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Human antibodies eluted from ligand-free Sepharose capable of binding bacterial polysaccharides and sulfated glycans

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ABSTRACT

Sepharose matrix without immobilized ligands binds antibodies from human blood serum or immunoglobulin preparations. The eluted antibodies bind bacterial polysaccharides having no structural similarity to agarose (Sepharose is a cross-linked polysaccharide agarose) with a high affinity. It is concluded that the identified antibodies are capable of recognizing spatial rather than linear epitopes of bacterial polysaccharides. This side activity of Sepharose matrix should be taken into account in isolating target antibodies and other proteins from human blood.

1. Introduction

At present, most laboratory protocols for affinity isolation of blood proteins and, presumably, of industrial technologies use Sepharose matrix, mainly its cross-linked version, Sepharose FF. Sepharose™ is a trademark of a material based on the algal polysaccharide agarose with the sketch structure shown in Fig. 1.

Agarose consists of alternating β-D-galactose and 3,6-anhydro-α-L-galactose units linked by β1→4 and α1→3 glycosidic bonds. In fact, some L-galactose units in the polymer may not contain the anhydro bridge, and some D-galactose and L-galactose units can be methylated, pyruvated, or sulfated at one of the four positions (http://www.fao.org/docrep/x5822e/x5822e03.htm Armisen and Galatas). In the Fast Flow (Sepharose FF) agarose, chains are additionally cross-linked with 2,3-brompropanol (Fig. 1). Thus, the affinity matrix itself contains a considerable proportion of 'side' ligands.

When studying naturally occurring human anti-glycan antibodies, we used a preliminary step (Korchagina et al., 2005; Obukhova et al., 2011a,b) of isolation of the antibodies from blood serum or intravenous immunoglobulin preparation (IVIG), followed by PGA profiling of the material eluted from the adsorbent (Korchagina et al., 2005; Blixt et al., 2004; Huflejt et al., 2009). If obtained, these specific antibodies were subjected to additional chromatography on a glycan-ligand-free Sepharose adsorbent; up to 50% of initially isolated antibodies were retained; this 'trash' material was found to be immunoglobulin (Obukhova et al., 2011a). Because humans have a wide repertoire of anti-glycan antibodies, including Abs against βGal-terminated glycans sulfated by galactose (positions 3, 4, and 6) (Huflejt et al., 2009; Schneider et al., 2015), as well as polysaccharides (Bovin, 2013), we assumed that the immunoglobulin binds to Sepharose as to an antigen, i.e., through the variable part of the Fab fragment, rather than non-specifically, in a random fashion (Hamilton and Adkinson, 1985). In this study, we profiled 'anti-Sepharose' antibodies using PGA containing ~400 glycans typical af mammals and ~200 bacterial polysaccharides, and found preferential binding to a limited repertoire of bacterial polysaccharides, as well as glycans 4-O-sulfated at the terminal Gal (NAc)β residue.

Abbreviations: PGA, printed glycan array; Ig, immunoglobulin; CIP, complex immunoglobulin preparation; IVIG, intravenous immunoglobulin; Abs, antibodies; RFU, relative fluorescence unit; PS, polysaccharide; PAA, poly(N-2-hydroxyethylacrylamide); Su, sulfate; Me, methyl

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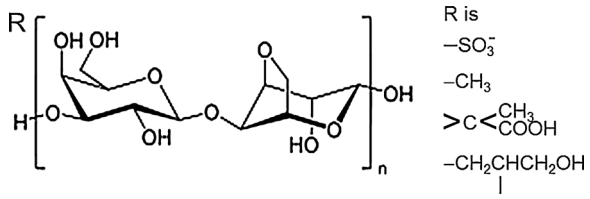

Fig. 1. Core structure of agarose, the polysaccharide used for fabrication of the Sepharose™ affinity chromatography medium.

