ISSN 0006-2979, Biochemistry (Moscow), 2007, Vol. 72, No. 5, pp. 485-494. © Pleiades Publishing, Ltd., 2007.
Published in Russian in Biokhimiya, 2007, Vol. 72, No. 5, pp. 603-614.

REVIEW

Unknown Functions of Immunoglobulins A

T. N. Kazeeva and A. B. Shevelev*

Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, 119071 Moscow, Russia; fax: (495) 954-2732; E-mail: shevelev@inbi.ras.ru

Received October 25, 2006
Revision received February 4, 2007

Abstract—Traditionally, the function of immunoglobulins A (IgA), the major type of secreted antibodies, has been thought to be restricted to binding antigens outside the epithelium basal membrane. Therefore, effector mechanisms eliminating IgA-opsonized targets have not been investigated so far. However, some indirect observations of infectious agents penetrating into tissues and blood from the environment suggest such mechanisms (analogous to IgG/IgM-dependent activation of complement and natural killers). In the present review, we examine details of IgA structure that might contribute to elucidation of IgA-dependent effector functions in human and animal immunity. Special attention is given to a putative transduction of signal about antigen binding in the active center of IgA from the Fab- to the Fc-superdomain via intramolecular conformational rearrangements. Different structure of the IgA subclasses (IgA1 and IgA2) is examined taking into account probable divergence of their functions in immune response.

DOI: 10.1134/S0006297907050045

Key words: secretory immunity, IgA, antibodies, effectors, IgA1-proteases, CD89, respiratory burst

For decades, the structural and functional organization of IgA has been the focus of interest of principle laboratories worldwide as well as that of other immunoglobulins. This has led to accumulation of an extensive database about this object. Many features in structure and function of IgA have become classics of biochemistry and immunology, being cited in textbooks [1]. Now interest in physical and chemical studies of IgA is considerably lesser. Apparently, since 1988 the opinion of leaders in this field includes that of a group of researchers headed by M. Kerr, J. Woof, and J. Mestecky, who have published a number of classical reviews [2-4].

However, in this field several fundamental problems remain to be solved, these mainly concerning the switching of the isotypic allocation, functions of the effector mechanisms in the secretory immunity, and a major divergence in the idiotypic specificity of the antibodies in different secretions of the same organism.

Abbreviations: a.a.) amino acid residue; CHα (Cα)) constant domain of heavy (α) IgA chain; Fab) variable antigen-binding superdomain of Ig; Fc) constant superdomain of Ig; FcαR) receptor specific for Fc-superdomain of IgA; Fd) component of the heavy chain within Fabα-fragment; Ig) immunoglobulin; NIP) 5-iodo-4-hydroxy-3-nitrophenylacetyl (hapten); pIgR) polymeric immunoglobulin receptor; SC) secretory component of Ig; S-IgA) secreted form of IgA; VH) variable region of Ig.

* To whom correspondence should be addressed.

In the present review, we will not analyze the commonly acknowledged data about structure, biosynthesis, and functions of human and animal IgA. We aimed to systemize the facts that might contribute to elucidation of new roles of IgA in immunity. We restricted data about IgA primary structure by unifying amino acid residue numbering and mass of fragments appearing from limited proteolysis. The relevance of these data is substantiated by the necessity of unifying nomenclature of the residues when data of different authors are compared. This problem is often complicated by occurrence of a variable region in the IgA structure that is nonstandard in length. Ambiguous allocation of variable/constant region borders by different authors leads to an average in the zero point of the sequences. Besides, IgA preparations from different natural sources are significantly heterogeneous in molecular mass, apparently due to nonuniform O-glycosylation of α- and J-chains. Variability of disulfide bond linkage is another factor of heterogeneity. Therefore, the nomenclature of the sequences, the glycosylation, and the disulfide exchange are emphasized.

Traditionally, IgA functions have been restricted by the primary antigen recognition in mucosal serous secretions. For this reason, probable secondary effector mechanisms of the elimination of the recognized targets are ignored. This is substantiated by poor transportability of the components of these putative mechanisms to the

outer space from the blood or tissue fluid. This point of view does not seem to us convincing, since in human and animal blood there is a great amount of IgA including antibodies against many relevant antigens. Lack of eliminating mechanisms for IgA-derived immune complexes automatically makes these complexes a safe refugee for different pathogens, which may use them as a masking competitor against IgG, IgM, and lymphocyte receptors.

Involvement of hypothetical effector mechanisms of IgA immune complex elimination is plausible only if free IgA are clearly distinguishable from the IgA complexes. Otherwise, the effector mechanisms would be nonspecific and dangerous for normal tissues of the host. This task (as well as in IgG) may be solved by signal transduction from Fab- to Fc-superdomain via conformational rearrangements.

We considered the structural data about IgA interactions with the known CD89 and the polymeric IgA receptors as an appropriate tool for solving the above-mentioned tasks. We tended to take into account differences in the studied human and animal IgA structure. Specialized IgA-recognizing proteins from pathogenic bacteria (IgA1 proteases and IgA-binding proteins) may serve as another relevant tool for visualizing conforma-

```
  CH1            Υ            ≠                      *            176
vslasptspk vfplslcstq pbgbvviacl vqgffpqqpl svtwsesgqg vtarbfppsq 1
                 d  p q       c                      n            2m1
                 d  p q       c                      n            2m2

                                                    Main hinge
                    ≠        ≠       *           ↓  ⊗   ⊗ ⊗ ⊗   ⊗ 236
dasgdlytts sqltlpatqc lagksvtchv khytnpsqbv tvpcpvpstp ptpspstppt 1
                    c pg     c                 c  pppp p--------- 2m1
                    c pg     c        s        cr pppp p--------- 2m2

   ↓ΥΥ CH2                  *  Υ                                 296
pspscchprl slhrpaledl llgseanltc tltglrdasg vtftwpstsg ksavqgpper 1
---                                                               2m1
---                                                               2m2

                                            *   ↓CH3
356
dlcgcysvss vlpgcaepwb hgktftctaa ypesktplta tlksgntfr pqvhllppps 1
                                 h  l      nit                    2m1
                                 h  l      nit                    2m2

             ≠                                                   416
eelalnelvt ltclargfsp kdvlvrwlqg sqelprekyl twasrqepsq gtttfavtsi 1
                                                                  2m1
                                                           y      2m2

                ≠                        ↓    *            Υ    472
lrvaaedwkk gdtfscmvgh ealplaftqk tidrlagkpt hvnvsvvmae vdgtcy     1
                                                                2m1
            e                                i         a        2m2
```

