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REVIEW

IgG-Binding Proteins of Bacteria

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Abstract—Proteins capable of non-immune binding of immunoglobulins G (IgG) of various mammalian species, i.e. without the involvement of the antigen-binding sites of the immunoglobulins, are widespread in bacteria. These proteins are located on the surface of bacterial cells and help them to evade the host's immune response due to protection against the action of complement and to decrease in phagocytosis. This review summarizes data on the structure of immunoglobulin-binding proteins (IBP) and their complexes with IgG. Common and distinctive structural features of IBPs of gram-positive bacteria (staphylococci, streptococci, peptostreptococci) are discussed. Conditions for IBP expression by bacteria and their functional heterogeneity are considered. Data on IBPs of gram-negative bacteria are presented.

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Key words: immunoglobulin G, immunoglobulin-binding proteins, Fc-fragment of IgG

Studies on molecular mechanisms of the pathogen–host interaction is one of the most important problems of biology, immunology, and medicine underlying searches for efficient agents for diagnosis, prevention, and treatment of infectious diseases. At different stages of the development of infection, a pathogen influences the host's defense systems using macromolecules with specific activities. Comparison of biological activities of these macromolecules with their effects in the host's body allows us to characterize these biopolymers as pathogenicity factors. Proteins capable of non-immune binding of immunoglobulins (Ig), i.e. without involvement of their antigen-binding sites, are important factors of pathogenicity of bacteria. Such non-immune binding of immunoglobulins with bacterial cells through immunoglobulin-binding proteins (IBP) is believed to protect bacteria against the action of complement [1, 2] and to decrease the opsonization and phagocytosis that, as a result, allows the microorganisms to evade the influence of the protective factors of the host's body [1-3].

IBPs of bacteria form a large group of proteins different in location in microorganisms, in molecular structure, and in binding properties. These proteins are found on the surface of bacterial cells, in the capsule, and in the culture medium [1, 2]. Molecular weights of IBPs vary from 20 to 350 kDa [3-15]. Among IBPs there are both monomers and oligomers. Moreover, IBPs are different in affinity for human and animal immunoglobulins of different classes and subclasses and can also be different in binding sites on the Ig molecule [4-7]. Many known IBPs are multifunctional. They can interact with different proteins of human and animal blood serum and also directly with immunocompetent cells of the macroorganism [16-20]. As a tool for influencing the host's immune system, a bacterial cell can concurrently possess several IBPs different in structure and functions [8-10]. The expression of IBPs in bacteria depends on the growth conditions and composition of the nutrient medium. Some strains of the same bacterial species display a variable expression of IBPs during laboratory passages or form cell populations different in contents and structure of IBPs [14, 15].

Information about IBPs of staphylococci and streptococci has been summarized in some reviews published in 1987-1998 [21-23]. Data on these proteins are also

Abbreviations: C_H, heavy chain constant region domain; Fab, antigen-binding fragment of Ig; Fc, crystallizable fragment of Ig; GAS, group A streptococci; GCS, group C streptococci; GGS, group G streptococci; IBP, immunoglobulin-binding protein; Ig, immunoglobulin; PpL, peptostreptococcal protein L; PsaA, pH-6 antigen; Sbi, second IBP of staphylococci; Sib, second IBP of group A streptococci; Skp, chaperon protein; SpA, staphylococcal protein A; SpG, protein G of group C and G streptococci; SSL, staphylococcal superantigen-like protein; V_H, heavy chain variable region domain; V_L, light chain variable region domain.

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presented in separate sections in reviews describing virulence mechanisms of pathogenic gram-positive cocci [24, 25]. Recently a review was published in Russian as well an English that discussed the structure and properties of proteins of gram-positive bacteria interacting with IgA [26].

We summarize here the available data on IgG-binding bacterial proteins. The main attention is paid to the structure of IBPs and their complexes with immunoglobulins, and less attention is paid to problems associated with their functional properties and biological activities. For the first time we present the literature data on IBPs of gram-negative bacteria.

IBPs OF GRAM-POSITIVE BACTERIA

Starting from the 1950s, abundant experimental material has been accumulated about the structure and functional properties of IBPs of gram-positive bacteria and also about mechanisms of their interaction with Ig. IBPs of staphylococci and streptococci of groups A, C, and G, and of peptococci are now rather well studied. Based on the ability of IBPs of gram-positive bacteria to bind human and animal IgG, these proteins are subdivided into several functional types (Table 1) [27]. Protein A (SpA) of staphylococcus, M-proteins and protein G (SpG) of streptococci (the best known IBPs) are assigned to types I-III, respectively. All IBPs of gram-positive bacteria except for protein L (PpL) bind with the Fc-fragment of the IgG molecule [22]. Despite similar specificity of binding, M-proteins, SpA, and SpG have different primary structures and are thought to be a result of convergent evolution of proteins of different species of pathogenic bacteria [28].

Table 1. Classification of IBPs based on their binding with human and animal IgG

Source of IgG	Functional types of IBPs (Fc-receptors)		
	I	II	III
Human	+	+	+
Rabbit	+	+	+
Guinea pig	+	–	+
Dog	+	–	–
Cat	+	–	–
Horse	–	–	+
Pig	+	+	+
Sheep	–	–	+
Cow	–	–	+
Chicken	–	–	–

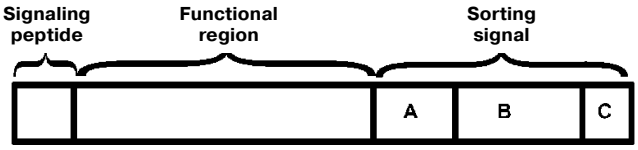

Fig. 1. Structure of IBP molecule. The sorting signal includes the LPXTG motif (a), the hydrophobic region (b), and a positively charged region (c).

The structure of all IBPs located on the surface of gram-negative bacteria is characterized by the presence in the molecule of three different regions: the *N*-terminal region containing the signaling peptide responsible for the translocation of the protein across the cytoplasmic membrane and then detached; the functional region consisting of several domains that determine the functional activity of the protein; and the *C*-terminal region denoted as a sorting signal responsible for anchoring of the protein in the cell wall (Fig. 1) [25].

The functional regions of IBPs are constructed on a single principle: they contain polypeptide repeats of one or several types. Highly homologous repeats of the same type can be organized as tandems. The number of repeats in IBP molecules can vary and determine the protein heterogeneity in molecular weight and functional activity. Structures of individual Ig-binding domains SpA, SpG, and PpL and their complexes with IgG molecule have been established by X-ray crystallographic analysis and NMR spectroscopy [29-32].

