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REVIEW

IgM and Its Receptors: Structural and Functional Aspects

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Abstract—This review combines the data obtained before the beginning of the 1990s with results published during the last two decades. The predominant form of the IgM molecule is a closed ring composed of five 7S subunits and a J chain. The new model of spatial structure of the pentamer postulates nonplanar mushroom-shaped form of the molecule with the plane formed by a radially-directed Fab regions and central protruding portion consisting of Cμ4 domains. Up to the year 2000 the only known Fc-receptor for IgM was pIgR. Interaction of IgM with pIgR results in secretory IgM formation, whose functions are poorly studied. The receptor designated as Fcα/μR is able to bind IgM and IgA. It is expressed on lymphocytes, follicular dendritic cells, and macrophages. A receptor binding IgM only named FcμR has also been described. It is expressed on T- and B-lymphocytes. The discovery of new Fc-receptors for IgM requires revision of notions that interactions between humoral reactions involving IgM and the cells of the immune system are mediated exclusively by complement receptors. In the whole organism, apart from IgM induced by immunization, natural antibodies (NA) are present and comprise in adults a considerable part of the circulating IgM. NA are polyreactive, germ-line-encoded, and emerge during embryogenesis without apparent antigenic stimuli. They demonstrate a broad spectrum of antibacterial activity and serve as first line of defense against microbial and viral infections. NA may be regarded as a transitional molecular form from invariable receptors of innate immunity to highly diverse receptors of adaptive immunity. By means of interaction with autoantigens, NA participate in maintenance of immunological tolerance and in clearance of dying cells. At the same time, NA may act as a pathogenic factor in atherosclerotic lesion formation and in development of tissue damage due to ischemia/reperfusion.

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Immunoglobulins class M (IgM) is the most ancient phylogenetic class of antibodies common to all vertebrates starting from cartilaginous fish [1]. They are the first to emerge during embryonic development [2] and immune system response to contact with antigens [3]. In the organism IgM exist as a component of antigen receptors on membranes of B-lymphocytes [4], circulating in blood molecules, and also on mucous membranes and excretory gland secretions as secretory IgM [5].

IgM are produced by two different B-lymphocyte populations. B1-cells synthesize IgM called natural antibodies (NA) as their production is not connected with immunization [6]. B2-lymphocytes produce IgM as a reaction to antigenic stimuli [7]. IgM induced by immunization differ from NA by structure of antigen-binding centers, affinity, specificity repertoire, and by the spectrum of their functions. Both serve as components of the organism's defense system against pathogens [8].

Data on the variety of IgM molecular forms and functions is necessary for understanding the broad spectrum of issues of fundamental and practical immunology – from hypothesis of the origin and development of adaptive immunity to the development of new vaccinal drugs and approaches to prophylaxis and therapy of infectious, parasitic, autoimmune, allergic, and other diseases.

Notions on IgM structure and role in immunity were established upon results obtained mainly during 1960-1980s. Reviews generalizing those studies [9-11] were

Abbreviations: a.a., amino acid residue; C1q, the 1st component of complement; Fab, antigen-binding antibody region; Fc, constant antibody region; Fcα/μR and FcμR, receptors binding Fc-regions of α- and/or μ-chains; FDC, follicular dendritic cells; Ig, immunoglobulins; IgA, IgG, IgE, and IgM, immunoglobulins of class A, G, E, and M, respectively; NA, natural antibodies; PC, phosphorylcholine; pIg, polymeric immunoglobulins; pIgR, polymeric immunoglobulin receptor; PTC, phosphatidylcholine; SC, secretory component; sIgA and sIgM, secretory IgA and IgM, correspondingly.

published before the 1990s. New data obtained during the last twenty years call for the necessity of reconsideration of some commonly accepted views.

The aim of this review is to combine the data of early publications with results published during the last two decades and to mention problems that have become obvious with the newer data.

IgM STRUCTURE

Similarly to antibodies of other classes, IgM molecules are composed of heavy chains forming homodimers, and light chains, each of them being bound to one of the heavy chains (Fig. 1a) [12].

Heavy μ-chain (56-60 kDa), which defines the isotype, contains 576 amino acid residues (a.a.). Variable domain (Vμ) contains 124 of those residues. The constant region consists of four domains (Cμ1-Cμ4) and contains 452 a.a. The μ-chain most of all resembles IgE ε-chain, which also contains one variable and four constant domains. The comparison of primary structure (Fig. 1b) reveals high similarity between μ- and ε-chains: 29% amino acid residues take identical positions, and 86% of positions are taken by amino acid residues of moderately or highly similar physicochemical properties [13].

In the Cμ1 domain the cysteine residue in position 140 forms a disulfide bond with a light chain. The carboxy-terminal region of the Cμ2-domain in position 337 contains cysteine that forms an S–S-bond with the second μ-chain [14-16]. The complex consisting of two light and two heavy chains (heterotetramer) is characterized by sedimentation constant of 7S and molecular weight of 180 kDa and represents a subunit (monomer) that exists independently or is included in polymeric structures [9, 17, 18].

Monomeric IgM, being a part of an antigen receptor of B-lymphocytes (Fig. 2a), is characterized by a polypeptide in the C-terminal region of the Cμ4-domain that is responsible for incorporation of the molecule into the membrane. It consists of 41 a.a., of those 14 belong to the extracellular part, 25 are included in transmembrane hydrophobic region, and three form a short cytoplasmic domain [19, 20]. The receptor has a hetero-oligomeric structure in which ligand binding and signal transmission are spatially separated [21]. The IgM monomer represents the ligand-binding part of the receptor. The component that is responsible for signal transduction consists of two glycoproteins—Igα (CD79α) and Igβ (CD79β). The molecular weights of these proteins in humans are 47 and 37 kDa, respectively, and in mice – 34 and 39 kDa. The cytoplasmic domains of Igα and Igβ contain tyrosine motifs responsible for transduction of signal from the receptor [22, 23].

In some conditions B-lymphocytes are able to produce monomeric IgM as a secretory product, but the structure and properties of these molecules are not well studied [24, 25]. IgM along with IgA is included in the group of polymeric Ig (pIg) [26]. The predominant and best studied form of the IgM molecule is a closed ring (Fig. 2b) consisting of five 7S-subunits and the J chain [27]. The pentamer contains 10 antigen-binding sites and is characterized by ~900 kDa molecular weight and 19S sedimentation constant [9, 14].

The subunits are associated in the pentamer by two cysteine residues. The first is localized in the so-called tail piece following the Cμ4-domain. The tail piece consists of 18 a.a., does not have a defined secondary or quaternary structure, and is not a part of the Cμ4-domain. It is a highly conservative element of secretory pIg. In the pentameric IgM molecule, cysteines-575 from the tail pieces form disulfide bonds between μ-chains of neighboring 7S-subunits and also between two of them and the J chain [28, 29]. The J chain, forming bonds with cysteines-575, incorporates between two neighboring 7S-subunits as an insertion in the place of a disulfide bridge [18, 30]. The second cysteine residue responsible for pentamer assembling takes position 414 [15, 16]. When it is substituted by a serine residue, tetra- and pentameric IgM molecules are synthesized irrespectively of the presence of the J chain [31-33].

