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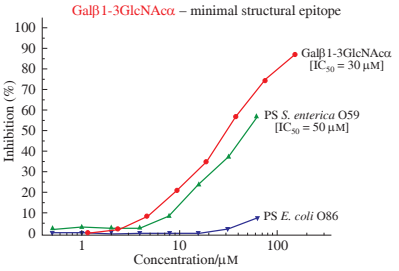
Human blood antibodies recognizing Galβ1-3GlcNAcα epitope in bacterial polysaccharide

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The disaccharide Galβ1-3GlcNAcα was synthesized and immobilized on Amino Sepharose 6 Fast Flow. Using this adsorbent, antibodies to the disaccharide were isolated from pooled donor blood serum and characterized. These antibodies bound to the polysaccharide from *Salmonella enterica* strain O59, in which the terminal fragment was Galβ1-3GlcNAcα.

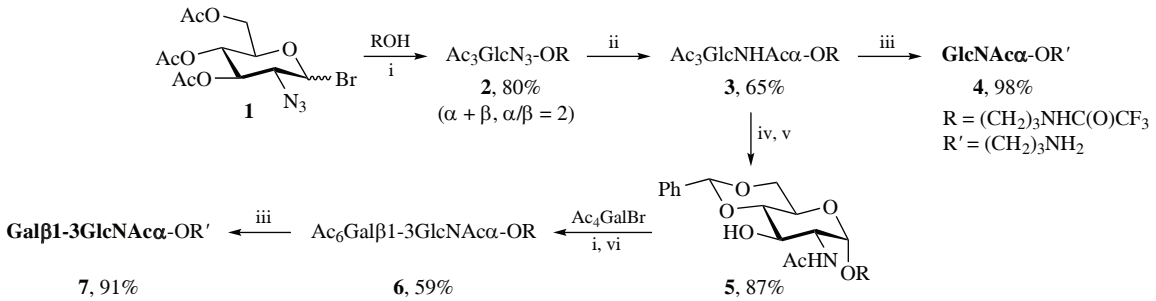


Keywords: glycan synthesis, natural antibodies, printed glycan array, epitope, bacterial polysaccharides, bispecificity.

The level of binding to the disaccharide motif Galβ1-3GlcNAcα turned out to be one of the highest among anti-glycan antibodies of human blood (as we determined using a printed glycan array), moreover, the antibodies were found in almost all healthy donors tested. Taken together, these facts indicate either a high frequency of human contact with the corresponding pathogen, most likely of bacterial origin[1] [more precisely with its polysaccharide (PS)], or that antibodies to Galβ1-3GlcNAcα belong to innate immunity,[2,3] that is, evolutionarily selected due to their importance. Oligosaccharides (OS) containing this disaccharide motif were previously obtained only as a byproduct of glycosylation with the glycosyl donor per-*O*-acetylated Galβ1-3GlcNAcBr.[4] The quantity of isolated oligosaccharides was sufficient for identifying antibodies to them using a glycoarray, but insufficient for isolating and studying the antibodies in detail. In this work, we synthesized the alpha-linked disaccharide as 3-aminopropyl glycoside, which was then immobilized on Sepharose (here and below 'Sepharose' is Amino Sepharose 6 Fast Flow). Using the resulting adsorbent, antibodies were isolated from human blood, and their specificity was characterized using bacterial polysaccharides and synthetic oligosaccharides.

As shown in Scheme 1, 3-aminopropyl glycoside **4** was synthesized in three steps from 3-(trifluoroacetamido)propan-1-ol and bromide **1** (prepared from peracetylated 2-azido-2-deoxy-D-glucose (Ac_4GlcN_3)[5] and $TiBr_4$[6]) according to the general procedure.[4] Acceptor **5** obtained from **3** in 87% yield was glycosylated with acetobromogalactose,[7] and converted into disaccharide **7**. Structures of the target products **4** and **7** were confirmed by mass spectrometry as well as ^{13}C and ^{1}H NMR spectroscopy. Specifically, the coupling constant value $J_{1,2}$ 3.4 Hz (for **7**) and 3.6 Hz (for **4**) for H^1 proton in GlcNAc residue corresponds to α-configuration, whereas $J_{1,2}$ 7.9 Hz for H^1 in Gal residue is characteristic of β-configuration.

Disaccharide **7** was immobilized on Sepharose similarly to the described protocol,[8] and using the resulting affinity adsorbent, antibodies (the total fraction, IgG : IgM ratio 3 : 1) were isolated from a pool of donor's blood serum. Their specificity was then profiled using a Printed Glycan Array (PGA) and additionally



Scheme 1 *Reagents and conditions*: i, AgOTf, TMU, CH_2Cl_2, MS-4 Å, room temperature, 20 h; ii, H_2, 10% Pd/C, then Ac_2O/Py; iii, 0.1 M MeONa/MeOH, 30 min, then NaOH (0.1 M aq.), 16 h; iv, MeONa (0.05 M in MeOH), 1 h; v, TsOH, $PhCH(OMe)_2$, MeCN; vi, AcOH (80% aq.), 70 °C, 2 h, then Ac_2O/Py.