The *C*-terminal regions of IBPs are highly homologous and contain a conservative fragment (the LPXTG motif) followed by a hydrophobic domain and a region that preferentially includes positively charged amino acid residues (the charged tail) (Fig. 1) [33, 34]. On the example of *C*-terminal region of the SpA of staphylococci, it was shown that separate fragments of the sorting polypeptide affect the protein fastening on the cell surface (Fig. 1). The full-size SpA was found only fastened in the cell envelope. To separate it from the bacterial surface, the cells must be treated with enzymes hydrolyzing peptide bonds in peptidoglycan (e.g. with lysostaphin). The LPXTG motif is directly involved in formation of a covalent bond with peptidoglycan of the bacteria cells envelope. Removal of this motif with retention of other fragments of the sorting signal results in the protein staying in the bacterial membrane [35]. To isolate this protein, the bacterial cells are treated with ionic detergents (e.g. SDS). The loss of the charged tail or of the charged tail together with hydrophobic fragments or of the whole sorting polypeptide results in the secretion of the protein into the medium.

Some IBPs, e.g. SpA, SpG, and PpL and their derivatives, are now used in medicine and are commercial preparations [22, 36-38]. Due to their high affinity for a

wide spectrum of immunoglobulins, IBPs are used in test systems for immune diagnosis and also as components of sorbents in the treatment of patients who need autoantibodies, immune complexes, or immunoglobulins to be removed.

IBPs of staphylococci. In 1958 Jensen observed that some strains of *Staphylococcus aureus* can precipitate immunoglobulins of human and animal non-immune sera [39]. Later the IgG precipitation was shown to be caused by the binding of SpA on the surface of staphylococci with the Fc-fragment of the immunoglobulins [40]. Due to this work, SpA was identified as the first protein binding human IgG in a non-immune manner.

According to data of electron microscopy, SpA is located in the outer layer of the cell wall of *S. aureus* and is evenly distributed on the cell surface [41]. SpA can be isolated from the cell with enzymes splitting the peptidoglycan layer. In this case, SpA contains a small amount of hexosamine that suggests its binding with peptidoglycan in the cell wall of staphylococci. SpA can be also found in the culture medium when the bacteria are cultured on a liquid nutrient medium [42]. Thus, SpA can be expressed in cells both in fastened in the cell wall and in extracellular forms. The two forms of the protein have similar physicochemical characteristics and immunochemical properties [43, 44].

The location and expression of SpA in the cell depend on the medium of the staphylococcus growth and on the bacterial strain. The presence in the medium of mannitol or sodium chloride at the concentration of 7.5% inhibits biosynthesis of SpA [45, 46]. However, a high yield of SpA is observed when staphylococci are cultured in the presence of blood or blood serum [45, 46]. All coagulase-positive strains of staphylococci simultaneously synthesize two forms of the protein, whereas strains unable to produce coagulase do not contain SpA [42]. Strains of staphylococci resistant to neomycin produce more SpA than the strains resistant to meticillin [47-49].

Strains of *S. aureus* are different not only in the amount of expressed SpA but also in its structure and functional activity. Analysis of SpA from 12 serologically different strains by SDS-PAGE and by Western-blotting revealed seven protein variants with molecular weights of 45-57 kDa. These SpA variants were different in structure according to data of peptide mapping and also in functional activity [50].

SpA proteins are highly resistant to denaturing factors. They are thermostable, resistant to a wide range of pH (1-12), and are not destroyed by trypsin [43, 51]. Optical spectroscopy shows that 50% α-helical and 10-20% β-sheet structure is present in SpA [52]. The SpA molecule structure is typical for IBPs of gram-positive bacteria. The molecule includes a signaling sequence (S-region) [53], five highly homologous IgG-binding domains (E, D, A, B, and C) each consisting of 58-62 amino acid residues [54, 55], and a *C*-terminal part (XM) containing 150 amino acid residues. The primary and secondary structures of this *C*-terminal part, which fastens the SpA molecule in the cell wall, determine its significant difference from the five functionally active domains [56, 57] (Fig. 2a). SpA can lack one of the IgG-binding domains and/or some parts of the *C*-terminal

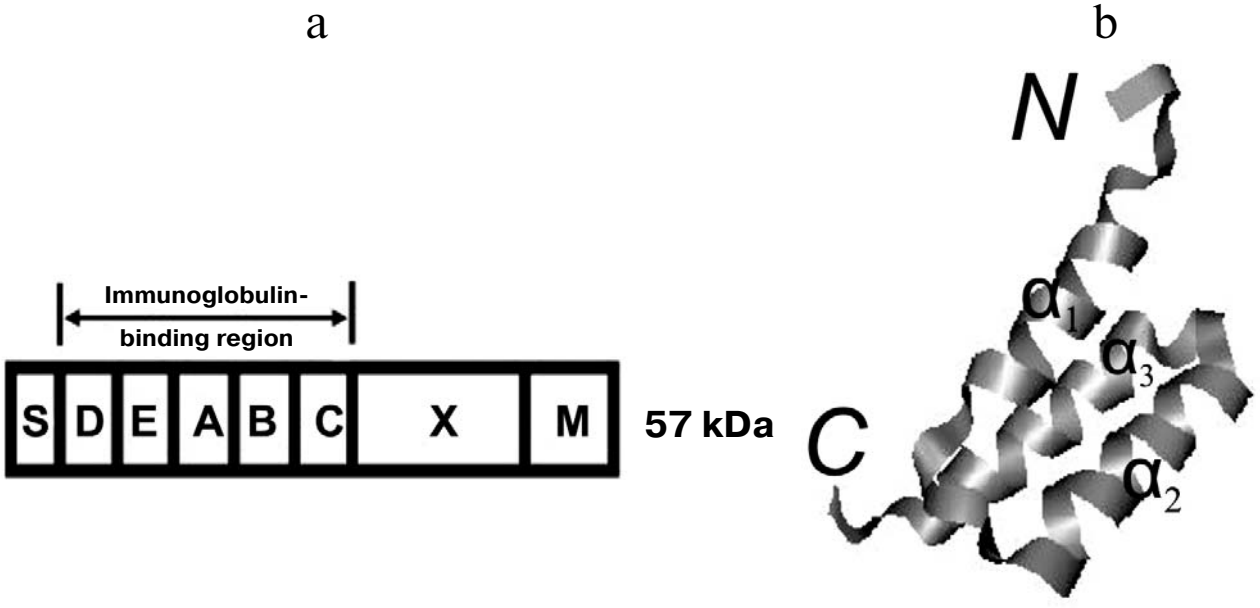

Fig. 2. Structure of the SpA molecule of staphylococcus. a) Scheme of SpA structure. S is signaling sequence; D, E, A, B, and C are IgG-binding domains; XM is the region fastening the SpA molecule in the cell wall. b) Spatial structure of the Ig-binding E-domain of SpA (PDB-code 1edi). The picture was prepared using the VMD 1.8.7 program.

region responsible for the protein fastening in the cell wall [58]. This explains the heterogeneity of SpA in molecular weight and properties.