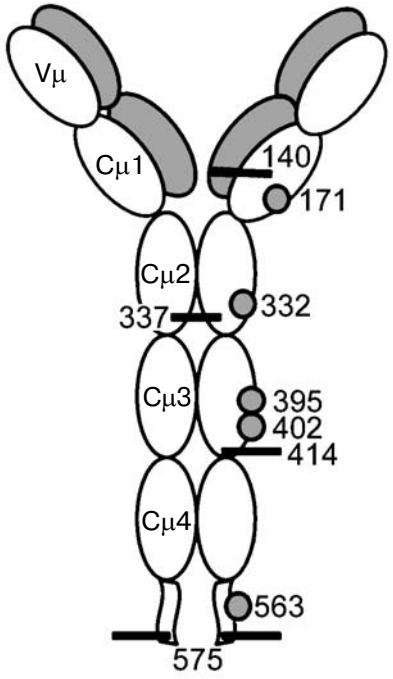
Ultracentrifugation and electron microscopy revealed IgM molecules consisting of six 7S-subunits instead of five. Hexameric IgM does not have J chain and is produced by cells that either lost the J chain gene or synthesize defective μ-chains unable to associate with J chain. It was found in experiments with cultured lymphoma cell lines that at low level or in the absence of J chain synthesis, cells predominantly produce hexameric IgM molecules [34].

The J chain takes part not only in IgM pentamer assembly. It also provides transport of the pentamer across epithelium to the secretions of mucous membranes and exocrine glands [11]. The molecules of secretory IgM (Fig. 2c) carry additional structure – secretory component (SC). It was shown in one of the first sIgM studies that the link between IgM and SC is quite unstable: ~40% of IgM molecules lost connection to SC during isolation and purification [35]. Additional studies revealed that the link between IgM and SC was predominantly established by noncovalent interactions [36]. The conformation of IgM pentamer is assumed to prevent the formation of disulfide bonds with the SC molecule [37].

No region enriched by proline and cysteine, similar to IgG hinge region, is found in μ-chain [38]. However, there are proline residues at the border of the Cμ1- and Cμ2-domains. During mild processing of IgM with denaturing agents, Cμ2-region shows some signs of conformational flexibility corresponding to the hinge region [39].

During IgM hydrolysis using papain Fabμ-fragments (3.7S, 48 kDa) were obtained, while the major part of the Fc-region was degraded to dialyzable peptides [40, 41]. A modification of the papain cleavage method increases the

a



b

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IgM (107) AELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQA
IgE (228) DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGITPGTINITWLEDGQVMDV----DLSTA
          . .**.*.:: :: **    *....*:*..:*::* .**::**.:*. :..    *...*

IgM (167) EAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFA
IgE (283) STTQEGE-LASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGV-SAYL
          .:.:.*. . ...*.**:.:..***:..:**:*..:* **::::..   ::.... ..:

IgM (227) IPPSFASIFLTKSTKLTCLVTDLTTYDS-VTISWTRQNGEAVKTHTNISESHPNATFSAW
IgE (341) SRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVT
          ..**  .:*:.**..:****.**:. .: *.::*:*.:*.:*:..*...*.:.*:*::..

IgM (286) GEASICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRE
IgE (401) STLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRA-APEVYAFATP-EWPGSRD
          :. :: ..**..**.:.*.***..**.:*:::.::..*.    *:** ::.: *  . *:

IgM (346) SATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEW
IgE (459) KRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQP--RKTKGSGFFVFSRLEVTRAEW
          . *::**:..*.*.*: ***::.. .*:..:..*:.*  .....: :*..* *.*:..**

IgM (406) NTGETYTCVVAHEAL-PNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
IgE (507) EQKDEFICRAVHEAASPSQTVQRAVSVNP
          :. :.:.* ..***  *::..:*:*. :.--------------------
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Fig. 1. a) Structure of IgM monomeric 7S-subunit. Dark ovals, light chain domains; light ovals, heavy chain domains. Straight lines and numbers indicate positions of cysteine residues and disulfide bonds formed by them; circles and numbers indicate positions of glycosylated asparagine residues. b) Alignments of amino acid sequences of μ- and ε-chains (data according to [13]). Asterisks indicate positions of identical amino acid residues; points and colons indicate positions of amino acid residues with moderately or highly similar physicochemical characteristics, respectively.

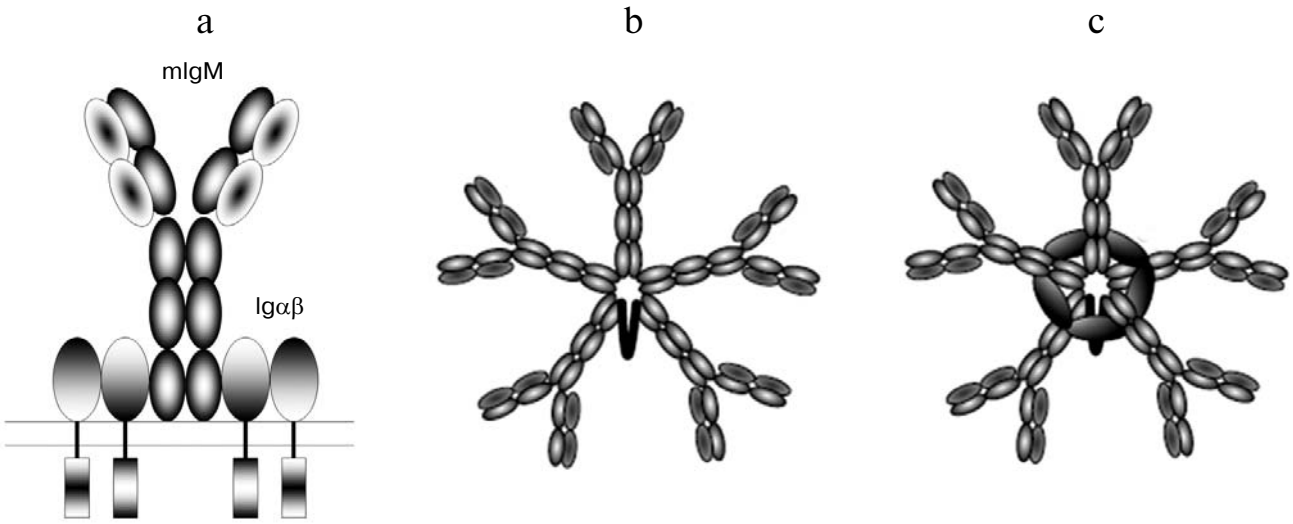

Fig. 2. Structure of B-cell receptor (a), IgM pentamer (b), and secretory IgM (c).

yield of Fc-fragments [42]. Trypsin cleavage at 60°C leads to total destruction of Cμ2-domains and formation of monovalent Fabμ-fragments and $(Fc\mu)_5$-fragments (10.5S, 340 kDa) containing interconnected Cμ3- and Cμ4-domains and J chain [43, 44]. This and other data indicated that Cμ3- and Cμ4-domains represent a genuine Fc-region of the IgM molecule, and the Cμ2-domain is a structure typical only for IgM, which was lost or transformed into hinge region during the evolutionary formation of other Ig classes [45].