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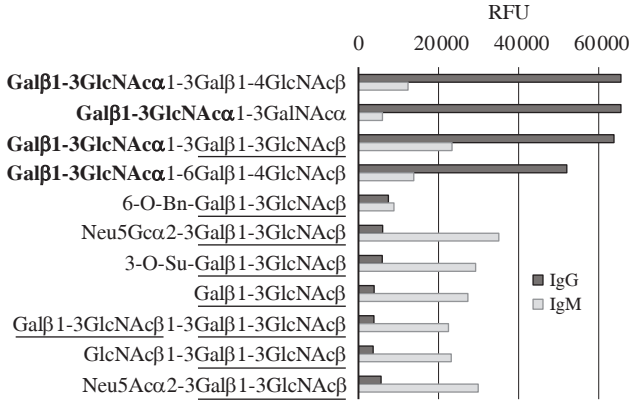


Figure 1 Data on PGA (containing oligo- and monosaccharides) analysis of affinity isolated antibodies, the total fraction (using a Galβ1-3GlcNAcα-Sepharose adsorbent), from the pooled blood serum of 113 donors; the measured RFU (relative fluorescence units) values are proportional to the binding intensity (the scale maximum is ~65 000). The Galβ1-3GlcNAcα fragment is highlighted in bold, the Galβ1-3GlcNAcβ fragment is underlined.

verified using an enzyme immunoassay (EIA). Class M antibodies (Figure 1) bind to both the disaccharides Galβ1-3GlcNAcα and Galβ1-3GlcNAcβ (the latter known[9] as Le^C), as well as to more complex oligosaccharides containing these disaccharide fragments. Of note, their binding to the monosaccharides Galβ and GlcNAcα was not observed. In contrast, IgG antibodies clearly prefer [see also the EIA data shown in Online Supplementary Materials, Figure S1(*a*)] the Galβ1-3GlcNAcα, *i.e.*, the epitope of the affinity adsorbent. Thus, as expected, class G antibodies are more specific.

Additional chromatography of the total Ig's fraction on Galβ1-3GlcNAcβ-Sepharose (antibody 'depletion') yielded a higher IgG content (IgG : IgM ratio 8 : 1) and eliminated binding to the Le^C disaccharide [the EIA data shown in Figure S1(*b*)]. This suggests that the ability of the initially isolated antibodies to interact with both disaccharides is explained not by their bispecificity, but by the presence of two subpopulations. It is believed that the driving force behind the development of innate antibodies to glycans is the priming of B1 lymphocytes by bacterial polysaccharides.[10] Therefore, antibodies to Galβ1-3GlcNAcα additionally depleted on Galβ1-3GlcNAcβ-Sepharose were analyzed using polysaccharide PGA (300 bacterial PSs). Figure 2 presents data for only a few of the PGA polysaccharides, namely, those containing the Galβ1-3GlcNAc fragment (α- or β-linked) in their repeating unit. Interaction was observed only with the polysaccharide from *S. enterica* O59,[11] the only one (CSDB, https://csdb.glycoscience.ru[12]) where the Galβ1-3GlcNAcα fragment is located in the terminal position. Direct and inhibitory EIA data confirm binding to PS from *S. enterica* O59 (Figure 3). No binding was observed with PSs that do not meet this criterion, for instance, *A. baumannii* capsular polysaccharides K21[13] and K82,[14] *S. enterica* O52,[15] *E. coli* O153,[16] *P. mirabilis* O33,[17] where disaccharide Galβ1-3GlcNAcα is located inside the PS chain.

The *S. enterica* O59 strain is not highly pathogenic, and the presence of antibodies against a fragment of its PS in all individuals rather suggests a shared epitope with more pathogenic bacteria. The similar affinity of these antibodies for the full-length PS and its disaccharide fragment opens up prospects for designing simple synthetic vaccines, provided that further research confirms the protective efficacy of antibodies targeting such a small epitope.

The interaction of antibodies with the terminal region of a polysaccharide chain is the rule rather than the exception, although we have previously described antibodies to the disaccharide Glcβ1-3GalNAc located within the PS chain.[19] It should be noted that antibodies to this glucose variant are present in virtually all individuals studied (*i.e.*, according to this criterion, antibodies are also classified as natural) and interact with bacterial polysaccharides.[19] Thus, the epitope of natural immunoglobulins in one case turned out to be internal, and in the other, the terminal disaccharide region. Whether the first case is regular or exceptional will be shown by further studies of natural anti-glycan antibodies.