Ig-binding domains of SpA are highly hydrophilic, resistant to proteolytic enzymes, and lack cysteine residues. Each of these five domains of SpA consists of three tightly packed antiparallel α-helices (Fig. 2b) [59]. The spatial structure of the domain is stabilized by hydrophobic interactions between the α-helices.

SpA interacts with human and animal immunoglobulins but the affinity for different Ig subclasses of the same species can be different (Table 2). The binding is strong between SpA and human subclasses IgG1, IgG2, and IgG4, whereas interaction with IgG3 is virtually absent [65]. In the case of mouse IgG, SpA strongly interacts with IgG2a, IgG2b, and IgG3, but binds weakly or does not react at all with IgG1 [69, 70]. SpA can also bind with other classes of Ig: IgM, IgA, and IgE. The binding sites with SpA are located on Fc- and Fab-fragments (outside the antigen-binding sites) of the Ig molecule [71].

The majority of *S. aureus* strains demonstrate IgG-binding activity, which was earlier believed to be only due to expression of SpA. However, recent genetic studies have revealed that *S. aureus* possesses another gene encoding the IgG-binding protein [72]. The full gene (*sbi*) encoding this protein (Sbi) has been cloned and sequenced [73]. Protein Sbi consists of 436 amino acids and demonstrates Ig-binding specificity similar to that of SpA. Moreover, Sbi is positively charged.

The functional region of the Sbi molecule consists of four domains (I-IV). Sbi can precipitate human IgG via interaction of two *N*-terminal domains of the protein (I and II) with the Fc-fragment of IgG [74]. These domains, Sbi I and Sbi II, are formed by three α-helices and are rather homologous in structure with the Ig-binding domains of SpA. Note that the highest homology was observed in the regions of these domains of the two proteins, which are involved in the binding with the Fc-fragment of IgG [75]. Seven of 11 amino acids of the B domain of SpA involved in the binding with IgG were identical to amino acids in the Ig-binding domains of Sbi. Similarly to many other IBPs, Sbi is multifunctional and can bind not only IgG but also other serum proteins. It binds the C3 component of complement through the Sbi III and Sbi IV domains and thus stimulates complement activation through the alternative pathway [76]. Sbi does not have the anchor sequence LPXTG, which is typical for proteins of the cell envelope of gram-positive bacteria. But *S. aureus* does not release this protein into the medium. The protein can be isolated by treatment of the cells with an SDS-containing buffer [73]. Therefore, it was suggested that Sbi should be located in the bacterial membrane similarly to mutant forms of SpA that lost the LPXTG motif in the sorting signaling peptide.

Two staphylococcal proteins, IsaB [77] and SSL10 [78], were recently shown to also have Ig-binding activity. The immunodominant protein IsaB of *S. aureus* was shown to interact with human IgM and IgG. SSL10 is a member of the family of staphylococcal superantigen-like (SSL) proteins, which are structurally similar to the toxin TSST1 and enterotoxins of staphylococci but lack the superantigenic activity inherent in these proteins. The homology of amino acid sequences among 14 proteins of this family is 36-67%. The function and role of the major-

Table 2. Characteristics of the best studied IBPs of gram-positive bacteria

IBP	M, kDa	Functional type	K_b, M^{-1}									Number of Ig-binding domains
			human					mouse	rat	goat	rabbit	
			Σ_{IgG}	IgG1	IgG2	IgG3	IgG4					
SpA	41-57 [50]	I	$4.4\cdot10^{10}$ [65]	$1.3\cdot10^{9}$	$2.1\cdot10^{9}$	n/b	$3.4\cdot10^{9}$	$2.6\cdot10^{10}$	$1.8\cdot10^{7}$	n/b	$5.2\cdot10^{10}$	4-5 [54, 58]
Protein H (M-protein family)	42.5 [60, 61]	IIa	$1.6\cdot10^{9}$ [66]	–	–	–	–	–	–	–	–	2 [68]
SpG	63 [62]	III	$6.7\cdot10^{10}$ [65]	$2.0\cdot10^{9}$	$3.1\cdot10^{9}$	$6.1\cdot10^{9}$	$4.7\cdot10^{9}$	$4.2\cdot10^{10}$	$1.4\cdot10^{9}$	$1.4\cdot10^{10}$	$7.0\cdot10^{10}$	2-3 [68]
PpL	76-106 [63, 64]	does not bind IgG Fc-fragment	$1.5\cdot10^{9}$ [67]	–	–	–	–	–	–	–	–	4-5 [68]

Note: K_b, binding constant with IgG; n/b, no binding.

ity of these proteins in pathogenesis of staphylococcal infection are unknown. SSL is characterized by the presence in the molecule of an *N*-terminal globular domain with β-barrel structure (the so-called "OB-fold" domain), which binds oligonucleotides or oligosaccharides; however, this domain is also shown to display protein-binding and enzymatic activities. In the molecule this domain is bound with the *C*-terminal domain, which has some common structural features with molecules of the staphylococcal superantigen.

SSL10 specifically binds IgG and inhibits the interaction between IgG and C1q-component of the complement that inhibits complement activation through the classical pathway. This protein contains a carbohydrate-binding domain, but the carbohydrate chains of the Ig molecule are not involved in its binding with IgG. The SSL10 binding site on the IgG molecule is still undetermined. Therefore, information is of interest about the high homology of SSL10 and SSL7, which also binds Ig independently of carbohydrates, but IgA and not IgG, and interacts with the IgA Fc-fragment in the region of contact of the $C\alpha_2/C\alpha_3$-domains through the "OB-fold" domain [79].

IBPs of group A streptococci. Human streptococcal infections are mainly associated with group A bacteria. The majority of known IBPs of group A streptococci (GAS) are M-proteins (from "mucoid" because colonies of M-positive strains have a mucoid consistency). These proteins were and still remain subject to intensive studies first of all because they are an important factor of the GAS virulence and a possible vaccine against streptococcal infections.

M-Proteins form a superfamily of proteins different in structure and functional properties. This superfamily includes three families of related proteins: Emm (classes I and II), Mrp (FcrA), and Enn. Genes encoding M-proteins form a regulon the transcription of which is controlled by the positive regulator *mga*. Mga-regulons of different GAS strains can include one, two, or all three families of M-genes, although the gene *emm* is always present. Based on the low expression of the *enn* genes in bacteria, it was suggested that they act mainly as reservoirs of sequences for recombination with the *mrp* and *emm* genes. No doubt, some unusual "mosaic" Enn-proteins including protein H, Enn 5.813, and Enn 64/14 (they all can bind IgG) are results of recombination of the *emm* and *enn* genes [25].