μ-Chains contain five regions of N-glycosylation. Totally, the carbohydrate component of IgM comprises from 7 to 12% of the mass of the molecule. Complex glycans are connected to Asn residues in positions 171, 332, and 395, and oligomannose glycans are attached at positions 402 and 563. The latter represent 23.4% of all serum IgM carbohydrates, ~100% of Asn402 sites and ~17% of Asn563 sites being glycosylated [46, 47].

A spatial model of the IgM molecule was proposed on the basis of glycosylation characteristics, according to which IgM has a disk shape with two qualitatively different surfaces. One contains Cμ4-domains and J chain, the other contains antigen recognition sites and complex glycans groups. Thereby, IgM molecules are able to bind antigenic determinants of bacteria and viruses and their lectin-like components such as hemagglutinin of influenza virus [48].

The analysis of homology of IgM and IgE constant domains and data of crystal structure of IgE Fc-region, complemented by observations of atomic force microscopy, produced a more detailed spatial model of the IgM molecule. It corresponds to the above-mentioned, but it has one significant difference. Assuming that all cysteine residues take part in the formation of disulfide bonds between subunits, the authors concluded that the pentameric IgM molecule is not planar [48, 49] but is mushroom-shaped (Fig. 3). Its flat part ("cap") is formed by radially-directed Fab-regions. The protruding part above it ("leg") is formed by Cμ4-domains rotated by 90° to the flat part. Thereby, antigen-binding sites and glycan groups are located on the surface opposite to the protrusion [13].

IgM RECEPTORS

Despite the fact that macroglobulin antibodies (the former name of IgM) were identified in the 1930-1940s and up to the beginning of the 1970s were already well studied, the question of existence of IgM receptors similar to analogous Fc-receptors of IgA, IgG, and IgE [50-52] was open for a long time.

Polymeric immunoglobulin receptor (pIgR). It was established in the beginning of the 1980s that SC, being a part of sIgA and sIgM, is a fragment of membrane protein receptor (pIgR) that is incorporated in the structure of pIg during transfer across mucous membranes [53]. pIgR is expressed on basolateral surface of mucous epithelium and ducts of excretory glands. The highest level of pIgR expression is observed in small and large intestines. pIgR is also present in kidneys, pancreas, lungs, and endometrium [54].

pIgR belongs to the Ig protein superfamily (Fig. 4a). It contains five extracellular domains stabilized by disulfide bonds (D1-D5) of 100-110 a.a. each, a linker, and transmembrane and cytoplasmic segments [55]. A short hinge-like segment is located between D1 and D2. The second flexible segment is located between D3 and D4 [56]. The linker region closest to a membrane contains amino acid residues that provide its flexibility and sensi-

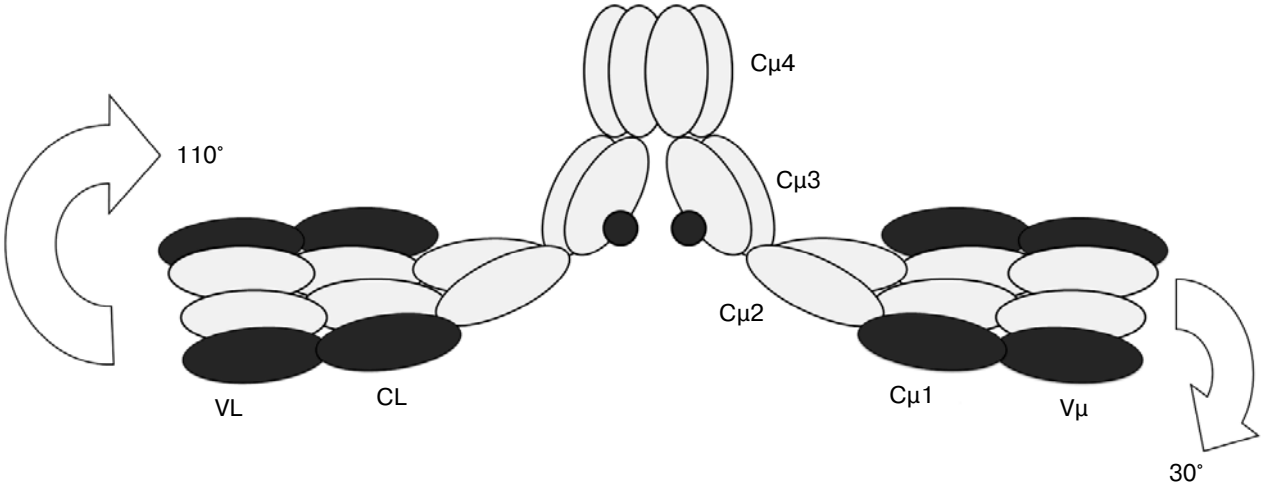

Fig. 3. Spatial disposition of domains in the pentameric IgM molecule according to the model proposed in work [13]. Two of five 7S-subunits are shown. Light ovals indicate domains of heavy chains; dark ovals indicate light chains; dark circles indicate localization of C1q binding sites; arrows indicate maximal rotation angle of Fab-regions relative to the horizontal plane.

tivity to proteolysis [57]. There are 20 cysteine residues in the human pIgR molecule. Their distribution provides the formation of one (in D2) or two (in D1, D3, D4, and D5) intradomain disulfide bonds. The pIgR molecule is highly glycosylated, the carbohydrates being up to 22% of its weight [58]. Carbohydrate composition is quite variable, but it does not influence the ligand-binding activity of pIgR [59].

An pIgR precursor with molecular weight 90-100 kDa is synthesized in endoplasmic reticulum and is incorporated into the membrane on the basolateral surface of epithelium cells [60]. Subsequent transformation of the pIgR molecule takes three stages. The first stage is ligand binding and endocytosis of the complex, which depend on two tyrosine-containing sites in the cytoplasmic pIgR domain [61]. The second stage is translocation in endosomes to the apical pole of the cell. It is determined by phosphorylation of serine residues and microtubule dynamics [62, 63]. The third stage is exit to the apical cell surface followed by proteolytic degradation of the extracellular part either in the form of free SC with molecular weight 80 kDa, or as SC bound to sIgA or sIgM. Proteolysis is performed by endopeptidase localized on the membrane and is independent from ligand binding [64]. The sedimentation coefficient of native and recombinant SC is close to 4.25S. The molecular weight of SC separated from different tissues can vary, probably due to characteristics of proteolytic degradation [65].

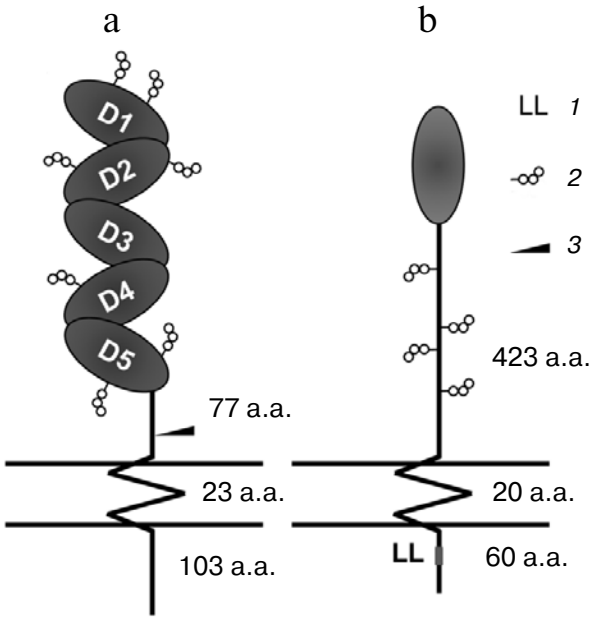

Fig. 4. Structure of pIgR (a) and Fcα/μR (b). *1-3*) Dileucine motif, carbohydrate groups, and proteolysis site, respectively.

