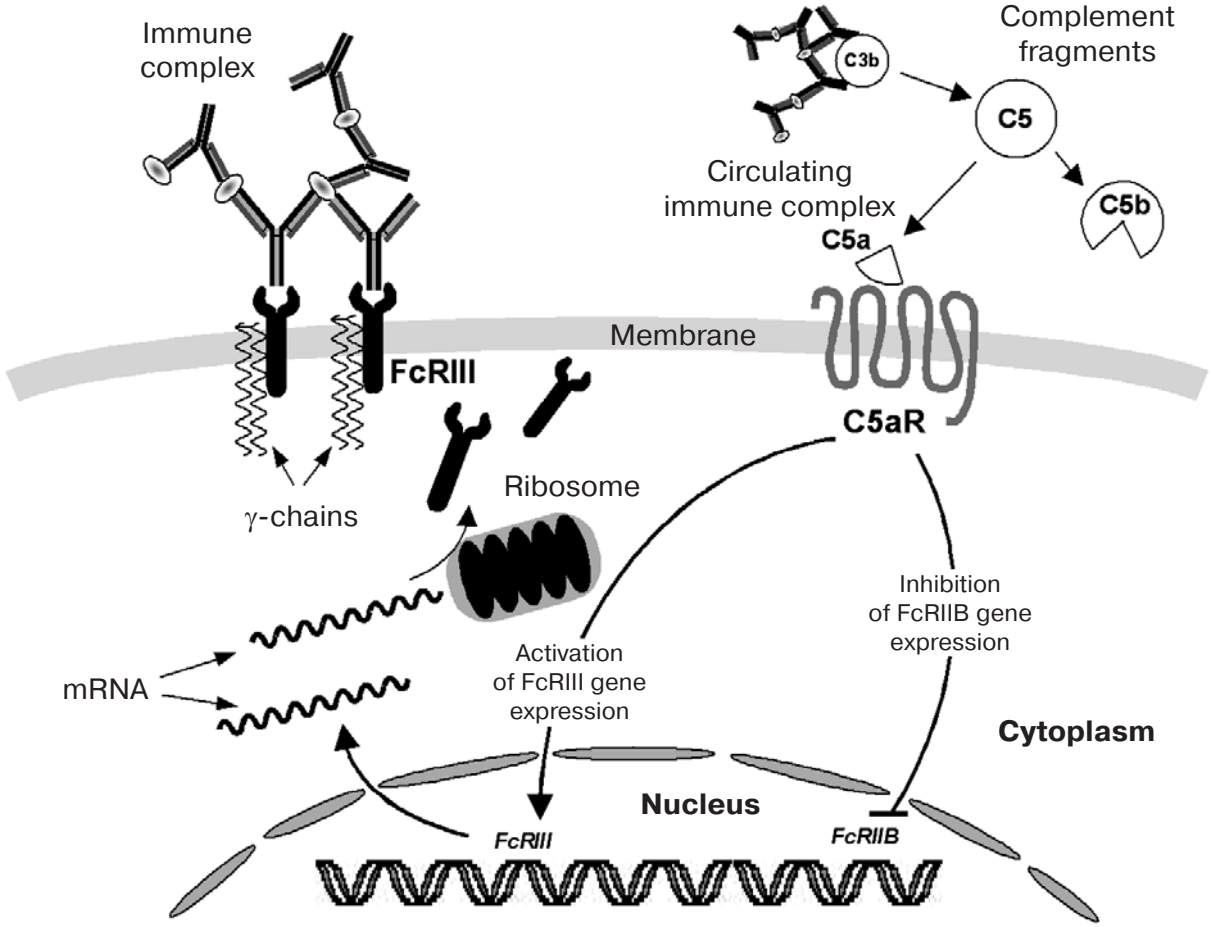

Fig. 3. Effect of C5a receptor on pathogenic signals of immune complexes. Intracellular signals transduced through C5aR are accompanied by suppression of inhibitory FcRIIB receptor expression and augmentation of activatory FcRIII receptor gene expression. This results in quantitative imbalance between macrophage receptors binding immune complexes towards structures generating proinflammatory signals.

main source of C5a in immune complex pathology [123]. These situations are frequently observed in autoimmune diseases, chronic infections, or in inherited defects of the complement system. These defects may be quite different and often they are latent. However, this emphasizes complement importance in safe removal of immune aggregates. Indeed, complement opsonizes immune complexes, suppresses immunoprecipitation of CIC (and causes their solubilization), and provides aggregate binding with erythrocytes. The complement system can also exhibit anti-inflammatory effects due to participation in immune complex elimination. For example, it has been demonstrated [124] that interaction of human macrophages with IgG–ovalbumin complexes lacking complement components was accompanied by release of arachidonic acid, a main source for synthesis of proinflammatory lipid mediators including PAF. The presence of iC3b in the complexes blocked this reaction, suppressed phosphorylation of Syk tyrosine kinase, and decreased production of proinflammatory cytokines IL-1β, MIP-1α, and MCP-1 [125].

In conclusion, it should be noted that immune complex pathology includes a large group of syndromes of various etiology, which, however, share common pathogenic mechanisms. There are systemic (autoimmune diseases, serum disease, chronic infections) and local (reactions to vaccination, local administration of antibiotics, γ-globulins) manifestations of this pathology. Answering the problems formulated in the beginning of this paper, we conclude that the most frequently type III hypersensitivity occurs during long-term persistence of antigens (oncoantigens, autoantigens, and infectious antigens) in the body. Causative factors may include certain properties of humoral immune response to antigens such as intensity of antibody production and antibody isotype and affinity. They may underlie formation of immune complexes of smaller size (compared with normal ones), weakly activating complement but readily penetrating into tissues, and effectively interacting with leukocyte and mast cell Fc-receptors. The other group of reasons is associated with peculiarities of complement functioning. Defects in proteins responsible for initial steps of complement acti-

vation via the classical pathway often result in the development of systemic lupus erythematosus [126]. Abnormalities can occur in complement receptors, its regulators, Fc-receptors, intracellular signaling pathways, etc. Thus, immune complex pathology usually has genetic predisposition, and this explains its appearance in some but not in all individuals. However, latent symptomless presence of CIC in blood circulation should not be considered as immune pathology. It appears that increase in CIC content may suggest the development of humoral immune response to antigen, and lack of inflammatory reactions reflects normal functioning of physiological clearance mechanisms.

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