IBPs were found among M-proteins of all the three families. And virtually all studied Emm proteins (class II), Mrp, and Enn bind immunoglobulins. Recombinant Enn-proteins expressed in *Esherichia coli* can usually bind IgA [80].

In the current literature, proteins of the M-protein superfamily are traditionally subdivided into M-proteins and M-like proteins based on manifestations of their antiphagocytic activity. But new data on this activity in M-proteins contradict this classification, and it seems reasonable to revise it [81, 82]. Therefore, this classification is not used in the present review.

According to data of electron microscopy, M-proteins are located on the microbial cell surface where they form structures similar to pili and fimbria of gram-negative bacteria [83]. M-Proteins are fibrillar proteins, and their molecules are dimers consisting of two long coiled-coil α-helices. This spatial structure of M-proteins is due to penetrating through nearly the whole amino acid sequence of their molecule of a repeating motif of seven amino acids with nonpolar first and fourth ones. This promotes the exposition of hydrophobic amino acids on the outer side of the α-helix, which serves as a surface for dimerization and is typical for the coiled-coil α-helix conformation [83, 84]. Seven-membered repeats are also found in highly variable *N*-terminal domains, but their distribution is not always optimal.

Cloning and sequencing of genes encoding various M-proteins have shown that their primary structure consists of several types (A-D) of polypeptide repeats organized in tandems (Fig. 3). The *N*-terminal region denominated as the region of A-repeats is a hypervariable part of the molecule and determines serotypic differences among M-proteins. Among M-proteins of three different families, the *N*-terminal region of Emmproteins displays the greatest variability. The central region (region of B-repeats) is also variable in individual serotypes [84], whereas the region of C-repeats contains a conservative sequence that is common in all serotypes [85, 86]. Similarly to the majority of surface proteins of gram-positive bacteria, the *C*-terminal region of the M-protein contains an anchor region (sorting signal) responsible for the protein fastening in the cell envelope [33, 86]. The size and number of repeats can vary within the amino acid sequence of M-proteins. Thus, as discriminated from the Emm and Enn proteins, conservative C-repeats can be absent in the Mrp-proteins. Instead of such repeats, these proteins have in a central region of the molecule other type repeats with IgG-binding sites presumably located on them [8]. The difference among M-proteins, including those with the same serotype, depends on the number of repeating units in the regions of A- and B-repeats [84]. Based on specific anti-

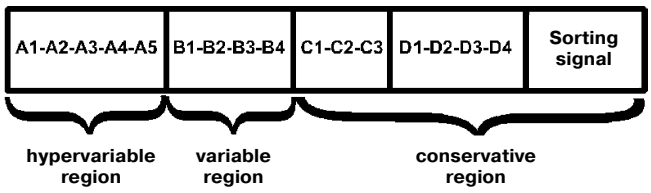

Fig. 3. Structure of M-proteins of group A streptococci. The protein molecule consists of four regions of repeated polypeptides (A-D) and sorting signal responsible for protein fastening in the bacterial cell wall.

Table 3. IgG-binding profiles of different IBPs of functional type II

IgG	IBP subtype				
	IIa	IIb	IIc	IIo	II'o
Human IgG1	+	–	+	+	+
Human IgG2	+	–	–	+	+
Human IgG3	–	+	–	+	+
Human IgG4	+	–	+	+	+
Rabbit IgG	+	–	+	+	+
Swine IgG	+	–	+	+	–
Horse IgG	+	–	+	+	–

genic features of M-proteins, >80 M-serovars of GAS have been identified, and with a few exceptions each of them contains a single M-protein variant.

The M-proteins capable of binding IgG have functional type II. But within this type the proteins are noticeably different in IgG-binding activity. Therefore, based on their ability to bind different subclasses of human and animal IgG, the type II proteins were subdivided into five subtypes (IIo, II′o, IIa, IIb, and IIc) (Table 3) [5]. Like the majority of IBPs, M-proteins are multifunctional. Each repeat type, except those located in the region responsible for the protein fastening to the cell envelope, is capable of binding different proteins of blood plasma: IgA [87-89], albumin [60, 90, 91], fibrinogen [8, 61, 90, 91], kininogens [92], the C4BP protein [93, 94].

Because some of M-proteins interact with human IgG1, IgG2, and IgG4 (subtype IIa), whereas other M-proteins bind only IgG3 (subtype IIb) or all four human IgG subclasses (subtype IIo), it was supposed that two or more IgG-binding sites should exist on molecules of M-proteins [95]. This hypothesis was confirmed by studies on H proteins (Enn family) and on M1 proteins (family Emm I), which are assigned, respectively, to subtypes IIa and IIb. These proteins were shown to differ in the primary structure of the binding sites and in their localization in the molecule (Fig. 4).

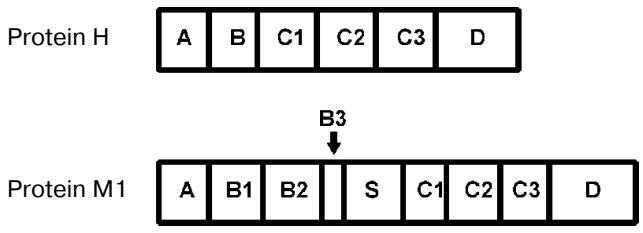

Fig. 4. Structure of M-proteins H and M1. The proteins are bound with the bacterial cell wall and the membrane by the *C*-terminal region D. Regions A, A and B, and C1-C3 of the protein H molecule are binding sites of fibronectin, IgG, and albumin, respectively. Regions A-B3, S, and C1-C3 of the protein M1 molecule bind fibrinogen, IgG, and albumin, respectively.

Protein H has two IgG-binding sites in the A and B regions located immediately on the *N*-terminus of the molecule, whereas the only IgG-binding site of the M1 protein in the S region is located approximately in the middle of the molecule. IBPs of the Mrp family were shown to have a region of the polypeptide chain homologous to fragments from the IgG-binding regions (A, B) of protein H, and therefore these regions could be identified as the binding site(s) of IgG1, IgG2, and IgG4, and Mrp-proteins were assigned to subtype IIa [60]. The protein Arp4 (Emm II family) similarly to protein M1 can bind only human IgG3 and has a region in the molecule that is homologous to the polypeptide fragment in the S-region of protein M1 [8]. This polypeptide can be considered as the binding site of human IgG3 (subtype IIb). Further studies on the structure and functions of IBPs seem promising for identification of IgG-binding sites of M-proteins of other IBPs of type II.