Some pIgR molecules do not split out at the apical surface but go back to the basolateral part of a cell [66]. This receptor "turn-over" allows using it as a target for monoclonal antibodies for targeted incorporation of genetic material into cultivated cells or *in vivo* [67].

The creation of a series of mutant IgM revealed that the pentameric form of the molecule, the presence of Cμ4-domain, and J chain are crucial for binding to pIgR [68]. Biochemical and molecular studies revealed that the ligand-binding region of pIgR is located in D1, most distant from the membrane. D1 is a necessary and sufficient element for IgM binding, though there are also other domains taking part in this process in some animal species [64, 69].

The study of recombinant non-glycosylated SC fragments containing only D1, D1-3, or D1-5 revealed that

all of them are able to bind IgM, but interaction affinity increases 20-fold when receptor contains other domains along with D1 [37].

As D1 is similar to V-domains of Ig it was assumed that structures, similar to complementarity-determining regions (CDR), may take part in the formation of ligand-binding sites of pIgR. Point mutations in rabbit pIgR regions corresponding to CDR1, CDR2, or CDR3 of antibody molecules significantly decreased the efficiency of binding [70]. The mutual exchange of regions between human and rabbit pIgR CDR showed that they are interchangeable, but CDR2 of human receptor is necessary for pIgM binding [71]. These data correlate with previous investigations showing that pIgR of some animal species (human, bovine) can bind pIgA and pIgM, whereas mouse, rat, and rabbit pIgR bind pIgA but not pIgM [72].

An important role in study of ligand-binding activity of pIgR belongs to the determination of the crystal structure of human D1 pIgR [73]. D1, similarly to V-domain of Ig, contains five main amino acid residues stabilizing domain structure. A relatively long CDR1 loop contains a single helical loop (Ser28-His32) consisting of highly conservative amino acid residues. Mutations in the CDR1 helical region of rabbit pIgR block its ability to bind pIg [70], stressing the crucial role of this structure in the properties of the ligand-binding surface.

Fcα/μ-receptor. For a long time there were no significant results in the search for Fc-receptor binding of IgM on immune cells since a gene encoding the target protein remained unknown [74-76]. The finding in 2000-2001 of a new protein binding IgA and IgM was not broadly recognized. Publications confirming the discovery of a novel receptor appeared only in 2007-2009.

A receptor called Fcα/μR was found during screening of an expression library of a cultivated line of mouse lymphoma for products able to bind IgM. Fcα/μR is a protein of the Ig superfamily with molecular weight about 70 kDa. It contains more than 500 a.a. with four regions of potential glycosylation (Fig. 4b). Cells, transfected with the Fcα/μR gene endocytose *Staphylococcus aureus* bacteria covered with IgM. The endocytosis process depends on a dileucine motif (Leu519, Leu520) in the cytoplasmic domain of the receptor. Transfectants bind mouse IgM with high affinity ($2.9 \cdot 10^9$ M^{-1}). The affinity of IgA binding was an order of magnitude lower ($3 \cdot 10^8$ M^{-1}). Along with mouse IgM, the receptor binds human and rat IgM and rat IgA [77].

A homolog of the Fcα/μR gene, 49% identical to the mouse gene, was discovered on human chromosome 1 (1q 32.3) close to genes encoding other Fc-receptors. Human Fcα/μR bind both IgM and IgA. The region of human Fcα/μR within the limits of a.a. 76-98 is homologous to the CDR1 region of pIgR and is predicted to be a part of a conservative ligand-binding site. Mouse and human receptors are of similar structure. They consist of one Ig-like domain similar in structure to D1 of pIgR (40% with identical positions of amino acid residues), adjacent to the membrane of mucin-like region, transmembrane and cytoplasmic domains [78].

Receptor has been found in all lymphoid tissues including lymphatic nodes and appendix. It is widely expressed in non-hematopoietic tissues, in kidney and intestine, and less in lungs, liver, and myocardium [79]. Cell studies revealed the presence of Fcα/μR on most B-lymphocytes and macrophages but not on T-cells, granulocytes, and natural killers. Cross-binding by Fcα/μR of IgM molecules dissolved or adsorbed on particles induces receptor internalization, which is critically dependent on the presence of the intact cytoplasmic domain [80].

Knockout mice with inactivated Fcα/μR gene were immunized by transfected cells expressing this receptor and used for obtaining hybridomas. Reagents partially or totally blocking binding of IgA and IgM with Fcα/μR were found among the resulting monoclonal antibodies. The same antibodies interacted with a peptide fragment reproducing an amino acid sequence common for Fcα/μR and pIgR. This implies that ligand-binding regions of these receptors have similar or identical structure [81].

Subsequent studies using monoclonal antibodies and PCR revealed that receptor is presented on the cell surface as a stable homodimeric complex with molecular weight 115-135 kDa. On most human B-lymphocytes Fcα/μR is present in quantities close to the detection threshold. The exception is a small population of B-cells in tonsillar tissue. This points out significant species differences in Fcα/μR expression – it is present on all circulating B-lymphocytes in mice. It is also found that the main cell type expressing Fcα/μR in humans is follicular dendritic cells of germinal centers [82].

Fcα/μR is also expressed in lamina propria cells of intestinal tissue and in germinal centers of some lymphoid follicles. It is found on intestinal macrophages, plasma cells, and Paneth cells. This implies that it takes part in both local and systemic immune reactions of mucous membranes. The variability of Fcα/μR expression in different parts of the intestine (small and large intestine and appendix) indicates possible differences in the function of this receptor in maintaining mucous membrane immunity [83].

Deeper study using a wide panel of IgA and IgM mutant forms showed that only polymeric variants of these molecules interact with Fcα/μR. The interaction of IgA with Fcα/μR is defined by the region on the border of the Cα2- and Cα3-domains, which is known as a recognition site of SC, FcαRI myeloid receptor (CD89) [84], and bacterial IgA-binding proteins [85]. Domains Cμ3 and Cμ4 appeared to be crucial for IgM binding. Free SC and bacterial proteins, as well as monoclonal antibodies against Cμ3 and Cμ4, inhibited ligand–receptor interaction [86]. The dependence of interaction of Fcα/μR with

IgM on binding of the latter with antigen was not studied specifically. The collection of data from the above-cited studies suggests that Fcα/μR binds free IgM as well as that bound in immune complexes.