Fig. 1. Amino acid sequences of the constant region of α-chains of human IgA1 and IgA2 and their covalent modifications. The main sequence is shown for the monoclonal immunoglobulin Bur (class IgA1, SwissProt Accession Number 763134A) from Val117 until the C-terminal residue Tyr472. For IgA2 allotypes 2m1 and 2m2, only residues different from IgA1 are shown, the gaps indicate missing residues (deletion). Borders of the domain and functional blocks in the sequence are shown with vertical arrows. Designations: *, N-glycosylation sites; ⊗, O-glycosylation sites; ≠, intrachain disulfide bonds (Cys145–Cys204, Cys196–Cys220 in CH1-domain; Cys266–Cys323 in CH2-domain; Cys369–Cys432 in CH3-domain); ϒ, interchain disulfide bonds linking the heavy chain to other subunits: with L-chain (Cys133 in IgA1 and Cys241 or Cys242 in IgA2m(2)), with the opposite α-chain (Cys241 and Cys299 in IgA1), with SC (Cys311 in secreted IgA), and with J-chain (Cys471 in dimeric IgA).

tional rearrangements in IgA induced by antigen binding. Occurrence of such proteins interacting mainly with IgA Fc-superdomains is an appropriate argument showing the existence of IgA-dependent target elimination mechanisms.

STRUCTURAL ORGANIZATION OF IgA

Primary structure of IgA heavy chains. In analysis of the structural organization of the IgA class, the occurrence of two isotypes (subclasses), IgA1 and IgA2 [1], derived from alternative splicing of the common primary transcript [2] must be taken in account. The structure of the constant region of the heavy chain in IgA1 is invariant for the whole human population. In contrast, IgA2 include three different allotypes: (IgA2m(1), IgA2m(2), and IgA2(n)). The primary structures of IgA1 and IgA2 in human and other species are uniform along the whole sequence except the hinge region between the Cα1- and Cα2-domains (Fig. 1) [1]. The unique region of IgA1 (18 a.a., Pro223-Ser240) involves a Pro-rich twice repeated motif of 8 a.a. (-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser)$_2$ [3]. The length of the respective region in IgA2 is 5 a.a. Taking in account the protein secondary structure, Val222-Pro244 [4] or even Val222-Ser238 [5] are included in the IgA hinge region. Occurrence of two Cys residues (241 and 242), which may form disulfide bonds between the opposite α-chains, is significant. The terminological ambiguity of the hinge region location is the only reason for different Cys number count in the hinge region [3-5]. It is noteworthy that contradictory results of protein mapping by different methods may be explained by random including of the neighboring regions to the hinge as well as variability of the molecular masses of the protein fragments caused by different extent of their glycosylation. For instance, work [4] reports unique determinants of the hinge region in IgA1 Bur myeloma protein spanning 26 a.a., which substantially exceeds the length derived from the sequence.

IgA1 amino acid sequences in human, mouse, and rabbit coincide by ~50%. In is noteworthy that the hinge region between IgA Cα1- and Cα2-domains in the studied mammals other than humans and hominids is much shorter and does not contain Pro-rich motifs [6, 7].

Domain organization of human IgA heavy chains. The IgA1 heavy chain, embracing 472 a.a., forms four globular domains named VH (variable), CHα1 (Cα1), CHα2 (Cα2), and CHα3 (Cα3) (constant) (Fig. 2) [3]. VH and CHα1 together with λ or κ light chains form the antigen-binding Fab-superdomain. CHα2 and CHα3 form the constant Fc-superdomain.

First, the domains were mapped based on the amino acid sequence alignment of many Ig-superfamily proteins. However, their allocation to the discrete autonomously stable globules in the tertiary structure was partially confirmed by data of *Proteus mirabilis* metalloprotease cleavage [8]. Therefore, the VH-domain is composed by 114-120 a.a, CHα1 and CHα2 encompass 93-100 a.a., whereas CHα3 length is ~123 a.a. As mentioned above, the spacers between CHα1 and CHα2 correspond to the main hinge region of IgA1 or IgA2. The spacers between CHα2 and CHα3 are 5 a.a. long in both IgA isotypes. Thus, the linkage between CHα1 and CHα2 is endowed with a great flexibility, whereas the 5-a.a. spacer between CHα2 and CHα3 domains cannot provide substantial movement [4].

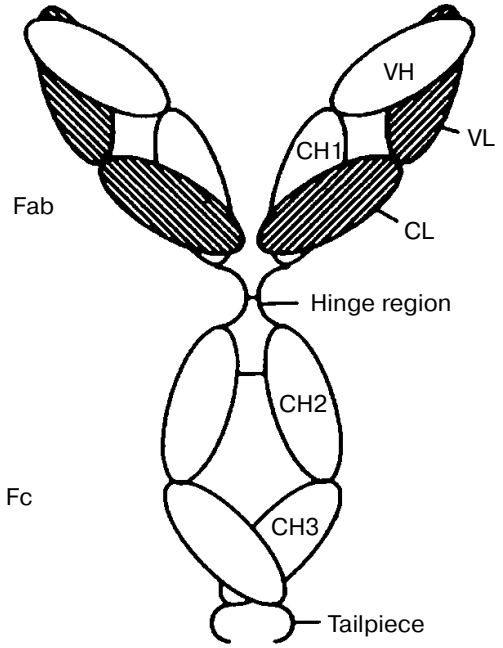

Fig. 2. Structure of human IgA1 (from [3]).

Cys133 in the CHα1-domain is responsible for disulfide bond formation between the IgA1 α-chain and the light chain (L-chain). In the case of IgA2 allotype m2, the functionally analogous bond involves Cys241 or Cys242 in the CHα2-domain. In IgA2 allotype m1, there is no disulfide bond between α- and L-chains. In contrast, both H- and both L-chains are paired with each other. In this case, a steady structure of the IgA monomer antibody is fixed by noncovalent interactions. Treatment of such antibodies with SDS results in dissociation of the monomeric molecule IgA2m(1) to H-chains and L-chain dimers, which can be observed with non-reducing electrophoresis in polyacrylamide gel as bands with apparent masses ~120 and ~55 kD, respectively [2, 4, 9]. Cys241, -242, -299, and probably -301 in the CH2-domain in human, mouse, and rabbit are responsible for covalent binding of the opposite α-chains in IgA1 and IgA2m(1). Cross-linking of the non-symmetric Cys residues in the opposite α-chains of IgA2m(2) (e.g. Cys241-Cys301 and Cys242-Cys299) is hypothesized [4].