2. Materials and methods

2.1. Reagents

The complex immunoglobulin preparation (CIP) was from Microgen (Russia). Alexa^{555}-labeled goat anti-human IgG and Alexa^{647}-labeled goat anti-human IgM were obtained from Invitrogen (USA). Phosphate-buffered saline (PBS), bovine serum albumin (BSA), and glycine were obtained from Sigma (USA). Tween-20 was from ICN (USA). Tris − OH was from Serva (USA). NaCl was from Helicon (Russia). Sodium azide was from Fluka (Switzerland). Glycan ligand free Sepharose (PAA-Sepharose 6 F F) was from Lectinity (Russia); it was synthesized by modification of aminated Sepharose 6 F F (GE Healthcare Bio-Science AB, Sweden) with poly[4-nitrophenyl acrylate] followed by quenching of nitrophenyl groups with ethanolamine. The microarrays were produced by Semiotik LLC (Russia).

2.2. Isolation of antibodies

2.2.1. Regular pH elution

CIP (35 mg/ml, 280 mg per 1 ml of adsorbent) was dissolved in PBS (pH 7.3) and applied onto a column containing PAA-Sepharose 6 F F equilibrated with the same buffer; the column was washed with PBS, 75 cm/h. The bound antibodies were eluted with 0.2 M TrisOH/0.5 M NaCl, pH 10.2, and then with 0.2 M glycine − HCl, pH 2.5, 15 cm/h, with immediate neutralization of the eluate with 2 M glycine − HCl (pH 2.5) and 1 M TrisOH (pH 10.2), respectively, to a final pH of 7.3–7.5. All the buffers used contained 0.02% NaN_3. Chromatography was monitored using a UV detector (Bio Rad, USA) at 280 nm. The antibodies were concentrated by centrifugation in plastic test tubes equipped with membrane filters with a molecular weight cut-off of 100 kDa (100 kDa MWCO, Millipore, USA) at 4 °C, 3000*g*, to a volume of 0.3–0.9 ml. The concentration of the antibodies was measured at 280 and 260 nm. The antibodies were stored at 4 °C.

2.2.2. Two-step elution

At the first step of elution, a gradient of L-rhamnose monosaccharide (0.01–1 M in PBS, pH 7.3) was used, and at the second step, regular elution was performed as described above.

2.3. Printed glycan array

Microarrays on glass microscope slides contained ~400 synthetic glycans (purity, 95%) and ~200 bacterial polysaccharides (for complete structures, NMR data, and related references see http://csdb.glycoscience.ru/bacterial). The isolated antibodies (10 μg/ml) were applied onto a microarray (pretreated with PBS containing 0.1% Tween-20 for 15 min) and incubated in a humidified chamber at 37°C for 1 h. The arrays were washed with PBS containing 0.05% Tween-20 and incubated with a mixture of labeled antibodies in PBS (8 μg/ml of goat anti-human IgG–Alexa^{555} and 8 μg/ml of goat anti-human IgM–Alexa^{647}) containing 0.1% Tween-20 for 45 min at 37°C. After washing with PBS containing 0.05% Tween-20 and deionized water, the arrays were scanned using an Innoscan 1100AL Fluorescence Scanner (Innopsys, France) at a resolution of 10 μm. The images were processed using the ScanArray Express 4.0 (fixed circle method) and the Microsoft Excel. The signals were measured as medians with interquartile deviations (Q1 and Q3).

3. Results and discussion

Most data were obtained with CIP as the source of human antibodies. CIP is prepared from > 1000 specimens of donor plasma, and, hence, serves as an averaged representative set of human Igs. Unlike in most other immunoglobulin preparations (von Gunten et al., 2009), the ratio between IgG, IgM, and IgA in these therapeutic Igs is close to the natural one. The version of PGA used here allowed us to measure the levels of both IgG and IgM separately in one experiment, with the use of a mixture of anti-IgG and anti-IgM Abs labeled with different fluorescent dyes.