Transcripts of the Fcα/μR gene were found in cultivated cells of human kidney mesangium. IL-1 stimulation increased the amount of transcripts [87]. Studies on mice showed that kidney tissue contains a special Fcα/μR isoform not present in lymphoid tissues. "Kidney" receptor type interacts with IgA and IgM, but is distinguished by the fact that it is expressed in tubular epithelium cells and not in glomeruli [88].

FcμR receptor. Receptor called FcμR is described in work [89]. It is a transmembrane protein with molecular weight 60 kDa expressed on T- and B-lymphocytes. Its gene is located in the same cluster 1q32.2 of chromosome 1, like genes of *pIgR* and *Fcα/μR*. Despite the resemblance between ligand-binding domains of all three receptors, FcμR is less similar to the other two. First, fewer amino acid residues take identical positions, i.e. the identity level with pIgR and Fcα/μR is much lower. Second, the length of the CDR1-region, which is predicted to contact with ligand (IgM), is shorter than that of pIgR and Fcα/μR, it contains only 5 a.a. Third, the CDR1-region contains only two charged amino acid residues. Histidine takes position 32 in all three compared molecules, but arginine in position 31 is present only in pIgR and FcμR. This residue is assumed to have direct contacts with the ligand, but there are only uncharged amino acid residues in this position in FcμR from seven different animal species. FcμR recognizes only IgM, while pIgR and Fcα/μR are able to bind both IgM and IgA [55, 82]. These data imply that the character of binding of the new receptor with IgM differs from that of other receptors binding with pIg. Data on the ability of Fc(μ)5-fragment, consisting of Cμ3/Cμ4-domains, to inhibit IgM binding to FcμR suggests that the ligand region providing FcμR binding is localized in these particular structures. In the case of pIgR this region is located in the Cμ4-domain [55, 68].

IgM-binding proteins from microorganisms. It is known that some viruses and bacteria express receptors on their surface that bind Fc-regions of IgA or IgG [90, 91]. These receptors are assumed to enable pathogens to escape from recognition by the host's immune system and destruction by effector mechanisms such as phagocytic ingestion and complement-dependent lysis [85, 92, 93]. Analogous receptors binding IgM Fc-region have not yet been found in bacteria and viruses. All known pathogenic microorganisms carrying such proteins are parasitic protists. IgM-binding proteins of *Toxoplasma gondii* [94] and pathogenic types of *Trypanosoma somatidae* are described [95]. Detailed data on molecular genetics of IgM-binding proteins found on erythrocytic membranes infected with *Plasmodium falciparum* have been published recently [96].

FUNCTIONAL ASPECTS

B-Lymphocytes – IgM producers. In humans immature B-lymphocytes with membrane-bound IgM emerge on eighth week, and mature B-cells able to synthesize IgM on the 12th week of pregnancy [97]. The first producers of IgM, known as B1-lymphocytes [2, 98], originate from embryonic liver cells [99]. They make up to 50% of lymphocyte population in newborn babies, and in adults their amount reduces to 20% [100]. In the adult organism they are mainly localized in abdominal and pleural cavities and represent a self-maintaining population [101, 102]. B1-cells are phenotypically characterized by high expression of IgM and low expression of IgD and CD11b. Depending on the presence of CD5 marker, they are divided into B1a-(CD5+) and B1b-(CD5–) subpopulations [103, 104].

B1a-lymphocytes lack mechanisms of V-gene rearrangement, isotype switching, and somatic hypermutation. IgM-antibodies synthesized by them are encoded by germline genes [105]. They appear without any obvious connection with outer antigen stimuli, and so they are referred to the category of natural antibodies (NA) [106].

A second population of IgM-producing cells is formed during postnatal period. B2-lymphocytes originate from hematopoietic stem cells of bone marrow [107] that migrate to spleen, differentiate, and occupy niches of the marginal zone and lymphoid follicles [108]. After contacting antigen and T-helper, follicle B2-cells start producing IgM-antibodies, then they undergo isotype switching and affinity maturation and turn into plasmocytes or memory cells [109]. B2-lymphocytes are distinguished by high expression of surface IgM and IgD and the absence of CD11b [110]. IgM-antibodies synthesized by B2-lymphocytes, unlike EA, are called immune or immunization-induced.

Recent studies revealed that B1b-cells represent a cell type combining characteristic of B1a- and B2-lymphocytes. They are localized in the abdominal cavity and produce IgM on the basis of germline transcripts, as B1a-cells. On the other hand, under the influence of cytokine or antigenic stimuli they can activate the mechanisms of somatic hypermutation and switching to IgA synthesis like B2-lymphocytes. B1b-lymphocytes are assumed to be precursors of plasma cells-producers of high-affinity IgA-antibodies found in lamina propria of the intestinal mucosa [111].

Immunity mediated by IgM was assumed to be short-term and thereby not playing an important role in protection against pathogens. This thesis is probably to be reconsidered because in a set of experiments it was revealed that mouse B1a- and B1b-cells are able to synthesize IgM-antibodies over a long period of time after experimental infection [103, 104, 112]. Using a new experimental model, the mechanism of maintaining long-term IgM production by means of re-initiation of germinal center formation was revealed [113].

On the whole, the available data correspond with the earlier proposed view that the mammalian immune system is constructed of two subsystems. The first, primordial, consists of B1-lymphocytes and γδT-cells and is concentrated in lymphoid tissue associated with mucous layers, primarily, the mucous layer of the gastrointestinal tract. The second, evolutionarily newer, contains B2- and αβT-lymphocytes of the central and peripheral lymphoid organs [114].

IgM interaction with antigens. Functionally IgM class antibodies have relatively low affinity constants. The presence of 10 or 12 (in hexamers) antigen-recognizing sites substantially increases the integral binding ability (avidity) of IgM, but because of steric constrains this potential is only realized in reactions with molecules having molecular mass not exceeding 1.5 kDa [115]. During interaction with antigens of larger size IgM behave as antibodies containing from two to five binding centers [116]. The high valence of IgM polymers allows binding antigens with a wide range of avidities (from 10^{-3} to 10^{-11} M^{-1}, usually from 10^{-6} to 10^{-7} M^{-1}) to cause agglutination and aggregation of particles and neutralization of toxins and other biologically active molecules [117, 118]. Additional increase in avidity may be due to the contacts of carbohydrate residues of IgM molecules located on the same surface as antigen-recognition sites with lectins of bacteria and viruses [13, 40]. The large size of IgM molecules promotes aggregation and agglutination of particles with low-density of antigenic determinants and binding of antigens with multiple repeating epitopes (virus particles, erythrocytes, and surface carbohydrates of bacteria and cells). Agglutination is considered as an important component of the complex of defense reactions mediated by IgM [115]. Agglutination ability of IgM exceeds that of IgG by 100-10,000 times.