As assessed by PAGE, molecular mass of IgA H-chains is ~60 kD. This value is much greater than in IgG, which might be explained by a bulky glycosylation of IgA. In the hinge region of IgA1, there are two putative N-glycosylation and five O-glycosylation sites. In IgA2, there is no O-glycosylation site. The O-bound sugar composition in myeloma serum IgA1 is relatively simple (a single site bears an N-acetylgalactosamine residue; four others harbor residues of galactosyl-(β1-3)-N-acetylgalactosamine). Glycosylation of myeloma-derived IgA may differ substantially from the functionally active antibodies of the same class. This may be illustrated by occurrence of sialic acid and fucose beyond N-acetylgalactosamine in the total hydrolyzate of the O-linked sugars from milk S-IgA. The N-linked sugars are structurally heterogenic even within monoclonal protein preparations and they are rather branched [3].

Like the μ-chain in IgM, the C-terminus of the α-chains in both IgA isotypes extends an 18 a.a. "tailpiece". The penultimate Cys471 residue in the tailpiece of either of two α-chains may be linked with the J-chain, which leads to formation of the dimeric IgA form. In circulating IgA monomers, this Cys471 is prone to be linked with the symmetric residue of the opposite α-chain. With a lesser probability, it may be linked with non-symmetric Cys residues of the opposite α-chain and even with non-immunoglobulin blood proteins such as albumin or α1-antitrypsin. In the most stable dimeric form, all Cys residues are involved in intramolecular disulfide bonds [3].

The J-chain gene is located in the 4q21 chromosomal region of the human genome. As derived from EST database search, it is expressed in T- and B-lymphocytes. The J-chain precursor protein embraces 158 a.a. (predicted mass 18 kD) from which 22 a.a. are involved in the leader peptide removed in the course of translocation to the endoplasmic region and lacking in the mature protein [10, 11]. Therefore, the predicted molecular mass of the mature peptide part of the J-chain is ~15 kD. However, N-glycosylation at Asn71 causes a considerable increase in J-chain mass and decrease in its migration rate in PAGE. The respective product exhibits molecular mass heterogeneity.

The J-chain contains eight Cys residues, six of which are linked within three intramolecular disulfide bonds (Cys35–Cys123, Cys94–Cys114, and Cys131–Cys156). Two others, Cys37 and Cys91, can be involved in linkage with the tailpiece of the IgA α-chains [12-14].

BIOSYNTHESIS AND BIODISTRIBUTION OF IgA IN HUMANS AND ANIMALS

IgA are the major element in humoral immunity in mucosal secretions such as saliva, colostrums and milk, and respiratory and urogenital tracts [4, 15-17]. Although in human blood serum there is 5-7-fold less IgA than IgG, IgA are presumed to be the most abundantly synthesized class of antibodies in humans. Daily production of IgA reaches ~65 mg/kg, which exceeds the total production level of all other antibody classes [3].

IgA compose a significant share of the serum antibodies in humans [3]. Antibodies are found at mucosal surfaces of different mammals, most of these belonging to IgA, not IgG or IgM, which dominate in serum (table) [3].

More than 80% of human serum IgA is four-chain basic monomers (heterotetramer formula 2α,2κ/λ). In contrast, IgA in serum of other studied animals occur predominantly as a dimer of the basic complex [4]. Although the dimer of the basic complex is usually considered to bear a single J-chain molecule, the share of complexes with namely this subunit number has not been thoroughly assessed. One may hypothesize that a certain number of the dimers is linked with two J-chains, whereas other basic monomers are directly cross-linked via Cys residues in the tailpiece. Complexes of these structures can be easily obtained *in vitro* with a treatment of natural IgA with mild reducing and oxidizing agents, and these are stable under storage [3]. The IgA dimers look as a

Contents of IgA and IgG in human blood serum and secretions (from [3])

Liquor	IgG, mg/100 ml	IgA, mg/100 ml	Polymeric IgA, %	IgA1/IgA2
Serum	1230	328	13	89 : 11
Colostrum	10	1234	96	65 : 35
Saliva	4.9	30.4	96	63 : 37
Intestinal secretion	34	27.6	95	70 : 30
Colonic secretion	86	82.7	–	35 : 65
Bile	18.2	10.5	65	74 : 26
Nasal mucous secretion	5.8	25.7	–	95 : 5
Bronchial secretion	1.8	–	82	67 : 33

paired Y-shaped structure in electron microphotographs. This confirms linkage of two monomer subunits in "tail-to-tail" manner involving C-terminal regions of Cα3 [1].

The secreted human IgA (S-IgA) may belong to either subclass (IgA1 or IgA2). Almost all are dimers with sedimentation coefficient 11S and molecular mass 385 kD. The dimers are readily found in serous secretions of the mucosa in a complex with a so-called secretory component (SC) [1]. The SC contains 20 Cys residues. The equilibrium constant of the purified SC versus the dimer IgA is rather high (10^{-8} M^{-1}). The SC is linked with α-chain with one or more disulfide bonds (the most probable counterparts for the linkage with SC in α-chain are Cys299, Cys301, and Cys311 in the CHα2-domain). Immunochemical competition tests exhibited the ability of CHα3-domain for covalent linkage with SC independent of CHα2 [4, 18].

The completely assembled S-IgA molecules are typically composed with two IgA monomers, a single SC (70 kD), and one J-chain (15 kD). The mechanism of the intramolecular interaction and order of the disulfide bond connection within S-IgA may be variable in conformity to the conditions of the complex formation. However, most authors acknowledge the theoretical S-IgA model delineated in Fig. 3 to be the most probable [3, 4, 16, 17].

The SC is extensively glycosylated as well as the J-chain. The latter contains 8% carbohydrates within a single N-linked chain, whereas SC bears 22% carbohydrates in 5-7 chains causing substantially greater molecular mass of S-IgA than predicted from the amino acid sequence [4, 18].