The experiments on isolation of antibodies from serum of an individual human donor or a pool of serum samples from several healthy donors yielded similar results, with individual variation (Shilova et al., 2015). It is noteworthy that the optimized conditions of chromatography, including the elution step, do not affect the antibody specificity. This follows from our numerous previous experiments (Huflejt et al., 2009; Obukhova et al., 2011a, 2011b) on isolation of anti-glycan antibodies; specifically, antibodies after the secondary loading–elution cycle do not lose titer (the titer means the resultant of Ab concentration and affinity) and do not display an extended profile (an increased number of positive glycans), as probed with 600-ligand PGA. Immunologists used to consider human natural antibodies to be polyspecific (polyreactive) immonoglobulins with a tendency to acquire new antigenicities in response to changing conditions (Hadzhieva et al., 2016). In our case, if the polyreactivity did occur, it was rather an intrinsic property than one acquired in the process of Ab isolation.

The yield of anti-Sepharose Ab isolation was 0.03% w/w of the total CIP, which was comparable with the typical yield of the isolation of specific anti-glycan Abs (Korchagina et al., 2005; Obukhova et al., 2011a). The concentration of antibodies applied onto the array was selected to correspond to the serum concentrations of the most abundant anti-glycan Abs, anti-Le^C and anti-A_{di} (Bovin, 2013), in order to compare properly the 'anti-Sepharose' Igs with specific anti-glycan Abs of native serum. The operation range of the array is ~65 000 RFU; some human antibodies have titers approaching this value. In order to analyze the most pronounced effects, only the signals exceeding 2000 RFU for IgG and 3000 for IgM were normally taken into consideration.

The results for IgG and IgM antibodies, shown, respectively, in Figs. 2 and 3, demonstrate a repertoire of anti-glycan antibodies capable of binding glycan-ligand-free Sepharose.

The titers of top-rank antibodies, especially IgM, approach the highest RFU values known for specific anti-glycan antibodies. Expectedly, the repertoire of the IgM component is wider compared to IgG; note that 10 out of 15 IgG binders are IgM-positive as well.

Only IgM antibodies have been found to bind sulfated glycans, most of the glycans having the 4-O-Su-Galβ1-4GlcNAcβ key motif. It is known that human serum contains antibodies, both IgG and IgM, against a variety of sulfated glycans, including anti-4-O-Su-Galβ1-4GlcNAcβ (Huflejt et al., 2009), whereas, as mentioned above, agarose has contaminant O-sulfation, therefore, the obtained result is not entirely surprising. It should be noted that glycan-ligand-free Sepharose contains less than 0.1% w/w of PAA; hence, its influence should be negligible. It is noteworthy that all but one IgG binders appear to be polysaccharides (GlcNAcβ1-4Mur-L-Ala-D-i-Gln-Lys is a fragment of a peptidoglycan, a derivative of poly-[GlcNAcβ1-4], i.e., a polysaccharide); most top-rank IgM antibodies are directed against PSs as well. It is interesting that polysaccharides (Table 1) are very diverse in structure, and only 4 of 17 (23%; underlined in Table 1) have a βGal or related motif, a major agarose structural unit. However, 9 of 17 (53%) polysaccharides (shaded in Table 1) have one or more L-Rha residues, known as the D-Gal isostere, which could rationally explain the binding