Strains of group A streptococci were recently shown to express IBPs other that the M-superfamily proteins. These IBPs called Sib can bind all subclasses of human IgG, its Fc- and Fab-fragments, and also IgA and IgM [16]. They are encoded by genes not belonging to the Mga-regulon. The Sib molecules do not contain regions of polypeptide repeats that are specific for the M-proteins and do not have a structural homology with M-proteins. However, similarly to M-proteins, the *N*-terminal sequences of Sib involved in the binding of immunoglobulins have α-helical structure. Sib are located on the cell surface and are partially released into the culture medium. They do not have an anchor possessing the LPXTG-motif, which is necessary for the protein fastening in the cell wall of bacteria [96].

IBPs of C and G group streptococci. In 1977 Myhre and Kronwall showed that streptococci of groups C and G (GCS and GGS) have type III IgG-binding activity (Table 1) [27]. The first IBP isolated from these bacteria was SpG [7, 97, 98], which could bind all four subclasses

of human and animal IgG (Table 2). SpG also interacts with albumin, kininogen, and the universal inhibitor of proteases α_2-macroglobulin [18, 99].

Although SpG and related proteins possess ligand-binding abilities similar to those of M-proteins of group A streptococci, their structure is different of that of M-proteins. Genes encoding SpG have been isolated and sequenced from various strains of the C and G group streptococci [62, 100-103], and this helped to establish the structure of these proteins (Fig. 5a). SpG molecules expressed on the bacterial surface contain repeating domains capable of binding immunoglobulins and serum albumin of various animals [104]. IgG-binding domains are located in the molecule nearer to the *C*-terminus, and a peptide fragment separates them from domains located in the *N*-terminal region and capable of binding albumin (GA-modules) (Fig. 5a). The total number of domains in the molecule can vary depending on the strain from which the protein was isolated. GA-modules are not present in all SpGs prepared from human clinical isolates [103, 105, 106]. Similarly to the majority of superficially expressed proteins of gram-positive bacteria, the *C*-terminal region of the SpG molecule has a fragment responsible for the fastening of the protein in the cell wall [62] (Fig. 5a). The IgG-binding SpG domains consist of approximately 55 amino acid residues packed as a β-sheet, which is built of two antiparallel and two parallel β-strands with the α-helix situated along its diagonal [107-110] (Fig. 5b).

In the mid-1990s SpG-like proteins were identified in some species of human and bovine streptococci of the C and G groups, such as *S. dysgalactiae* (proteins MIG and MAG) [19, 111, 112], *S. zooepidemicus* (now called *S. equi* subsp. *zooepidemicus*) (ZAG-protein) [113]. Similarly to SpG, the proteins MIG, MAG, and ZAG are IBPs of functional type III and can bind with the Fc- and Fab-fragments of the IgG molecule. Proteins SpG, MIG, MAG, and ZAG contain some highly homologous (70-100% homology of amino acid sequences) Ig-binding domains, the number of which varies from one domain in MAG to five domains in MIG. Similarly to SpG, these three proteins can bind α_2-macroglobulin but only within its complex with protease, whereas MAG and ZAG also bind albumin. The interaction with α_2-macroglobulin occurs through the variable *N*-terminal domains unique for MIG, MAG, and ZAG (homology of amino acid sequences in these domains is 10-30%). In SpG molecules the location of α_2-macroclobulin-binding domains is not established.

In addition to SpG and SpG-like proteins, clinical isolates GCS and GGS also contain such IBPs as FOG, Dem A, Dem B, and FgBP of the family of M-proteins found in GAS [114-116]. These proteins have Ig-binding properties similar to those of IBPs of the group A streptococci of functional subtype IIa and bind only the Fc-fragment of human IgG. These proteins have amino acid sequences specific for the surface proteins of gram-positive bacteria, and their spatial structure, predicted by

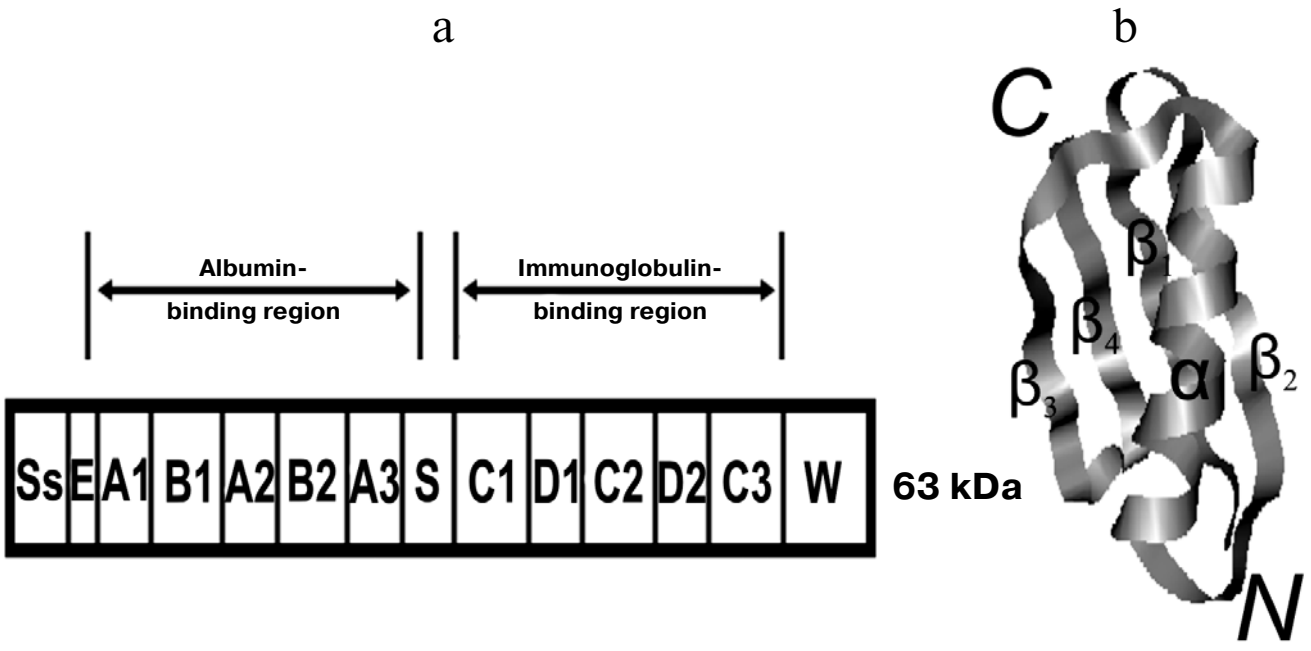

Fig. 5. Structure of SpG molecule of streptococci of C and G groups. a) Scheme of the SpG molecule. Ss, signaling peptide; A1-A3 and C1-C3, albumin- and IgG-binding domains, respectively; (B1, B2) and (D1, D2) are regions of the polypeptide chain separating, respectively, albumin- and IgG-binding domains; S, region of the polypeptide chain separating functionally different regions; W, fragment fastening the protein in the cell wall. b) The spatial structure of the IgG-binding domain of SpG (GB3) (PDB-code 1p7f). The picture was prepared using the VMD 1.8.7 program.

computer analysis, contains coiled-coil α-helices and is typical for M-proteins.