Interdomain disulfide bonds of IgA molecule are highly conserved. However, some Cys residues involved to the bonds within certain domains may have varying location in different animal species. Intrachain disulfide bonds in the myeloma protein IgA1 Bur are composed between Cys145 and Cys204, Cys196 and Cys220 in CH1-domain, Cys266 and Cys323 in CH2-domain, Cys369 and Cys432 in CH3-domain [4].

In contrast to J-chain, SC is an ectodomain of the polymeric-Ig receptor (pIgR), which is synthesized in the epithelial, not plasmatic cells that are responsible for IgA uptake from the blood circulation and their excretion [13, 19, 20]. The dimeric IgA bearing J-chain only exhibit affinity to pIgR, and this is prerequisite for their transportation to the secretions. The monomeric IgA abundant in the blood are not subjected to secretion [21-24]. IgMs bearing J-chain are also able to interact with pIgR. The polymeric IgA interact with the ectodomain pIgR in the course of the absorption from the blood and constantly remain in the complex with it. pIgR processing leading to ectodomain disjunction from the transmembrane domain occurs after the complex internalization to the cytoplasm of excretory gland epithelial cells. The IgA–ectodomain complex is addressed to duct lumen at the apical side of the epitheliocyte [22, 25].

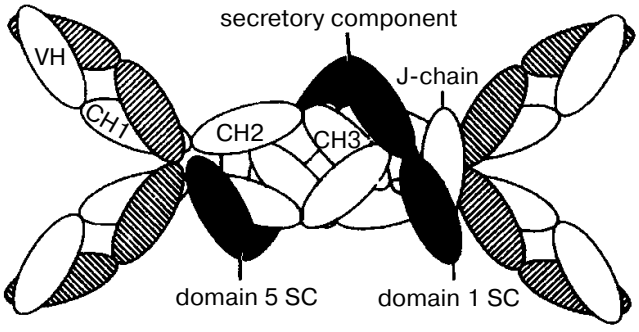

Fig. 3. Presumed model of S-IgA structure (from [3]).

Isolated Fc-fragments of IgA and IgM heavy chains conserving polymeric structure due to junction with the J-chain do not loose ability to interact with pIgR. Specificity analysis of chimeric IgA/IgM molecules obtained by expression of plasmid constructs in eukaryotic cells with mutually exchanged C-terminal domains of the heavy chains provided mapping of IgA regions directly involved in pIgR binding. A chimeric IgA molecule with Cα3 replaced for Cμ4 conserved the ability to form a pentamer joined around a J-chain. On the other hand, it formed a complex with pIgR ectodomain (SC). In both IgA and IgM, the 18 a.a. tailpiece contains Cys residues (Cys575 in μ-chain or Cys471 in α-chain) essential for the disulfide bond stabilizing the polymer [13].

As mentioned above, J-chain is essential for IgA–pIgR complex formation. pIgR is a member of Ig structural superfamily. It contains five Ig-like domains, membrane-associated segment (23 a.a.), and a cytoplasmic domain (103 a.a.) [26]. This glycoprotein (110 kD) binds polymeric IgA at the basolateral surface of the epithelium cell. The receptor–IgA complex is internalized and reaches the apical surface by transcytosis. Due to a proteolytic cleavage of the receptor ectodomain at the apical surface, the secretory IgA (S-IgA = $(IgA)_2$ + J + SC) is released to the lumen duct of an excretory gland. Random degradation of the free receptor results in formation of five extracellular Ig-like domains (D1-D5), so-called free SC. The residual membrane-associated 20 kD fragment is degraded intracellularly and does not appear in the circulation [22].

IgA FUNCTIONS AS A RECOGNIZING ELEMENT OF THE HUMORAL IMMUNITY

Antigen binding in serous secretions is traditionally considered as the natural IgA function. This thesis is also acknowledged in the recent reviews 2005-2006 [4, 16, 17]. On the other hand, the anti-adhesive role of IgA in the secretions itself does not exclude involvement of the serum IgA in human immunity reactions intended for

containment of the activity of microorganisms after their infiltration through the mucosa basal membrane.

Different ability of monomeric and polymeric IgA for intermolecular interactions with other proteins may not be ignored from the point of view of their role in the organism protective reactions such as switching effector mechanism of the target elimination. High contents of the monomeric serum IgA in human is its species peculiarity not found in other mammals. Traditionally used laboratory animals contain much less IgA than humans, and these IgA occur mainly in polymeric form [3].

Immunohistochemical assay of lymphatic nodes and nasal mucosa revealed tending of IgA1-producing plasmatic cells to be allocated to the excreting glands. Therefore, delivery of the antibodies to those sites where the probability of their facing a specific antigen is maximal. This tendency was not applicable to IgA2 producing cells. Such a distribution may partially substantiate the elevated contents of IgA1 in the secretions in comparison with IgA2 [27]. An unbalanced distribution of antibodies of the two subclasses is logically to be concerned with different role of the IgA isotypes upon switching the effector reactions eliminating the recognized targets [28-31]. In turn, such a fact may contribute to an adaptive strategy of pathogens producing IgA1-converting enzymes (*Neisseria meningitidis*, *Streptococcus pyogenes*, *Bacteroides* sp.). Enzymes of all these pathogens cleave IgA1 class only including S-IgA [32-36]. *In vitro* data demonstrate that the hinge cleavage blocks the protective function of S-IgA at the stage of interaction with effectors although resulting monovalent Fab_{α}-fragment conserves the antigen-binding capacity [37, 38]. Considering putative models of the IgA-dependent effector mechanisms of target elimination, we must emphasize their rather speculative character. Analyzing opinions in the recent reviews [4, 16, 17], these models can be clustered into three groups.

The first group of models is characterized by "passive immunity", when the antigen binding itself impairs its functionality. This mechanism is particularly efficient if it is directed against signal proteins such as receptors, growth factors, or enzymes. Namely, protective significance of blocking the bacterial adhesins by IgA is doubtless since this prevents bacterial adhesion to the epithelium surface [39, 40]. Plausibility of efficient virus neutralization by blocking envelope structures by IgA is also evident. High neutralization activity of human and murine IgA class antibodies towards influenza B, measles, Epstein–Barr viruses, and rotaviruses has been demonstrated [41-45]. On the other hand, activity of murine monoclonal IgA antibodies in a passive neutralization test was lower than those of IgG1 and IgG2 with the same antigenic specificity [41]. Although this particular observation abrogates the hypothesis about domination of the passive neutralization function of IgA in application to the considered model, the great number of other above-mentioned examples does not allow doubt in the significance of IgA as an anti-adhesive and neutralizing agent at the mucosal surfaces.