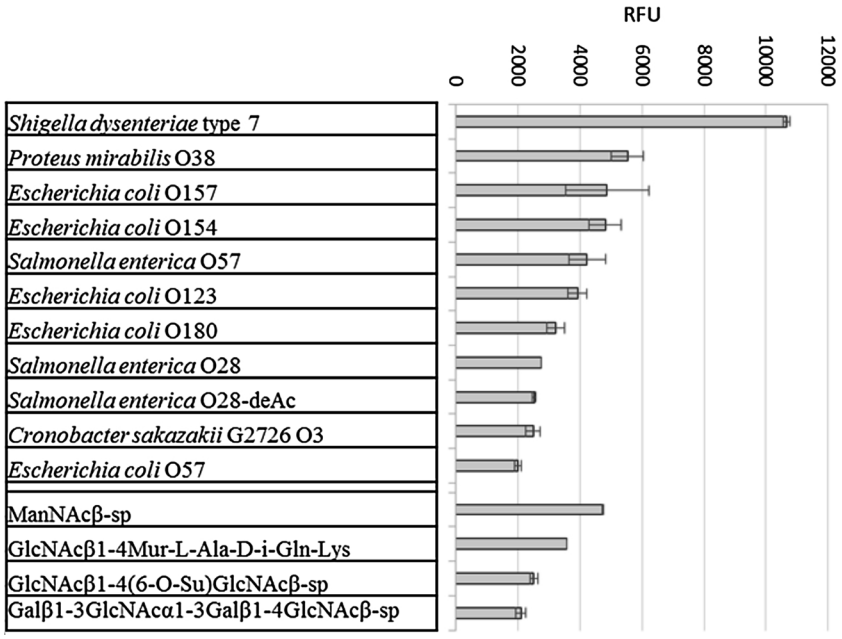

Fig. 2. The profile of IgG eluted from ligand-free Sepharose, PGA data. sp is an aminoethyl, aminopropyl, or glycylamide spacer.

of 'anti-Sepharose' antibodies to the polysaccharides. Rhamnose in its L-form has the same cluster of three hydroxyls as D-galactose, and the same position of this cluster relative to the O5 atom (Fig. 4).

However, six polysaccharides (Table 1) have neither βGal nor αRha fragments; therefore, we cannot explain binding of 'anti-Sepharose' Abs to these PSs as a direct molecular similarity of linear epitopes.

Next, we changed the experimental design at the stage of elution of the Sepharose-bound material. Specifically, we carried out the first elution with 0.01–1 M L-Rha monosaccharide in PBS and the second, regular pH-caused elution (as in previous experiments) (see the *Materials and Methods* section). The ratio of L-Rha to the alkaline-buffer-eluted material was ~1:10; i.e., L-Rha specifically eluted a sufficient amount of 'anti-Sepharose' Abs. Note that no tested material bound with the L-Rha monosaccharide immobilized on the array; therefore, the specificity of the Abs is more sophisticated than 'anti-Rha'. In the two-step isolation procedure, one of the top-rank PSs (Table 2, from *P. mirabilis* O13) contained no Rha motif. When IgG was analyzed, 4 of 11 (36%) Rha-eluted PSs and only 3 of 21 (14%) non-Rha-eluted PSs had