IBPs of *Peptostreptococcus magnus* (now called *Finegoldia magna*). The gram-positive anaerobic microorganism *P. magnus* is a common human symbiote that can also cause such diseases as vaginitis and urethritis. Approximately 10% of clinical isolates of *P. magnus* express on the surface a unique IBP that manifests an unusual ability to bind variable parts of light chains of the κ-type IgG, the protein PpL (Table 2) [4, 67, 117-119]. Although this binding occurs near to the antigen-binding site of the IgG molecule, it does not include hypervariable regions of antibodies involved in the immune recognition (binding) of the antigen. This unusual binding specificity allows PpL to bind antibodies of any subclass, and this determines the great interest in this molecule as a promising tool for isolation and study of IgG.

The ability of PpL to bind with the light chain of Ig seems to be physiologically significant. The addition of *P. magnus* strain 312 and also of the recombinant PpL to a culture of human basophils stimulated the release of histamines, whereas the addition of PpL-deficient strains failed to cause such effect [20]. This effect is supposed to be to the PpL binding with IgE, which is present on membranes of this type of cells and can occur in the host's body determining the role of PpL in pathogenesis of infection induced by *P. magnus*.

Similarly to the majority of surface proteins of gram-positive bacteria, PpL is bound with the cell wall through the *C*-terminal region. Similarly to SpA and SpG, PpL has a domain structure (Fig. 6a). The number of domains in the molecule can differ in proteins isolated from different strains [63, 64, 120]. Depending on the strain expressing the protein, the molecular weight of PpL is 76-106 kDa and it contains four or five highly homologous successively located Ig-binding domains. Moreover, the PpL molecule has repeating domains with unknown functions and can include albuminbinding domains similar to GA-modules of SpG [63, 64].

The threedimensional structure of domains binding the light chain of IgG has been determined by NMR spectroscopy and X-ray crystallographic analysis [121, 122]. Studies on the structure have revealed that the domain consists of a β-sheet formed by four β-strands and a central α-helix (Fig. 6b) [123]. The domains are stabilized by hydrophobic interactions and some hydrogen bonds, and their melting temperature is ~75°C [122, 124]. Despite the low homology of the amino acid sequence, IgG-binding domains of PpL have structural features common with the corresponding domains of SpG (Fig. 6b), which bind IgG in the region of the Fc-fragment of the heavy chain [121]. The main difference in their structures is determined by orientation of the α-helix respectively to the β-strands forming the β-sheet: in PpL it is nearly parallel to the β-strands, whereas in the SpG it is located along the β-sheet diagonal.

Structure of IBP complexes with IgG. IBPs can bind with different regions of the Ig molecule located in the heavy and light chains of Ig. Structural characteristics of the interaction between these proteins were determined

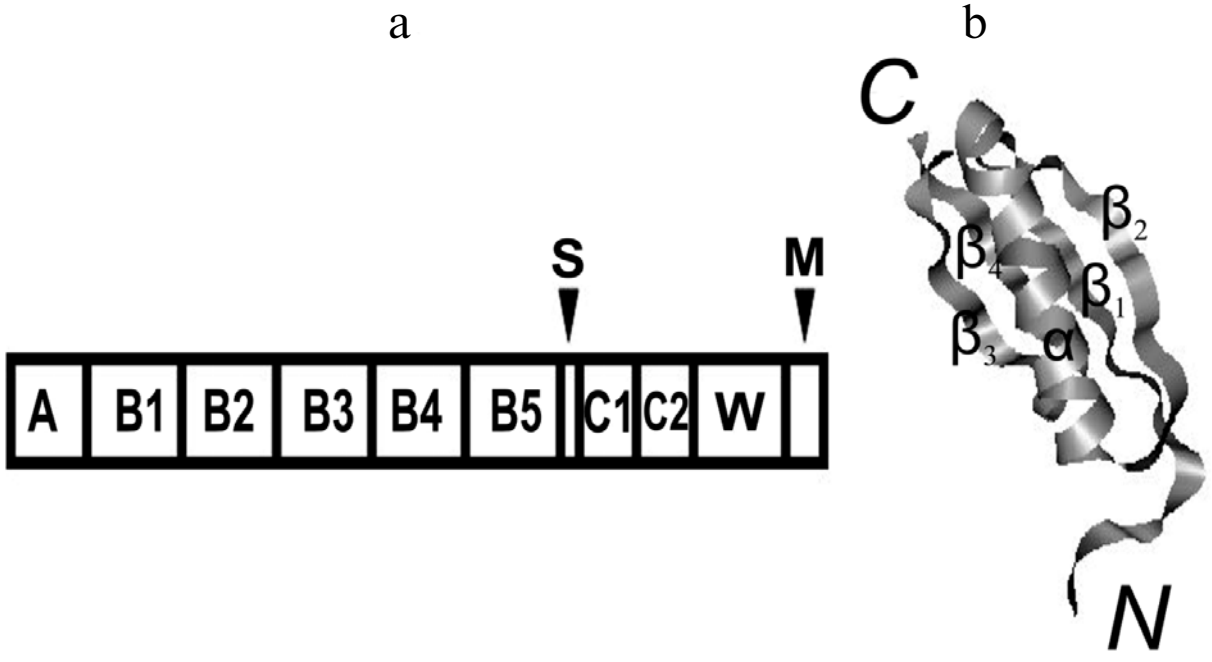

Fig. 6. Structure of the PpL molecule from *P. magnus*. a) Scheme of protein PpL. M, the region fastening the protein in the cell wall; the B1-B5 domains bind the light chain of Ig; functions of the A- and S-fragments and of the C1 and C2 domains are unknown. b) Spatial structure of the domain B1 binding the light chains of IgG molecule (PDB-code 1hz6). The picture was prepared using the VMD 1.8.7 program.

based on results of studies of complexes of isolated Ig-binding domains of IBPs with the Fc- and Fab-fragments of Ig.