The second group joins models of "IgA-depending complement activation". This model tends to reconstruct a parallel with IgG action. An obvious difficulty of the complement interaction with IgA immune complexes is caused by a spatial isolation of S-IgA and the complement in the organism. The complement is a physically unbound ensemble of factors; therefore, it cannot exit the blood circulation conserving functionality. In contrast, most of the IgA immune complexes are isolated from the blood plasma by walls of the vessels and by the basal membrane (*lamina propria*). Experimental testing the cytotoxic action of the complement against *N. meningitidis* opsonized by the specific IgA revealed a minor effect towards the pathogen. However, weakness of this signal allows a presumption that it was caused by traces of IgG or IgM in the IgA preparation used [3, 46]. Moreover, the hypothesis about putative IgA-dependent complement activation is not in good agreement with physiological data showing suppression of the pro-inflammatory effect of IgG immune complexes by IgA, whereas IgA immune complexes did not induce inflammation upon local application. A synergism was seen between the specific IgA and lysozyme in growth suppression of bacteria from the Enterobacteriaceae family [47].

The third group of models can be denoted as "IgA involvement into the cell reactions mediated by FcαR (CD89) and pIgR". Now all reactions mediated by serum or secretory IgA are traditionally concerned to occurrence of one of two known IgA-receptors at the effector cell surfaces (either FcαR (CD89) or pIgR) [39, 48]. As mentioned above, the main function of pIgR is the antigen-independent IgA translocation from the circulation to the secretions. Since dimeric IgA only with J-chain are excreted, this receptor is denoted as the polymeric immunoglobulin receptor [22]. It is noteworthy that regardless of the antigen independent manner of the IgA binding and translocating activity of pIgR, work [41] hypothesized and partially confirmed a putative pIgR involvement in excretion and intracellular ("active") neutralization of measles virus. The intracellular neutralization is specified as impairing the replication, encapsulation, and natural transport of the virus particles within the infected cell. These mechanisms may be involved into human protection from the influenza virus and even HIV infection [49, 50].

In contrast to pIgR, FcαRI (known as CD89 in humans) was originally discovered as an element of the IgA-dependent effector system of target elimination [51]. The mechanism of the antigen dependence of FcαRI was first reported in detail in [52], although an earlier work [8] had described the possibility of antigen-independent IgA binding by FcαRI and even inducing some cell reactions by reception of free IgA. The human FcαRI was predominantly studied on neutrophils, although it occurs also on

monocytes, eosinophils, and macrophages [4]. The receptor from neutrophils has been purified to homogeneity and characterized *in vitro* [53-55]. The 3D-structure of the free receptor and its complex with IgA has been resolved by X-ray crystallographic analysis [56].

FcαR (CD89) is homologous to receptors specific for IgG Fc-domains (FcγRI, FcγRII, FcγRIII) and a high-affinity IgE receptor (FcεR). All these belong to the Ig structural superfamily and have two or three (in FcγRI) extracellular Ig-like domains responsible for ligand binding. The ligand-binding α-chain of FcαR has two extracellular Ig-like domains. Multimerization of the α-chains is operated by a provisional γ-subunit linked by disulfide bonds with a transmembrane domain of each from them. This structure is a signal component of many Fc-receptors. The signal motifs in the intracellular domain of the γ-chain are presumed to be the key point in the mechanisms of the signal transduction from the receptor. These motifs (ITAM) occur in many other eukaryotic receptors from different types and take part in different reactions. They have a consensus sequence Tyr-Xaa-Xaa-Leu-Xaa_7-Tyr-Xaa-Xaa-Leu (Xaa being any residue). Tyr residues in this sequence are subjected to phosphorylation by protein kinase C, which is essential for to the cascade signal transduction to the nucleus, translation machinery, caspase system, and to other global systems of cellular activity regulation [39, 48].

By using mutant IgA heavy chains, FcαR-binding sites were mapped (Leu257-Gly259 in CHα2-domain and Pro440-Phe443 in CHα3-domain) [57-60]. These sites differed from the respective FcγR-binding sites in IgG regardless of an obvious homology between the respective ligands and receptors. When bound, FcγRI, FcγRII, and FcγRIII overlap a portion of the hinge and most of the CHγ1-domain in IgG. However, the CD89 binding site in heavy chain of IgA is homologous with FcεR binding site in IgE (in both cases it is located in the N-terminal part of the CH3-domain). Unusual location of Fc- versus Fab-arms in IgA1 (T-like molecule structure) not found in other Ig may result in a decreased accessibility of Fc-fragment N-terminus and cause certain differences in the receptor-binding region in the inner part of Fc.

Respiratory burst induction in neutrophils is usually considered as the only physiological effect of IgA-immune complex binding with FcαRI [55, 60-62]. This is in a good agreement with a rather complete inability of the neutrophils to phagocytosis and synthesis of lymphokines. On the other hand, IgA-reception by FcαRI presumably induces a chemotaxis, which is another significant mechanism of physiological activity regulation in neutrophils besides the respiratory burst. However, the environment of the IgA-immune complexes facing neutrophils remains to be studied.

It is noteworthy that a common problem of all recent studies on IgA interaction with neutrophils and their receptors is lack of data about conformational rearrangements caused by IgA binding with the antigen. Moreover, many reports operated with free IgA instead of the IgA complexes in effector mechanism studies. Meanwhile, neutrophils activation by IgA in the absence of a physiologically significant modulation by the antigen would induce an oxidative burst in scale of the entire circulation that would result in fatal consequences for viability.

An isolated Fc-fragment obtained by cleavage of the serum monomeric IgA1 with a protease from *N. meningitidis* retained an ability to cause respiratory burst in the neutrophils [8]. These data suggest that Fc-fragment completely preserved its biological function after cleavage from Fab — undamaged serum IgA1 and Fc-fragment possess the same activity on stimulation of cytotoxic response of the neutrophils. At the same time, cleavage modifies the effectiveness of S-IgA1 — in intact form they stimulate neutrophils like serum IgA1, whereas their $(Fc)_2$-SC-fragments obtained after cleavage by IgA1 protease from *N. meningitidis* loose this ability.