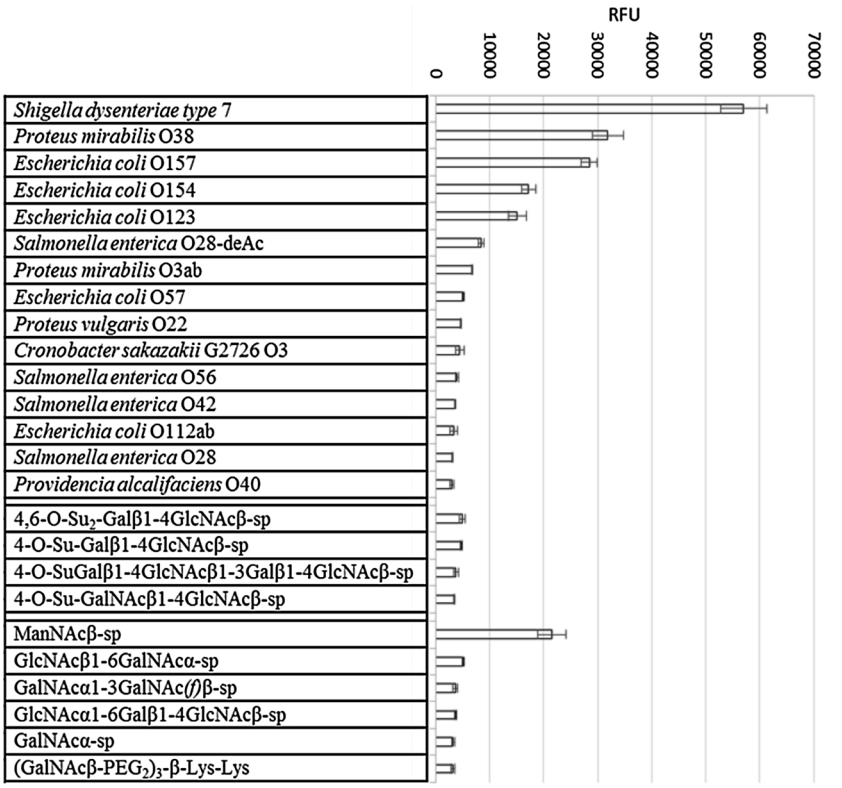

Fig. 3. The profile of IgM eluted from ligand-free Sepharose, PGA data. sp is an aminoethyl, aminopropyl, or glycylamido spacer; f is furanose.

Table 1
Structure of polysaccharides[a] (PSs) capable of binding antibodies eluted from ligand-free Sepharose. + + + The score corresponds to PS with the highest RFU values. αRha residues are highlighted; βGal, underlined.

Structure of O-polysaccharide or LPS oligosaccharide	Bacteria strain	IgG	IgM
-GalNAcA3Ac6NH$_2$α1-4GalNAcAα1-3GlcNAc-	*Shigella dysenteriae type 7*	+ +	+ + +
-3(EtNAc-1P6)GlcNAcα1-3Qui4N(Asp2Ac4-)1-6Glcα1-4GalAα1-	*Proteus mirabilis O38*	+	+ + +
−3GalNAcα1-2PerNAcα1-3Fucα1-4Glcβ1-	*Escherichia coli O157*	+	+ + +
−2-(ManNAcα1-3)Rhaα1-3Rhaα1-3Rhaα1-3GalNAcβ1-	*Escherichia coli O154*	+	+ + +
S-3HOBut1-2-D-Ala1-4 | -3-D-Qui4Nα1-6GlcNAcα1-3-L-QuiNAcα1-GlcNAcα6(30%)Ac1-	*Escherichia coli O123*	+	+ +
−4-D-Qui3NAcα1-3Rhaα1-4**Gal**β1-3(Glcβ1-4)GalNAcα1-	*Salmonella enterica O28de-Acetyl*	+	+
−2-D-Rha4NAcα1-3Fucα1-4Glcβ1-3GalNAcα1-	*Escherichia coli O57*	+	+
−4Qui3NAcα1-3Rhaα1-6GlcNAcα1-4GlcAβ1-3(Glcβ1-4)GalNAcα1-	*Cronobacter sakazakii G2726 O3*	+	+
−4-D-Qui3NAcα1-3Rhaα1-4**Gal**β1-3(Glcβ1-4)GalNAcα1-	*Salmonella enterica O28*	+	+
−3(GlcNAcβ1-2)Rhaα1-2Rhaα1-4Glcα1-3GalNAcβ1-	*Salmonella enterica O57*	+	
−4ManNAc3NAcAβ1-2Rhaα1-3Rhaβ1-4GlcNAcα1-	*Escherichia coli O180*	+	
GalAα6(L-Lys)1-4Glcα1-2 || -6GalNAcβ1-4GlcAβ1-3GalNAcβ1-	*Proteus mirabilis O3ab*		+
−3GlcNAcβ1-3(Qui3NAc2(65%)Ac4Acα1-2)Rhaβ1-4Rhaα1-4GlcAβ1-	*Proteus vulgaris O22*		+
-3-(L-Ser2Ac1-4)-D-Qui4N-β1-3Rib$_f$β1-4GalNAcα1-3GlcNAcα1-	*Salmonella enterica O56*		+
−3(ManNAcβ1-2)Rhaα1-2Rhaα1-2**Gal**α1-3GlcNAcβ1-	*Salmonella enterica O42*		+
−4(GlcNAcβ1-3)GalNAcα1-4Glcα1-4-L-IdoAα1-3GalNAcβ1-	*Escherichia coli O112ab*		+
−4-D-Qui3NFoβ1-3**Gal**α1-3GlcAβ1-3GalNAcβ1-	*Providencia alcalifaciens O40*		+