IgM level in serum of newborns and animals grown in sterile conditions on an antigen-free diet does not differ from that of normal animals [119]. This implies that most circulating IgM molecules are represented by NA synthesized by B1-lymphocytes [120]. Being a product of non-rearranged germline genes, NA have conformationally labile active center structures [121-123] and, as a consequence, relatively low affinity ($K_d = 10^{-4}$-10^{-7} M^{-1}). A second feature of EA is their polyreactivity, i.e. the ability to interact with a high range of structurally different antigens. NA react with both allogenic and many autoantigens [124-126], and so they are called natural autoantibodies [127, 128]. NA recognize phylogenetically conserved structures that are thymus-independent antigens [129, 130]. These antigens can bind with B1-lymphocyte receptors and induce synthesis of antibodies exclusively of IgM class, independently of T-helpers [131]. Some thymus-independent antigens (I type) activate B1-lymphocytes by means of binding Toll-like and analogous receptors without classical second co-stimulator signal [132]. Other thymus-independent antigens (II type) are structures with multiple repeating epitopes, such as membrane polysaccharides or bacterial flagella [133].

IgM antibodies induced by immunization comprise a relatively small fraction of circulating molecules. They are monoreactive, have higher affinity (10^{-7}-10^{-11} M^{-1}), and their variable regions contain point mutations evidencing a bypassed process of somatic hypermutation [134].

Interaction of IgM with complement. Complement activation through the classical pathway is thought to be the main and is the most studied IgM effector function [135]. The activation process is launched by the binding of a C1q molecule to IgM in an immune complex [136]. Mapping with the use of directed mutagenesis of C1q binding sites showed that they are located in the Cμ3 domain, within the range of a.a. 330-340 [137]. Proline in position 436 [138] and glycosylated serine 406 [139] play critical roles. The Cμ1-2 and Cμ4 domains also take part in complement activation [140], most probably through noncovalent interactions stabilizing the IgM–C1q complex.

The above-described model of IgM spatial structure (Fig. 3) postulates the location of C1q binding sites on the surface of the molecule opposite to the protrusion formed by Cμ4-domains [13]. Thermodynamics-based analysis of molecule segmental flexibility revealed that due to steric constraints Fab regions are able to deviate from the protrusion to an angle of not more than 30°. However, the bend to the opposite side may be up to 110°. This implies that during multivalent binding with antigen, deviating Fab regions disclose the surface of C1q binding sites and make them available for ligand interaction. Thus, the proposed model concretizes a previous hypothesis [136] according to which conformational reorganization of the IgM molecule for exposing C1q binding sites is necessary for complement activation.

The C1q molecule consists of six identical subunits. Each contains three globular domains in the C-terminal region that recognize IgM and IgG ligand regions [141]. From two to four C1q molecules contact IgM, which is sufficient for initiation of the classical activation pathway [142]. One molecule of pentameric IgM connected with an erythrocyte is able to activate complement and provide immune hemolysis [143]. In this regard its efficiency is equivalent to 1000 IgG molecules [144]. Monomeric IgM molecules do not activate complement.

Hexameric IgM exerts 10-100-fold ability to activate complement compared to pentameric IgM [145]. It may be that during reaction of hexameric IgM with C1q, which is also hexameric, stoichiometric correspondence provides a more stable complex or transformation of C1q to conformational state, more effectively activating the subsequent chain of events.

C1q binding to antigen–IgM complex initiates activation of C1-proteases cleaving C4 and C2. C4b and C2a

fragments assemble in C3-convertase, a heterodimeric complex covalently bound with antigen. Each molecule of the complex is able to activate up to 1000 C3 molecules, which initiates a chain reaction process and promotes lysis of the antigen target [146]. C3 proteolysis exposes a thioester bond concealed by hydrophobic surrounding in the native molecule [147] and reveals chemically active C3b and C3d fragments covalently bound to antigen [148]. These fragments play the role of molecular tracers that opsonize immune complexes and transform them into ligands of complement receptors. Receptors expressed on macrophages, neutrophils, and dendritic cells (CR3 and CR4) belong to integrin family (CD11/CD18) [149]. They promote phagocytosis and degradation of immune complexes. CR1 (CD35) and CR2 (CD21) receptors fix conjugates of immune complexes with C3b and C3d on membranes of B-lymphocytes and follicular dendritic cells (FDC) in spleen and lymphatic nodes and initiate formation of germinal centers and development of humoral immune response [150].

Immune complexes containing IgM are primarily located in the spleen where with participation of CR1 and CR2 they bind with B-lymphocytes of the marginal zone. These cells later migrate to follicles and transport immune complexes to the surface of FDC, which fix antigen on the membrane or adsorb it for subsequent presentation [151].

Along with effector functions, interaction of IgM with complement plays a role of a regulating factor of immune reactions [152]. In the early 1980s it was found that antibodies can either enhance or suppress immune response depending on isotype [153]. When specific antibodies of IgM class are introduced together with antigen being recognized (malarial parasite, erythrocytes, or the high molecular weight protein hemocyanin), enhancement of immune response was observed [154, 155]. This is revealed in the dynamics of primary and anamnestic reactions and in acceleration of affinity maturation [156, 157]. The effect of passive IgM immunization is blocked by C3-factor from cobra poison [152] and also by mutations of the gene encoding complement receptors CR1/CR2 [158, 159]. Monomeric IgM forms not activating complement [160] do not have an enhancing effect [161].

C3d fragment is a key part of the complement system, which enhances immune response by IgM-antibodies. This molecule together with IgM acts as a molecular adjuvant. Providing cross-binding of immune complexes with B-cell receptor and CR2, C3d decreases B-lymphocyte activation threshold [162]. Antigen–C3d conjugate is fixed on the FDC surface using CR2, and it stays connected with them for a long period of time [163]. Repeated contacts of B-cells with antigen fixed on FDC are necessary for isotype switching [164], somatic hypermutation [165], and probably for the formation and maintenance of memory B-cells [166, 167].

The immune response of IgG and IgE antibodies is modulated through Fcγ and Fcε cell receptors [153]. In the case of IgM the role of such mediators was given to complement receptors. It is still not known to what extent adjuvant effects of IgM and C3d depend on participation of Fcα/μ- and/or Fcμ-receptors.

The first studies of mice with Fcα/μ-receptor gene knockout revealed that the response to T-dependent antigens was equal in mutant and wild-type animals. The mutants showed accelerated formation of germinal centers, affinity maturation, and formation of memory B-cells in response to thymus-independent antigens. The absence of Fcα/μR also revealed prolongation of antigen retention on B-cells of the marginal zone and FDC, meaning that in the normal organism Fcα/μR regulates interactions of immune complexes with the indicated cellular elements [168].

Role of IgM in defense against pathogens. Despite long-term and intensive studies, the role of IgM in mechanisms of protection from pathogens is still not studied well for two reasons. First, rapid switch from the production of low-affinity IgM in the first phase of immune response to the synthesis of high-affinity IgA and IgG implies that the antibodies of the two latter classes performed the major protective function. Second, most circulating antibodies of IgM class refer to NA. For a long time NA were thought to be autoantibodies, and their interaction with alien antigen was assumed as a side effect of their polyreactivity, lacking biological significance. Only within the last 10-15 years has evidences for a role of NA in defense against infection been found [169].