These data presume a hypothesis that loss of ability to stimulate the neutrophils in $(Fc)_2$-SC in contrast to the free Fc form is caused by conformational changes induced by removal of the Fab superdomain. Cleavage of S-IgA1 predominantly occurring at the mucosa surface results in loss of their effector functions as activators of specific cytotoxic cell receptors. This enables the pathogen to avoid an attack of the nonspecific cellular immunity and to be easily disseminated over the entire host organism.

Discussing the protection caused by passive immunity mechanisms, a structural peculiarity of the antigen-binding centers of IgA should be taken in consideration. Both IgA isotypes have the disulfide bonds between the heavy chains located near the N-terminal residue of CH2-domain. But in contrast to IgG, there are no linkages of this type in CH1. Therefore, the enhanced mobility of the main hinge region of IgA allows them easy changing of the angles between the antigen-binding centers in Fc-superdomain (so-called Y- or T-conformations) [63]. In contrast, in IgG there are two disulfide bonds between the heavy chains located in N- and C-terminal parts of the main hinge region. Mutual fixation of the heavy chains in the exit point of the hinge peptide from the globular part CHγ1-domain makes the antigen-binding "fork" more rigid. This model is in conformity with data of low-resolution X-ray scattering (the distance between the apexes of Fab-fragments in IgA is 23 nm, which is much more than in IgG – 13-16 nm). High mutual mobility of Fab-superdomains in monomeric IgA1 facilitates simultaneous binding of two antigen molecules by the same IgA, in comparison with other Ig (e.g. IgG). This peculiarity may be physiologically relevant to the anti-adhesive Ig function towards bacterial and viral pathogens [63].

Cleavage by the protease from *P. mirabilis* between the CHα2- and CHα3-domains resulted in loss of ability

of the serum monomeric IgA1 to interact with FcαR, which confirms the established localization of the IgA binding site with the receptor [8]. This result confirms the relevance of the CH3-domain for IgA1–FcαR complex formation besides the primary binding site in CH2. Overlapping of the linkage between CHα2- and CHα3-domains by a streptococcal protein M resulted in impaired IgA1–FcαR (CD89) binding and prevented respiratory burst in neutrophils. Hence, a former model where SC masked both Fc fragments preventing its proteolysis should be revised (complete ability of S-IgA1 to bind FcαR must be taken in consideration).

SPECIFIC FACTORS OF PATHOGENS AS A TOOL FOR NOVEL IgA FUNCTION INVESTIGATION

The relevance of IgA in development of the protective immune response is confirmed by occurrence of the specialized systems of IgA blockage in pathogens. These systems include specific IgA-proteases (such as specific IgA1-proteases unable to cleave substrates other than human IgA1) as well as the IgA-binding proteins.

Most proteases converting human IgA1 are highly specific bacterial enzymes acting toward certain peptide bonds in the main hinge region. IgA1-protease production is found in common human bacterial pathogens such as *N. gonorrhoeae*, *N. meningitidis*, *Haemophilus influenzae* (serine protease), *Streptococcus pneumoniae*, *S. sanguis* (metalloproteases), *Bacteroides* (thiol protease), and some others [32, 33]. Occurrence of the enzymes completely unrelated in structure and enzymatic mechanism but almost identical in ability to cleave the hinge region of the human IgA1 (not IgA2) in taxonomically remote groups of pathogens elucidates an important adaptive role of such a cleavage for the pathogen survival in the host organism. It is noteworthy that the anti-adhesive function of IgA1 remains almost unaffected by the IgA1-protease treatment since the antigen binding ability (including the adhesin blocking one) of the resulting Fab fragments negligibly differs from that of the antigen binding ability of the intact IgA1. Moreover, mutant meningococci depleted in the IgA1-protease are not able to induce infection in experimental animals even if administrated intraperitoneally, when their anti-adhesive capacity is irrelevant [32]. Hence, one can speculate that IgA1 cleavage by the proteases of the pathogens is pivotal in the inner medium of the organism in contact with cells and humoral mechanisms of immunity. In this case breaking the linkage between Fab- and Fc-superdomains of IgA1 by the IgA1-proteases may cause the blockage of the signal transduction about the antigen binding between the domains and further to the unknown mechanism of the target elimination.

High species specificity of the studied IgA1-proteases of meningococci and related bacteria raises a question of appearance of this tool in evolution. Evidently, human pathogen strains distinguish the human IgA1 from other mammalian IgA by the unique structure of the hinge region. However, principal differences in the action mechanisms of the human and animal secretory immunity are doubtful. Therefore, occurrence of proteases adapted to the respective IgA structure is expected in animal pathogen strains. *Haemophilus influenzae* and *Streptococcus dysgalactiae* strains causing mastitis in pigs and cattle are the most promising models from this point of view. However, these tasks have not been experimentally solved, and thus they are not surveyed in this review.

Potential effector functions of IgA may be blocked by the IgA1-proteases as well as by the IgA-binding proteins found in dangerous human pathogenic strains (staphylococci and streptococci group A (*S. pyogenes*) and B) [61, 64]. These proteins are structural homologs of broadly known IgG binding proteins A and G (from *S. aureus* and *S. sanguis*, respectively). The site of recognition of these proteins with IgA was determined in experiments with hybrid IgA/IgG molecules. The key role of Leu257, Pro440, and Phe443 in the CHα2- and CHα3-domains in the interaction with the streptococcal protein was demonstrated. Therefore, recognition by these proteins of the same site in IgA as by FcαR is established (Fig. 4).

The competition between the streptococcal proteins and FcαR for the binding IgA was demonstrated. The mechanism of the cytotoxic reaction can be represented with the following scheme. An antibody molecule of IgA class specific to a certain antigen at streptococcus surface is bound with the target. The streptococcal IgA-binding protein immobilized on the cell wall surface forms a complex with the Fc-superdomain of the antibody, conserving the contact with the bacterial cell. Thus, the IgA is excluded for the interaction with FcαR that prevents elimination of the pathogenic bacterium by the immune system cells (e.g. neutrophils) [64]. The major advantage of this mechanism in comparison with the protein M secretion to the extracellular space is little IgA-capturer requirement. This is possible due to a precise addressing of the protein M to specific antibodies against superficial self-antigens of the bacterium, whereas nonspecific Ig are ignored.