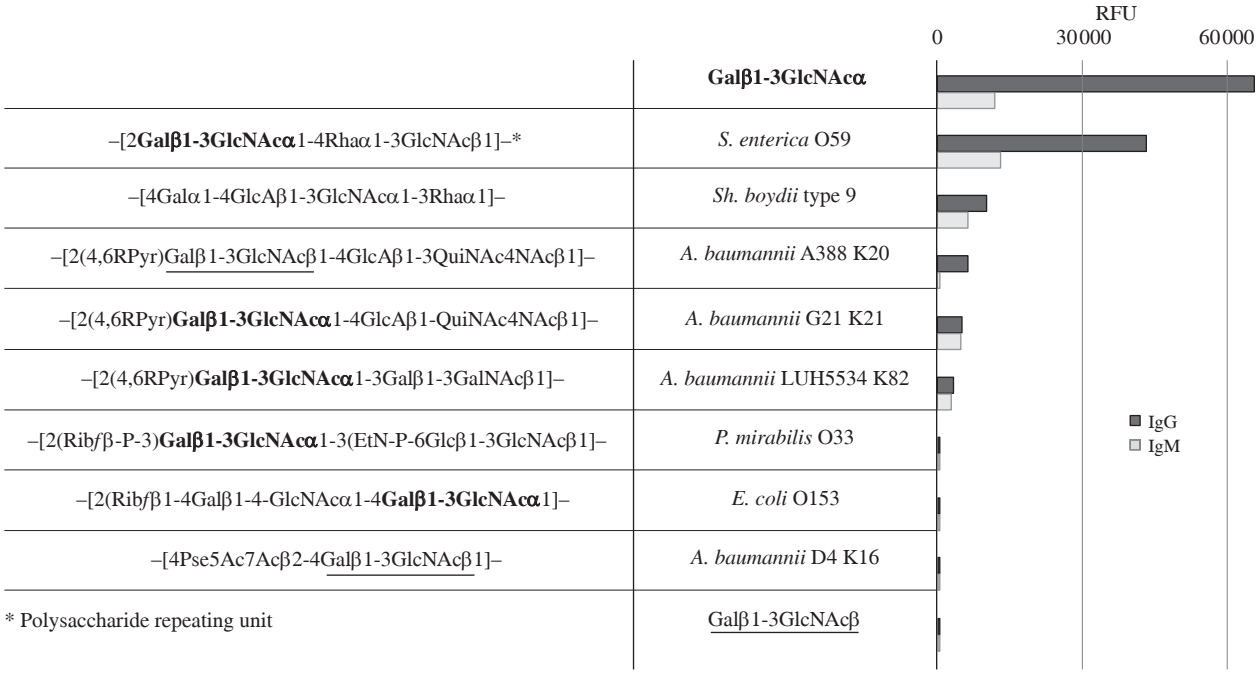


Figure 2 Binding of isolated anti-Galβ1-3GlcNAcα Abs additionally depleted on Galβ1-3GlcNAcβ-Sepharose to bacterial polysaccharides, PS-PGA data, the structure of polysaccharide repeating units is shown in the left part in glycan form, monosaccharides are pyranoses by default, rhamnose is L-sugar, all others are D-sugars. All abbreviations were used according to the database of polysaccharides https://csdb.glycoscience.ru/database. The Galβ1-3GlcNAcα fragment is highlighted in bold, the Galβ1-3GlcNAcβ fragment is underlined.

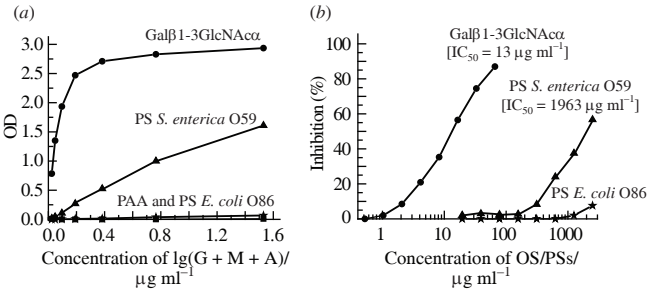


Figure 3 Binding of isolated anti-Galβ1-3GlcNAcα Abs additionally depleted on Galβ1-3GlcNAcβ-Sepharose to the disaccharide Galβ1-3GlcNAcα and bacterial PSs. (*a*) Direct EIA, Galβ1-3GlcNAcα-PAA (synthetic glycan-polyacrylamide conjugate), PAA and bacterial PSs were coated on the plate. PS *E. coli* O86[18] and PAA itself were taken as negative controls. (*b*) Competitive EIA, only Galβ1-3GlcNAcα-PAA was coated on the plate. The antibodies were analyzed at a concentration of 0.03 μg ml^{-1}, in the presence of inhibitors (OS or PS). PS *E. coli* O86 was taken as negative control.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.8127.

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