SpG binds with the polypeptide chain region connecting the second and third domains (C_H2 and C_H3) of the heavy chain of human IgG Fc-fragment [110]. The Fc-fragment region between the C_H2 and C_H3 domains is also the SpA binding site [125]. The M-protein FgBP of streptococci also interacts with this region of the horse IgG Fc-fragment, and for complete binding both domains are required [126]. Some residues included into the binding sites of proteins SpA, SpG, and FgBP on the Fc-fragment are common for these sites, and this explains the competition of these proteins for binding with IgG. Note that SSL7 interacting with IgA binds Fc also in the region of contact of the C_H2 and C_H3 domains.

Thus, the junction region of the C_H2 and C_H3 domains of the heavy chain of the Ig Fc-fragments is a target for IBPs independently of the structure of the proteins, their bacterial source (staphylococci or streptococci), and also of the class (IgA or IgG) and species of Ig (human, horse, etc.). Convergent evolution seemed to be favorable for the arising of bacterial proteins capable of binding the surface of the contact between the C_H2 and C_H3 domains of IgA and IgG. Evolutionary reasons for retention of such rather vulnerable sites on the surface of Fc-fragments of Ig are thought to be complex and associated with their role as sites of interaction with the key receptors of the host's body [126].

On formation of the SpA complex with the Fc-fragment, the binding with IgG involves residues of the second and mainly of the first (*N*-terminal) α-helices of the Ig-binding domain of SpA, whereas the third helix untwists and takes an elongated conformation [75, 127]. The SpG residues involved in the binding of the Fc-fragment of IgG are located in the *C*-terminal part of the α-helix, the *N*-terminal part of the third β-strand, and the loop region connecting these two structural elements. The α-helices of SpA and SpG in the complex with the Fc-fragment were earlier supposed to occupy the same position and thus to determine the competition of these proteins for the binding sites on the Fc-fragment region of IgG molecule. However, superposition of the two complexes reveals that locations of the SpA and SpG α-helices in complexes with the Fc-fragment do not coincide, whereas the third β-strand in SpG is located approximately in the same region as the first Fc-binding α-helix of SpA, and this seems to induce the competition of these proteins for binding with IgG [110].

The nature of intermolecular interactions stabilizing IBP complexes with Fc-fragments can be different. Thus, in the case of SpG the binding is mainly due to charged and polar residues producing a complicated network of hydrogen and ionic bonds, whereas the complex of the Fc-fragment with SpA is mainly stabilized by hydrophobic interactions and some polar contacts. This is confirmed by biochemical data that the binding of SpA and SpG with IgG differently depends on pH [65]. Although regions recognized on the Fc-fragment by three different proteins (SpA, SpG, and FgBP) are noticeably overlapped, the binding sites of these proteins are markedly different, which determines their different affinities and specificities for IgG subclasses. Thus, SpG can bind more IgG subclasses and with higher affinity than SpA (Table 2). Significant differences of these proteins in Ig-binding activities were found by studies on their interaction with horse IgG, which are characterized by an unusually high number of subclasses [128].

SpA and SpG interact not only with Fc-fragments, but also with Fab-fragments of IgG. The binding of SpA with Fab-fragments involves the second and third α-helices of IBPs and four β-strands of the variable region of the heavy chain (V_H) of Ig. The two interacting surfaces are formed by polar amino acids. Comparison of crystalline structures of free Fab-fragment and its complex with the Ig-binding domain of SpA has shown that the SpA binding with V_H does not affect the Fab-fragment conformation and therefore must not influence the availability of the antigen-binding site [129].

Similarly to SpA, SpG interacts with a region of the Fab-fragment heavy chain, but its binding site is located not in the variable but in the constant domain (C_H1). The structure of the complex of the Ig-binding domain of SpG with the Fab-fragment of IgG has been established by X-ray crystallography [30, 32, 109]. On the formation of the complex the α-helix of SpG does not interact with the Fab-fragment. The binding involves two β-strands that form an antiparallel β-sheet with the last β-strand of the C_H1 domain of the Fab-fragment.

Similarly to SpA of *S. aureus*, PpL of *P. magnus* binds with variable regions of the Fab-fragment, but these regions are located in the κ-type light chains of IgG (V_L) [118]. Studies on the crystal structure of the complex of the Ig-binding domain of PpL with the Fab-fragment has revealed in PpL two independent binding sites that interact with two similar variable regions of the light chains but with different affinities [130, 131]. The first site is located on the second β-strand and on the α-helix and the second site includes amino acid residues from the third β-strand and α-helix, and these sites do not overlap, and they bind with the V_L-region with different affinities. In the crystal of the complex, PpL is incorporated between the V_L-regions of two Fab-fragments. The second β-strand of the PpL domain interacts with the β-strand A of the first Fab-fragment producing an intermolecular β-sheet. A similar type of the binding with production of an interprotein β-sheet occurs between the Fab-fragment and SpG molecules. The third β-strand forms contacts mainly with β-strands A and B (as in the first interaction) of the second Fab-fragment. The binding of both PpL and SpA with the variable region of Ig does not influence the availability of the antigen-binding site.

IBPs OF GRAM-NEGATIVE BACTERIA

Proteins capable of IgG binding in a non-immune manner are also found in various species of gram-negative bacteria of different genera, but information about them is scarce and fragmentary. Studies on IBPs were often performed on the level of whole cells, bacterial lysates, and/or mixtures of outer membrane proteins. Individual IBPs were isolated only from some species of bacteria, and then some of their physicochemical and biological properties could be studied.

In the majority of cases, IBPs of gram-negative bacteria are found in the cell envelope [6, 13, 132, 133], but similarly to IBPs of gram-positive bacteria they can be also found in the capsular material and in the cultural medium [2, 9]. Thus, in some *E. coli* strains the IgG-binding activity was due to surface proteins maximally expressed in the stationary phase of the bacteria growth at 37°C [13]. In the case of *Haemophilus somnus* (now called *Histophilus somni*) proteins interacting with bovine IgG were isolated from the culture medium [9, 133]. Note that bacteria can possess one or several IBPs, including ones markedly different in molecular weight.

Among pathogenic bacteria of the *Yersinia* genus, which cause in humans and animals some severe diseases, only *Y. pestis* and *Y. pseudotuberculosis* have been shown to have proteins capable of nonimmune binding with IgG. IBP of *Y. pestis*, which is known as pH-6 antigen, or PsaA, is a high molecular weight oligomer consisting of subunits with the molecular weight of 15-16 kDa, which forms pili on the surface of the plague microbe [134]. This protein is thermostable and is rather resistant to proteases and denaturing agents [12]. Similarly to the majority of IBPs of gram-positive bacteria, PsaA has an isoelectric point in the acidic region (p*I* 5.8) but is differentiated by a large fraction of β-structured regions [135].