The described mechanism employing the immobilized protein M provides blocking of the protective function of the antibodies of the specialized IgA-dependent

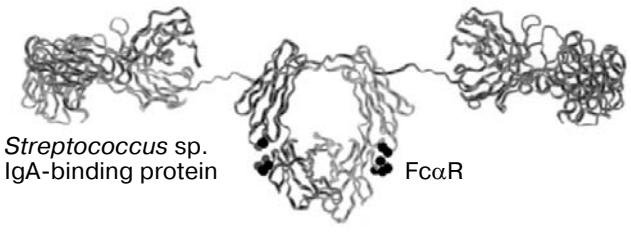

Fig. 4. Streptococcal Ig-binding protein and FcαR recognition sites in the human IgA1 molecule have similar localization (from [64]).

effector subsystem but seems to be useless from the point of view of "passive immunity". Thus, the mentioned features of the IgA binding mechanisms by the pathogens convincingly prove existence of the specialized IgA-depending effector systems (e.g. starting the respiratory burst in the neutrophils).

Considerably higher ability of the IgA2 immune complexes to induce the respiratory burst in the neutrophils in comparison with the immune complexes of IgG1, IgG2, and particularly IgG3 has been demonstrated in experiments with murine and human monoclonal antibodies against the hapten 5-iodo-4-hydroxy-3-nitrophenylacetyl (NIP) [65]. This parameter for the IgA2 immune complexes was at the same level with the activity of IgE immune complexes. The established model of obtaining the immune complexes of IgA with NIP conjugates was used to prove that the streptococcal protein Sir22 binds the Fc-superdomain within the immune complexes of IgA1 much better than within free IgA1 [66]. This observation allows considering Sir22 as a specific tool of the bacterium, providing blockage of the signal transduction about the antigen binding from the Fc-superdomain of IgA1 to the unknown receptor. Thus, Sir22 may serve as a useful tool for investigation of the conformational rearrangements in the Fc-superdomain of IgA caused by the antigen binding.

The authors are thankful to B. I. Shevelev, E. Yu. Varlamova, and E. E. Efremov for discussion and provided materials.

This work was partially supported by the Russian Foundation for Basic Research (grants 07-04-00245-a and 07-04-00124-a) and Russian Federation President grant MD-284.2007.4.

REFERENCES

1. Roitt, I., Brostoff, J., and Male, D. (2000) *Immunology* [Russian translation], Mir, Moscow.
2. Mestecky, J., and Russell, M. W. (1986) *Monogr. Allergy*, **19**, 277-301.
3. Kerr, M. A. (1990) *Biochem. J.*, **271**, 285-296.
4. Woof, J. M., and Mestecky, J. (2005) *Immunol. Rev.*, **206**, 64-82.
5. Biewenga, J. (1995) *Adv. Exp. Med. Biol.*, **371A**, 575-579.
6. Sumiyama, K., Saitou, N., and Ueda, Sh. (2002) *Mol. Biol. Evol.*, **19**, 1093-1099.
7. Chintalacharuvu, K. R., Raines, M., and Morrison, S. L. (1994) *J. Immunol.*, **152**, 5299-5304.
8. Almogren, A., Senior, B., Loomes, L., and Kerr, M. (2003) *Infect. Immun.*, **71**, 3349-3356.
9. Chintalacharuvu, K. R., Yu, L. J., Bhola, N., Kobayashi, K., Fernandez, C. Z., and Morrison, S. L. (2002) *J. Immunol.*, **169**, 5072-5077.
10. Bertrand, F. E., Billips, L. G., Gartland, G. L., Kubagawa, H., and Schroeder, H. W. (1996) *J. Immunol.*, **156**, 4240-4244.
11. Atkin, J. D., Pleass, R. J., Owens, R. J., and Woof, J. M. (1996) *J. Immunol.*, **157**, 156-159.
12. Krugmann, S., Pleass, R. J., Atkin, J. D., and Woof, J. M. (1997) *Biochem. Soc. Trans.*, **25**, 323S.
13. Braathen, R., Sorensen, V., Brandtzaeg, P., Sandlie, I., and Johansen, F. (2002) *J. Biol. Chem.*, **277**, 42755-42762.
14. Krugmann, S., Pleass, R. J., Atkin, J. D., and Woof, J. M. (1997) *J. Immunol.*, **159**, 244-249.
15. Mestecky, J., Russell, M. W., Jackson, S., and Brown, T. A. (1986) *Clin. Immunol. Immunopathol.*, **40**, 105-114.
16. Woof, J. M., and Kerr, M. A. (2006) *J. Pathol.*, **208**, 270-282.
17. Snoeck, V., Peters, I. R., and Cox, E. (2006) *Vet. Res.*, **37**, 455-467.
18. Perrier, C., Sprenger, N., and Corthesy, B. (2006) *J. Biol. Chem.*, **281**, 14280-14287.
19. Strong, R. K. (2004) *Structure*, **12**, 1919-1920.
20. Chang, Y., Lee, T. C., Li, J. C., Lai, T. L., Chua, H. H., Chen, C. L., Doong, S. L., Chou, C. K., Sheen, T. S., and Tsai, C. H. (2005) *Head Neck.*, **27**, 873-882.
21. Johansen, F. E., Braathen, R., and Brandtzaeg, P. (2001) *J. Immunol.*, **167**, 5185-5192.
22. Kaetzel, C. S. (2005) *Immunol. Rev.*, **206**, 83-99.
23. Braathen, R., Sandvik, A., Berntzen, G., Hammerschmidt, S., Fleckenstein, B., Sandlie, I., Brandtzaeg, P., Johansen, F. E., and Lauvrak, V. (2006) *J. Biol. Chem.*, **281**, 7075-7081.
24. Lewis, M. J., Pleass, R. J., Batten, M. R., Atkin, J. D., and Woof, J. M. (2005) *J. Immunol.*, **175**, 6694-6701.
25. Brandtzaeg, P., and Johansen, F. E. (2001) *Trends Immunol.*, **22**, 545-546.
26. Hamburger, A. E., West, A. P., Jr., and Bjorkman, P. J. (2004) *Structure*, **12**, 1925-1935.
27. Brandtzaeg, P., and Johansen, F. E. (2005) *Immunol. Rev.*, **206**, 32-63.
28. Mestecky, J., Lue, C., and Russell, M. W. (1991) *Gastroenterol. Clin. North. Am.*, **20**, 441-471.
29. Tarkowski, A., Moldoveanu, Z., Koopman, W. J., Radl, J., Haaijman, J. J., and Mestecky, J. (1991) *Clin. Exp. Immunol.*, **85**, 341-348.
30. Mestecky, J., Moldoveanu, Z., Prince, S. J., Kutteh, W. H., Kulhavy, R., McGhee, J. R., Moro, I., and Crago, S. S. (1991) *Adv. Exp. Med. Biol.*, **310**, 123-129.
31. McGhee, J. R., Fujihashi, K., Lue, C., Beagley, K. W., Mestecky, J., and Kiyono, H. (1991) *Adv. Exp. Med. Biol.*, **310**, 113-121.
32. Plaut, A. (1983) *Ann. Rev. Microbiol.*, **37**, 603-622.
33. Mistry, D., and Stockley, R. A. (2006) *Int. J. Biochem. Cell. Biol.*, **38**, 1244-1248.
34. Gilbert, J. V., Plaut, A. G., and Wright, A. (1991) *Infect. Immun.*, **59**, 7-10.
35. Mulks, M. H., Plaut, A. G., Feldman, H. A., and Frangione, B. (1980) *J. Exp. Med.*, **152**, 1442-1447.
36. Plaut, A. G. (1988) *Meth. Enzymol.*, **165**, 117-120.
37. Kirkeby, L., Rasmussen, T. T., Reinholdt, J., and Kilian, M. (2000) *Clin. Diagn. Lab. Immunol.*, **7**, 31-39.
38. Mansa, B., and Kilian, M. (1986) *Infect. Immun.*, **52**, 171-174.
39. Monteiro, R. C., and van de Winkel, J. G. (2003) *Ann. Rev. Immunol.*, **21**, 177-204.
40. Morton, H. C., and Brandtzaeg, P. (2001) *Arch. Immunol. Ther. Exp. (Warsz)*, **49**, 217-229.