[a] PSs are represented in a simplified form for clarity of comparison with oligosaccharides: monosaccharides are pyranoses by default; fucose and rhamnose are L-sugars by default, all the others are D-sugars.

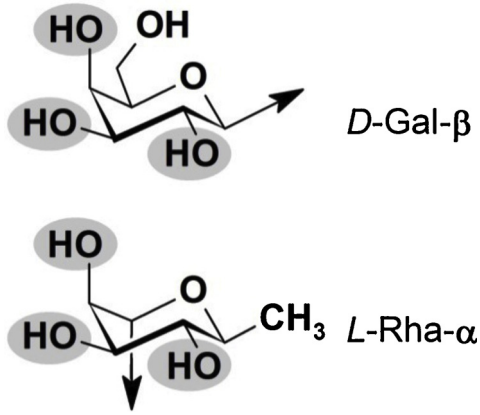

Fig. 4. Stereochemical similarity of the α-L-Rha and β-D-Gal residues. The mutual positions of 2 − OH, 3 − OH, and 4 − OH in D-Gal coincide with those of 4 − OH, 3 − OH, and 2 − OH in L-Rha, respectively. The arrows are glycosidic bonds.

rhamnose residues. In the case of IgM, the respective values were found to be 33% and 22%. Two anti-PS activities of IgG were completely eluted with Rha: that against the Rha-containing structure -[**2Rhaα1-2Rhaα1-3Rha**α1-3GlcNAcβ1]$_n$- (*Shigella flexneri type Y*) and that against the Rha-free -[3Gal$_f$2(30%)Acβ1-3Galα1]$_n$- (*Salmonella enterica O67*). The IgM antibodies completely eluted with rhamnose recognized -[2Rha4NAcα1-3Fucα1-4Glcβ1-3GalNAcα1]$_n$- (*Echerichia coli O157*), a rhamnose-free structure.

Taken together, the results of the elution of agarose-bound immunoglobulins suggest that some Abs recognize a linear epitope with a definite impact of the L-Rha moiety, and others are capable of recognizing spatial epitopes.

Interestingly, all PSs (Tables 1 and 2) without exception contain two or more Me groups as fragments of rhamnose, fucose, N-acetylhexosamine, or other monosaccharides or a pendant substituent (e.g., lactoyl or pyruvate) per repeating unit. Methyl residues are known as the most common components of antigenic determinants, especially of carbohydrate nature, due to enthalpy gain in stacking with aromatic amino acid residues of the antigen-binding site of immunoglobulins (Kogelberg et al., 2003; Vandenbussche et al., 2008). As agarose is randomly methylated (Armisen and Hispanagar, 1987), we could explain interaction of 'anti-Sepharose' Abs by selective isolation of methyl-sensitive species on the adsorbent containing a variety of methylated -[β-D-galactose-3,6-anhydro-L-galactose]- units. Assuming as little as 0.1% w/w of contaminant Su or Me groups in agarose, one can calculate that their content as an 'affinity ligand' will be ~1 μmol per milliliter of the affinity media; this is of the same order of magnitude as in the case of the typical affinity adsorbent with a specifically immobilized ligand; therefore, affinity isolation due to the small admixture of methylated (or sulfated) agarose is a logical explanation. However, the binding of serum Abs to the sulfated, methylated, and galactosylated epitopes of agarose cannot explain completely the observed high diversity of specificities, particularly towards polysaccharides. The structure of the PSs is variable and does not resemble the uniform linear epitopes of the agarose chain. To explain this fact, we should recall the hypothesis of the origin of natural anti-glycan Abs.