Two IBPs with molecular weights of 14.3 and 16.1 kDa (IBP-14 and IBP-16, respectively) have been isolated from cell envelopes of *Y. pseudotuberculosis* [136, 137]. IBP-14 is a thermostable hydrophilic protein with a high index of polarity (55.3%). Circular dichroism spectroscopy assigns it to combined α/β-proteins. The other protein from *Y. pseudotuberculosis*, IBP-16, was identified as the chaperon protein OmpH/Skp that was earlier predicted based on analysis of the full genomic sequence of *Y. pseudotuberculosis* [137]. According to the theoretical model of the spatial structure obtained by comparative modeling, the Skp molecule of *Y. pseudotuberculosis* includes a compact mainly β-structured central domain formed by the *N*- and *C*-terminal regions of the molecule and a domain consisting of two elongated α-helical segments that form a hairpin with ends in the central domain. According to computer-aided modeling of the Skp complex with human IgG1, the predicted binding sites of Skp with the C_H2- and C_{H3}-domains of the Fc-fragment are located in the α-helical region. Amino acid residues involved in the formation of the complex are mainly hydrophilic. And the contact region in the SpA complex with the Fv-fragment of IgG is also hydrophilic [138]. Molecular docking of *Y. pseudotuberculosis* Skp with the heavy chains A and B of IgG1 showed that the C_H1 domains of the Fab-fragment heavy chains can be involved in the interaction together with the Fc-fragment [137].

IBPs of *E. coli* were recently shown to form a family of so-called Eib-proteins consisting of six members: EibA, -C, -D, -E, -F, and -G [139-141]. Four recombinant proteins of this family bind with IgG in the Fc-fragment region and do not react with the Fab-fragment of IgG [142, 143]. In the human paradontosis-inducing bacterium *Fusobacterium nucleatum*, the Ig-binding activity is associated with polypeptides with molecular weights of 40 and 42 kDa. The *N*-terminal sequence of these proteins is homologous to that of the bacterial porin Fom A [144]. The above-mentioned bacterium *H. somnus* also has some proteins capable of non-immune binding with Ig (41, 120, 270, and 350 kDa) [9, 144]. Four IBPs of *H. somnus* have related antigens [133, 145], and the authors supposed that high molecular weight IBPs of this bacterium could be oligomers of the 41-kDa polypeptide.

As in gram-positive bacteria, the expression of IBPs in gram-negative bacteria is regulated by environmental factors. Thus, the maximal expression of IBP of *Y. pestis* (PsaA) is observed at 37°C and pH < 6.5 [134, 146]. The ability of *Y. pseudotuberculosis* cells to bind Ig also depends on the environmental conditions (temperature, pH, medium composition) and on the bacterium growth phase [147]. The highest activity is manifested by bacteria in the stationary growth phase and cultured on liquid medium at pH 6.0 and 4°C. The maximal expression of the *E. coli* IBP was observed during the stationary growth phase at 37°C [13].

IBPs of both gram-negative and gram-positive bacteria are different in their abilities to interact with different subclasses of human and animal Ig. Thus, four IBPs of *H. somnus* display some differences in their binding activities. The IBP with the lowest molecular weight poorly interacts with subclasses of bovine IgG and also with IgA and IgM, whereas the three high molecular weight proteins bind bovine IgG2, IgA, and IgM with high affinities [9, 145, 148]. Moreover, the 270-kDa IBP not only interacts with bovine immunoglobulins, but it strongly binds IgG of horse, rabbit, pig, cat, dog, and sheep. However, it poorly reacts with mouse, rat, chicken, human, and guinea pig IgG. IBPs of other species of gram-negative bacteria also have characteristic Ig-binding profiles. IBP of *Prevotella intermedia* interacts with human, monkey, swine, and bovine IgG but is unable to bind with rabbit, mouse, rat, and sheep IgG [6]. A 30-kDa protein isolated from *Pseudomonas maltophilia* (present name *Stenotrophomonas maltophilia*) interacts with high affinity with Fc-fragments of four subclasses of human IgG, with rabbit IgG,

and also with IgG and IgA of mouse [149]. PsaA of *Y. pestis* binds only human IgG (subclasses IgG1, IgG2, and IgG3) and does not react with rabbit, mouse, and sheep IgG. Similarly to the majority of bacterial IBPs, IBPs of *Y. pseudotuberculosis* interact with human and rabbit IgG in the Fc-fragment region on the IgG molecule. The formation of the IBP-14 complex with IgG depends on the reaction medium pH and is maximal at pH 6.0 [136]. Some IBPs of gram-negative bacteria are multifunctional, similarly to IBPs of gram-positive bacteria [135].

Note that gram-negative bacteria have also been shown to have IBP capable of non-immune binding with Ig not through the Fc-fragment, but through Fab. On the *Helicobacter pylori* surface a 60-kDa IBP was detected and identified as a heat shock protein (Hsp60) by Amini et al. [132, 150]. This protein interacted with human IgG1, IgG3, and IgM but did not react with human IgA and with rabbit and mouse IgG. The binding with Ig occurred in the Fab-fragment region and was inhibited by light κ-chains of human IgG [150]. Similar binding with light κ-chains was earlier described only for PpL of some strains of *P. magnus* [4, 67, 117-119].

Thus, pathogenic bacteria species have a large group of proteins with different structure but possessing in common the ability to bind human and animal IgG. Notwithstanding the structural differences, these proteins are mainly responsible for recognizing and binding the same region on the Fc-fragment of an IgG molecule, and this suggests a high importance of this mechanism for survival and functioning of the pathogen in the host's body. Note that IBPs are multifunctional: in addition to immunoglobulins, they also bind other proteins of the host. Structural determinants responsible for these interactions are much less studied than immunoglobulin-binding domains. It is unclear how these functionally different regions of a multifunctional protein interact with one another on realization of its biological activity in the organism. IBPs which bind Fab-fragments of Ig are superantigens for B-cells. However, data on their influence on the host's immune response and their contribution to pathogenesis of autoimmune diseases, which are very interesting both fundamentally and for practice, are rather scarce [151-153]. Further studies are required to obtain additional information about molecular basics of IBP binding with target molecules and to understand the role of these proteins in host–parasite interactions.

IBPs are widely used as a tool in immunology, biochemistry, medicine, and bioengineering for isolation and purification of Ig and also in various laboratory test systems. Based on Ig-binding domains of the SpA, SpG, and PpL proteins, hybrid proteins A/G, G/L, and L/A have been constructed that are more efficient and universal IBPs and can be used as powerful agents for binding, determination, and purification of antibodies and their fragments [154-157]. Applied works associated with use of IBPs for medical purpose seem to be very promising for further development.

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