41. Yan, H., Lamm, M. E., Bjorling, E., and Huang, Y. T. (2002) *J. Virol.*, **76**, 10972-10979.
42. Asahi-Ozaki, Y., Yoshikawa, T., Iwakura, Y., Suzuki, Y., Tamura, S., Kurata, T., and Sata, T. (2004) *J. Med. Virol.*, **74**, 328-335.
43. Pal, K., Kaetzel, C. S., Brundage, K., Cunningham, C. A., and Cuff, C. F. (2005) *J. Gen. Virol.*, **86**, 2347-2357.
44. Schneeman, T. A., Bruno, M. E., Schjerven, H., Johansen, F. E., Chady, L., and Kaetzel, C. S. (2005) *J. Immunol.*, **175**, 376-384.
45. Dotzauer, A., Brenner, M., Gebhardt, U., and Vallbracht, A. (2005) *J. Gen. Virol.*, **86**, 2747-2751.
46. Russell, M. W., Reinholdt, J., and Kilian, M. (1989) *Eur. J. Immunol.*, **19**, 2243-2249.
47. Jarvis, G. A., and Griffiss, J. M. (1989) *J. Immunol.*, **143**, 1703-1709.
48. Van Spriel, A. B., and van de Winkel, J. G. (2001) *J. Biol. Regul. Homeost. Agents*, **15**, 179-181.
49. Mazanec, M. B., Coudret, C. L., and Fletcher, D. R. (1995) *J. Virol.*, **69**, 1339-1343.
50. Bomsel, M., Heyman, M., Hocini, H., Lagaye, S., Belec, L., Dupont, C., and Desgranges, C. (1998) *Immunity*, **9**, 277-287.
51. Mazengera, R. L., and Kerr, M. A. (1990) *Biochem. J.*, **272**, 159-165.
52. Morton, H. C., Pleass, R. J., Woof, J. M., and Brandtzaeg, P. (2004) *J. Biol. Chem.*, **279**, 54018-54022.
53. Morton, H. C., Pleass, R. J., Storset, A. K., Brandtzaeg, P., and Woof, J. M. (2005) *Immunology*, **115**, 74-84.
54. Morton, H. C., Pleass, R. J., Storset, A. K., Dissen, E., Williams, J. L., Brandtzaeg, P., and Woof, J. M. (2004) *Immunology*, **111**, 204-211.
55. Pleass, R. J., Dunlop, J. I., and Woof, J. M. (1997) *Biochem. Soc. Trans.*, **25**, 327S.
56. Herr, A. B., Ballister, E. R., and Bjorkman, P. J. (2003) *Nature*, **338**, 921-941.
57. Pleass, R. J., Dehal, P. K., Lewis, M. J., and Woof, J. M. (2003) *Immunology*, **109**, 331-335.
58. Pleass, R. J., Dunlop, J. I., Anderson, C. M., and Woof, J. M. (1999) *J. Biol. Chem.*, **274**, 23508-23514.
59. Mattu, T. S., Pleass, R. J., Willis, A. C., Kilian, M., Wormald, M. R., Lellouch, A. C., Rudd, P. M., Woof, J. M., and Dwek, R. A. (1998) *J. Biol. Chem.*, **273**, 2260-2272.
60. Pleass, R. J., Anderson, C. M., Dunlop, J. I., and Woof, J. M. (1997) *Biochem. Soc. Trans.*, **25**, 328S.
61. Pleass, R. J., Areschoug, T., Lindahl, G., and Woof, J. M. (2001) *J. Biol. Chem.*, **276**, 8197-8204.
62. Podbielski, A., Schnitzler, N., Beyhs, P., and Boyle, M. D. (1996) *Mol. Microbiol.*, **19**, 429-441.
63. Boehm, M. K., Woof, J. M., Kerr, M. A., and Perkins, S. J. (1999) *J. Mol. Biol.*, **286**, 1421-1447.
64. Woof, J. M. (2002) *Biochem. Soc.*, **30**, 491-494.
65. Zhang, W., Voice, J., and Lachmann, P. J. (1995) *Clin. Exp. Immunol.*, **101**, 507-514.
66. Johnsson, E., Areschoug, T., Mestecky, J., and Lindahl, G. (1999) *J. Biol. Chem.*, **274**, 14521-14524.