According to G.F. Springer (Springer et al., 1959; Springer, 1971), anti-glycan Abs, including anti-A/B allo-agglutinins and Abs against the glycoprotein O-chain glycans Galβ1-3GalNAcα (TF) and GalNAcα (Tn), appear due to the contact of the newborn immune system with the intestinal microbiota. The key role in this phenomenon is thought to be played by bacterial PSs, the structure of which mimics ABH (Springer et al., 1961; Stowell et al., 2010), TF/Tn (Henderson et al., 2011; Klaamas et al., 2002), or related mammalian glycans. According to a more recent concept (Khasbiullina and Bovin, 2015), natural anti-glycan Abs are generated due to priming of B-1 lymphocytes by bacterial molecular patterns (spatial epitopes) rather than classical linear glycotopes. Indirect evidence for this type of molecular mimicry is the interaction of galectins^{-4}, -8, and -9 with bacterial PSs having no structural similarity with LacNAc, Lac, or related motifs (Knirel et al., 2014), e.g., with rhamno-polysaccharides. Since the moment of birth, the gastrointestinal tract and respiratory system of mammals are actively colonized by bacteria. About 10^3 species of nonpathogenic (commensal) bacteria form the basis of the normal intestinal microbiota (D'Argenio and Salvatore, 2015), the total number of species is estimated at > 10^6. The microbiota is vitally important and has many functions, including the contribution to intestine physiology, immune system development, and others (Cash and Hooper, 2005; Kozakova et al., 2006; Sekirov et al., 2010). This variety of bacteria possesses

Table 2
Polysaccharides[a] capable of binding Abs obtained by elution from ligand-free Sepharose with the use of the two-step procedure (Rha elution followed by pH elution).

Rha-eluted (IgG and IgM)	
• -**2Rhaαl-2Rhaαl-3Rha**α1-3GlcNAcβ1-	*Sh. flexneri type Y*
• -3Gal$_f$2(30%)Acβ1-3Galα1-	*S. enterica O67*
• -2Rha4NAcα1-3Fucαl-4Glcβ1-3GalNAcα1-	*E. coli O157*
PSs recognized by antibodies (IgG) insensitive to elution with Rha	
– 3(Rib$_f$β1-4GlcAβ1-4)Galαl-6Manαl-2Manα1-3GalNAcβ1-	*Sh. boydii type 10*
– 3(GlcAβ1-4)Galα1-6Manαl-2Manαl-3GalNAcβ1-	*Sh. boydii type 6*
– 2Gal3,4(RPyr)β1-4Manβ1-4Galαl-3GlcNAcβ1-	*Sh. dysenteriae type 9*
– 3(R-Lac2-4Glcβ1-6Glcαl-4)Ga1β1-6Gal$_f$β1-3GalNAcβl-	*Sh. dysenteriae type 3*
– 3(S-Lac2-4)GlcNAcβl-2Rhaαl-2**Rhaα**l-3(Glcβl-2)**Rhaα**l-3GlcNAcβl-	*E. coli O150*
– 4Qui3NAcα1-3**Rhaα**1-4Ga1β1-3(Glcβ1-4)GalNAcα1-	*S. enterica O28*
– 3GlcNAcβ1-3(S,R-CetLys2-6GalAα1-4)Galα1-	*P. mirabilis O13*
– 6(R-Lac2-4)Glcβ1-4GalNAcα1-3GalNAcβ1-	*Sh. boydii type 17*
– 2(ManNAcα1-3)**Rhaα1-3Rhaα1-3Rhaα1**-3GalNAcα1-	*E. coli O154*
– 4GalN6Acα1-3DFuc2Acα1-3(EtN1-P-6)GlcNAcβ1-3Galαl-	*P. vulgaris O19ab*
– 3LQuiNAcα1-3GlcNAcα1-6(S-Lac-1-3)GlcNAcα1-	*P. mirabilis O31*
– 2-DRha4NAcα1-3Fucα1-4Glcβ1-3GalNAcα1-	*E. coli O57*
pH-eluted IgM insensitive to elution with Rha	
– 3(S-3HOBut1-2DAlαl-4)Qui4Nβ1-6GlcNAcα1-3LQuiNAcα1-3GlcNAc6(30%)Acα1-	*E. coli O123*
– 4Qui3NAcα1-3**Rhaα**1-4Galβ1-3(Glcβ1-4)GalNαl-	*S. enterica O28de-Ac*
– 3(Ser2Ac1-4)Qui4Nβ1-3Rib$_f$β1-4GalNAcα1-3GlcNAcα1-	*S. enterica O56*
– 4Qui3NAcα1-3**Rhaα**1-6GlcNAcα1-4GlcAβ1-3(Glcβ1-4)GalNAcα1-	*C. sakazakii G2726 O3*