An important step in studying the character of natural IgM was made simultaneously by two independent groups using mutant mice lacking secretory IgM. For this, by means of directed mutagenesis of germline cells they inactivated the μ-chain exon encoding the tail piece that is crucial for assembly and secretion of polymeric IgM. Mice homozygous in this mutation ($\mu s^{-/-}$) were totally lacking IgM in their blood serum. The B-lymphocytes in mutants expressed membrane IgM and IgD and supported class-switch to IgA or IgG synthesis and immunity maturation. The mutants differed from normal animals by enhanced response against thymus-independent antigens, and delay in emergence of high-affinity IgG-antibodies against thymus-dependent antigens. They also displayed decrease in antigen uptake by FDC and decrease in germinal centers in spleen. Moreover, the number of B-1 lymphocytes in the abdominal cavity was three times higher than normal [170, 171].

NA-deficient animals turned out to be highly sensitive to viral and bacterial septic infection. Transfusion of normal mouse serum compensated immunodeficiency and restored the resistance to the normal level [172, 173]. Therefore, NA were shown to be full participants in the mechanism of defense against infectious agents.

The study of a wide panel of monoclonal NA IgM class secreted by mouse hybridomas showed that these agents are able to bind gram-negative as well as gram-positive bacteria, lyse them, enhance phagocytosis, and neutralize the action of bacterial endotoxins. Wide antibacterial activity of normal mouse serum decreased a dozen times after withdrawal of polyreactive NA and dramatically increased after enrichment with polyreactive NA [174].

NA polyreactivity and their constant presence in the circulation in sufficient concentrations are probably advantages allowing the organism to immediately react to a wide variety of potential pathogens.

A newly published review on the participation of IgM-antibodies in defense against viruses and extracellular and intracellular bacterial infections describes new data on IgM antibacterial activity at early stages of the infectious process and possible long-term synthesis of those antibodies. The authors point out the necessity of reconsidering existing views on the role of IgM in immune defense, and particularly on possible promise for production of vaccines inducing production of IgM class antibodies [175].

The number of etiological agents of human infectious diseases is close to 200 [176]. Contacts with them are episodic and do not always lead to the development of disease. These pathogens represent a small part of the variety of protozoa, bacteria, and viruses that make contact with multicellular organisms. The second part of the whole, which is in constant contact with our organism, is a consortium of 500-1000 species of symbiotic bacteria living on the mucous layers and in the intestinal tract [177]. Secretory IgA is thought to be the main factor of adaptive immunity providing interaction between organism and environment [178, 179]. IgM is also referred to secretory antibodies [5, 180]. It is proved to be transported to the surface of mucous layers [181]. However, its role in co-existing of organism with microbiota is still to be investigated. So far, we only realize the function of IgM as a component of immune defense in the neonatal period and in IgA deficiencies as a compensating factor [182].

Role of IgM in homeostasis and development of non-infectious pathology. IgM molecules of NA category (IgM-NA) interact with multiple antigens. The role of NA in the healthy organism and in the development of autoimmune processes has been actively studied and discussed [183]. The creation of mice deficient in secretory IgM ($\mu s^{-/-}$) provided some clearness regarding this problem. Isolated IgM deficiency in mice carrying only the $\mu s^{-/-}$ mutation predispose to the development of lupus-like syndrome and increase in autoantibody production against DNA after injection of lipopolysaccharide [184]. Incorporation of the same mutation into the genome of mice inclined to development of autoimmune lupus-like syndrome (lpr mutation) showed that in the absence of secretory IgM, the production of IgG-antibodies against double-stranded DNA and histones is enhanced, as well as the development of immune complex glomerulonephritis with deposition of complexes in glomeruli [185]. These data imply that IgM-NA represent a homeostatic factor taking part in maintaining immunological tolerance and regulation of synthesis of "pathogenic" IgG autoantibodies accompanying autoimmune diseases [186, 187].

The search for possible mechanisms of the participation of IgM-NA in maintaining immunological homeostasis has generated a set of hypotheses, one of them originating from widely known NA tendency to interact with phospholipid haptenic groups of proteins and polysaccharides. Phosphorylcholine (PC), phosphatidylcholine (PTC), and other phospholipids are included in cell walls of *Pneumococcus*, *Salmonella*, *Escherichia*, and other bacteria. NA against phospholipids are known to take part in reactions against bacteria of intestinal microflora and pathogenic microbes [188]. Monoclonal variants of antiphospholipid NA are well studied, as myeloma cell lines producing them were already obtained in the 1950-1970s [189, 190]. While studying these antibodies, it was shown that single amino acid substitutions in the variable region of heavy chain result in a loss of the ability to bind PC, but trigger the emergence of affinity to a set of phosphorylated macromolecules such as double-stranded DNA, protamine, and cardiolipin [191]. These data revealed a mechanism for transformation of antibodies involved in immune defense to "pathogenic" autoantibodies [192].

Attention to the nature of NA against phospholipids significantly increased when PC and PTC were shown to be exposed on membranes of damaged erythrocytes [193] and apoptotic cells [194]. NA perform one of the major homeostatic functions: elimination of aged erythrocytes, dying cells, and altered forms of serum proteins [195]. It is sufficient to mention that there are 10^9 apoptotic cells to be removed every day only within the immune system. During apoptosis phosphatidylserine is translocated to the outer membrane, and phospholipases are activated, leading to the formation of lyso-PC, which is a ligand for NA. Soluble lyso-PTC form diffuses to the outer medium and serves as an attractant for migrating phagocytes. Opsonization of apoptotic cells by IgM and complement promotes their fast absorption by phagocytes. If this process does not take place, cells undergo necrosis, which induces inflammation [196]. One of the proofs of the participation of NA in the clearance of dying cells was obtained by creating hybridomas based on lymphocytes from intact mice. A clone producing IgM-antibodies that increased phagocytosis of apoptotic cells by macrophages was found among those hybridomas. These antibodies bound lyso-PTC as well as some other autoantigens. Gene segments encoding variable regions of heavy and light chains appeared to be close in configuration to germline genes, one of the regions containing point

mutations. These data imply that the antibody refers to the EA repertoire, recognizes apoptotic cell marker, and may work as opsonin during macrophage absorption [197].

Studies in recent years revealed that NA, recognizing phospholipids, are not the only cells participating in clearance of apoptotic, aging, and damaged cells; IgM molecules binding autoantigens, in particular cytoskeleton and erythrocytic membranes, are also involved in this process [198].

Antiphospholipid EA were also found to recognize altered epitopes originating upon conjugation of oxidized low-density lipoprotein products with proteins and other molecules [199]. The participation of NA in the development of atherosclerotic damage of blood vessels is widely discussed [200]. For instance, an inverse correlation was described between the level of IgM-NA against oxidized lipids and the level of coronary artery stenosis [201]. Still, high level of IgM-NA against PC is assumed to be a protective factor decreasing the risk of atherosclerosis of the carotid artery in patients with hypertonic disease [202].

Recent studies show that IgM-NA and the complement cascade activated by them are responsible for damages originating after blood flow restoration in ischemic tissues (ischemia–reperfusion). These damages are most likely to occur during stroke, myocardial infarction, and blood flow disturbances in intestine and transplanted organs [203] and may lead to significant complications including lethality. Experiments carried out on a set of experimental models revealed that acute inflammatory process after ischemia–reperfusion was initiated by autoreactive IgM-NA and complement activation. Thus, knockout mice with C3 and C4 deficiency do not experience muscle and intestinal injury after experimental ischemia. Serum transfusion from wild-type animals restored their ability to develop disturbances [204, 205]. An analogous result was found during a study on mice with ($RAG^{-/-}$) mutation determining innate Ig deficiency.