[a] The Rha motif of PSs is boldfaced and underlined. For clarity of comparison with oligosaccharides, PSs are represented in a simplified form: monosaccharides are pyranoses by default; fucose and rhamnose are L-sugars by default, all the others are D-sugars.

millions of antigens that are able to prime B lymphocytes genetically selected for the synthesis of natural Abs (Butler et al., 2000; Khasbiullina and Bovin, 2015). It has been shown that up to 90% of the immunoglobulin-secreting cells of the conventional mouse intestine produce natural Abs that are absent in germ-free mice (Bos et al., 1980; van der Heijden et al., 1989). The key idea here is that *spatial epitopes* (fragments of molecular patterns) are the driving force for the generation of all or most of Ab specificities. There is no direct experimental proof for this hypothesis; however, the following evidence supports it. First, the natural Abs observed by us can bind to an antigen whose structure is entirely different from that of the affinity sorbent ligand. This can be explained by molecular mimicry in special cases, but this cannot be a general phenomenon. Second, as we know from the literature, polyreactivity is a common characteristic of natural Abs, and the affinities for both the "main" and the "secondary" antigens are low. All these facts are well explained by the model according to which the size of the direct antigen-binding site of natural Abs is wider than that of high-affinity antibodies; it is so extended that two (or more) linear epitopes can be placed in it without overlapping. If these epitopes are located on the cell surface or in the bacterial PS in close proximity, then they can be recognized by the antibody as a whole; this is what we mean by the spatial epitope from the point of view of immunology or the molecular pattern in terms of structural chemistry. Either way, the identification of ~20 specificities (in, presumably, thousands of existing species of anti-glycan Abs (Bovin, 2013; von Gunten et al., 2009)) of Abs capable of binding to spatial epitopes in polysaccharide agarose in our study is not unexpected.

4. Practical precautions

The capacity of glycan-ligand-free Sepharose for binding anti-glycan Abs was observed earlier (Hamilton and Adkinson, 1985; Osborn et al., 1994). Here, we have demonstrated that the repertoire of 'anti-Sepharose' human Abs is rather wide. The obtained results warn us to take into account the possibility of elution of completely unexpected proteins, namely Igs, during affinity isolation from serum with the use of Sepharose adsorbents. For example, we had to re-evaluate earlier data on the isolation and profiling of human natural Abs isolated on Sepharose with immobilized 4-O-SuGalβ1-4GlcNAc, Tn, Fs, and P_1, P^k oligosaccharides, because the profiles of ligand-specific and matrix-bound Abs partly overlap. In biotechnology, we expect the appearance of contaminant Abs in the process of affinity isolation of specific therapeutic Igs, carried out with the use of hapten-specific affinity chromatography. An evident way to avoid this is second affinity chromatography of the primarily eluted material using carbohydrate-ligand-free Sepharose.

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