To test the initiating role of IgM-NA, a screening test was carried out on a panel of mouse monoclonal IgM synthesized by hybridomas based on peritoneal cells enriched with B1-lymphocytes. The product of one of the clones restored the ability of Ig-deficient mice ($RAG\text{-}1^{-/-}$) to develop intestinal tissue damage after ischemia–reperfusion. In the damaged tissue deposition of IgM, C4 and C3, similar to that of wild-type mice, was observed. The analysis of nucleotide sequences encoding variable domains of heavy and light chains of the studied antibody revealed that they are highly homologous to germline genes and common for the repertoire of B1 cells producing NA [206]. This proves direct participation of IgM-NA in postischemic tissue damage and reveals possibilities for identifying specific autoantigens responsible for development of damage and the search for prophylaxis of this pathology.

It is not known whether this mechanism of postischemic damage acts in humans. When purified human IgM was injected into mice with innate Ig deficiency and intestinal ischemia followed by reperfusion was produced, the animals manifested typical tissue damage with C4 deposition in intestinal villi, which could be revealed immunohistochemically [207]. Therefore, human IgM-NA were shown to be able to produce damage to postischemic tissue in experimental models. Clinical studies where inhibitors of complement activation were used for prophylaxis of such complications did not give such clear results as animal studies [208]. Probably, the species characteristics of the development of postischemic damages in humans need to be studied more precisely (in particular, lectin, alternative, and classical pathways of complement activation could participate in this process to different extents).

Antitumor activity of NA. Studies of the pattern of tumor growth led to the assumption that glycans have a key role in progression of neoplasms. Glycans are found to participate in regulation of tumor cell proliferation, their invasion into surrounding tissues, and metastasis [209]. Transformed cells of epithelial tumors (carcinomas) often synthesize and express on their surface glycoproteins and glycolipids with abnormal structure [210]. Molecules of such type can serve as ligands for NA of IgM class.

The use of heterohybridoma technique and hybridoma creation on the basis of human cells allowed obtaining and studying of multiple antibody variants synthesized in the organism of patients suffering from malignant tumors [211]. Lymphocytes for hybridization were obtained from regional lymphatic nodes or from tumor tissues themselves. All discovered antibodies specific to tumor cells reacted with carbohydrate epitopes of antigens, which passed posttranslational modification, belonged almost entirely to IgM class, and are the products of non-mutated germline genes. This implies that all studied antibodies belong to the NA category. Monoclonal NA with no signs of mutations displayed wide cross reactivity with carcinoma samples obtained from different patients, whereas a few antibodies with mutation signs reacted only with a limited number of tumors. Among those studied were NA interacting with cells of stomach, lung, large intestine, and pancreatic carcinomas. The activity of these antibodies against malignant cells was manifested in induction of apoptosis and inhibition of proliferation [212]. These data imply, according to the authors, that IgM-NA play an important role in mechanisms of immunological surveillance upon emergence of malignant tumors of epithelial nature, and they may turn out to be effective anticancer agents [213]. Tumor cells are known to be able to escape from immunological surveillance. So, the strategy of antibody use as anti-tumor agents has to be developed, taking this property into account [214].

The data reviewed above indicate the necessity to correct some notions concerning IgM nature and to search the answers to number of questions.

According to common views, all Ig molecules are products of the adaptive immunity system of vertebrate animals [215]. There are IgM of two classes present in the adult organism that are different in origin and properties. IgM induced by immunization carry signs of somatic hypermutation, and therefore they should be assumed to be products of the adaptive immunity system.

A significant part of IgM molecules corresponds to polyreactive low-affinity NA that originate irrespectively of immunization and are encoded by non-rearranged Ig genes. The repertoire of antigens recognized by NA is limited and does not depend on external antigenic stimuli [216]. It is formed during realization of the genetic program of B1-lymphocyte development [119, 217]. Therefore, NA should be considered as one of the variants of innate immunity recognizing molecular structures (patterns) common for pathogenic and non-pathogenic microorganisms and components of autologous cells [218]. It should be mentioned that PC, being a hapten recognized by antiphospholipid EA, is simultaneously a ligand of classical factors of innate immunity, C-reactive protein and serum amyloid P [219]. NA are most probably to be assumed as a transitional form from invariable receptors of the innate immunity system to the highly variable antibody molecules. Evolutionary and physiological mechanisms determining the origination and conservation of genes encoding NA in the genomes of all vertebrates call for further investigation.

According to common views, synthesis of IgM-antibodies during immune response goes on from 3 to 5 days and then it is switched to production of antibodies of other classes. The concentration of IgM in blood serum of healthy individuals is maintained within the range of 1.5-3 g/liter, only 20-30% of which being constantly produced NA. What is the nature of the remaining 70-80% of circulating IgM molecules? What are the mechanism maintaining such high concentration of IgM in blood? The search for the answers to these questions calls for deeper studies.

The participation of J chain in IgM molecule assembly is still not fully defined. Along with accepting the crucial role of the Cys575 residue, the participation of a glycan group bound to the highly conservative Asn563 residue of the μ-chain "tail piece" in polymer formation is being discussed. In the absence of this component, cells produce hexamers and polymers of higher order that have low affinity to antigen [220]. We can propose that the ability of IgM for oligomerization due to noncovalent interactions is based on the structure of heavy chains, and the role of J chain is to regulate this process. Moreover, J chain is involved in secretory Ig formation. It takes part in the formation of the ligand region interacting with pIgR determining quaternary structure of pIg molecules and, possibly, directly contacting receptor protein [221].

Data on hexameric IgM production and their ability to activate complement was obtained mainly during studies of cell lines producing IgM. Data on hexamer production in an intact organism evidences their presence in serum of patients suffering rare diseases such as cold agglutinins syndrome and Waldenstrom's macroglobulinemia. It is not clear whether production of hexameric IgM is possible in normal organisms or during more widely spread pathological conditions. Studies in this direction were limited because ultracentrifugation was the only method to distinguish pentameric molecules from hexameric. Creation of monoclonal antibodies recognizing J chain in pentameric IgM allows the measurement of amount of hexamers, lacking J chain, in the pool of those molecules [222]. It allows for a new approach in studying hexameric IgM production in the whole organism.

As discussed above, pIgR was the first studied Fc-receptor binding IgM. Its expression on epithelial cells points out the participation of IgM in immune defense of mucous layers. However, specific data on IgM functioning in mucous layers is not present in the available literature, including the latest publications [223, 224].

The picture of IgM-antibody interaction with antigens, complement, and immune system cells was established in the period when the existence of Fc-receptors to IgM was not proved. Complement receptors were assumed to be the major mediators between humoral reactions with the participation of IgM and the cellular compartment of the immune system. Data on the existence of Fcα/μR and FcμR may significantly contribute to or alter present views. For instance, the mechanism of immune complex translocation from B-lymphocyte membrane to the surface of FDC has not been identified. The presence of Fcα/μR and FcμR on these cells implies that these molecules may be the missing link in a chain of events following formation of IgM–antigen complex and complement activation.

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