

Supplementary Material 2

1 Some Human Anti-Glycan Antibodies Lack the Ability to 2 Activate the Complement System

3 Nadezhda Shilova ^{1,2,*}, Alexey Nokel ^{1,2}, Alexander Lipatnikov ¹, Nailya Khasbiullina ², Yuri Knirel ³,
4 Ludmila Baidakova ⁴, Alexander Tuzikov ¹, Sergei Khaidukov ^{1,2}, Polina Obukhova ^{1,2}, Stephen Henry ⁵,
5 Batozhab Shoibonov ⁶, Emin Salimov ⁷, Robert Rieben ⁸ and Nicolai Bovin ¹

6 ¹ Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Science,
7 117991 Moscow, Russia; anokel@internet.ru (A.N.); alex.9508@yandex.ru (A.L.);
8 alextuzikov@yandex.ru (A.T.); khsergey54@mail.ru (S.K.); anruma@yandex.ru (P.O.);
9 professorbovin@yandex.ru (N.B.)

10 ² National Medical Research Center for Obstetrics, Gynecology and Perinatology of the Ministry of
11 Health of the Russian Federation, 117991 Moscow, Russia; crosbreed@list.ru

12 ³ Zelinsky Institute of Organic Chemistry Russian Academy of Science, 119991 Moscow, Russia;
13 knirel@gmail.com

14 ⁴ Branch of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy
15 of Sciences, 142290 Pushchino, Moscow Region, Russia; lionessa7591@gmail.com

16 ⁵ School of Engineering, AUT University, 92006 Auckland, New Zealand; stephen.henry@aut.ac.nz

17 ⁶ Federal Research Center for Original and Promising Biomedical and Pharmaceutical Technologies,
18 125315 Moscow, Russia; shoibonov@mail.ru

19 ⁷ Clinical Center of Sechenov First Moscow State Medical University of the Ministry of Health Care
20 of the Russian Federation, 119435 Moscow, Russia; dc13@mail.ru

21 ⁸ Department for BioMedical Research, University of Bern, 3008 Bern, Switzerland;
22 robert.riegen@unibe.ch

23 * Correspondence: pumatnv@gmail.com

24 **1 The effect of complement on the interaction of blood immunoglobulins with secondary** 25 **antibodies Introduction**

26

27 **1.1 Method**

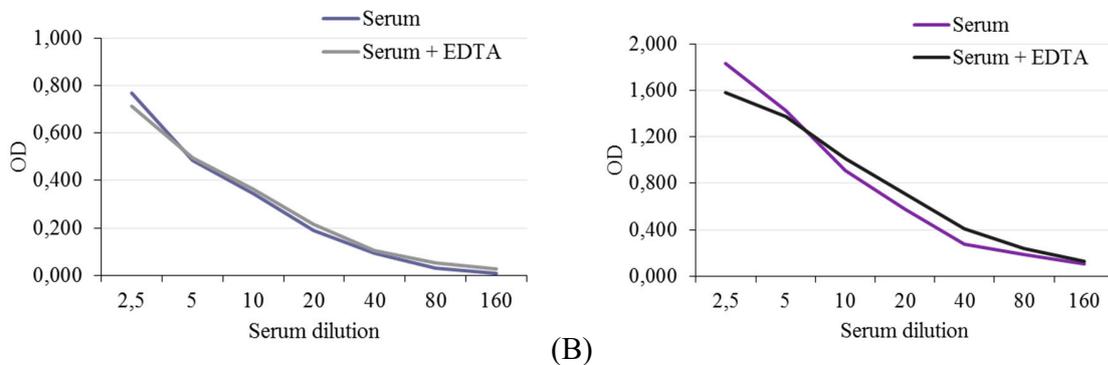
28 All inorganic salts were from (Sigma-Aldrich, USA).

29 MaxiSorp microtiter plates (NUNC, Denmark) were coated with glycan–PAA conjugates
30 (GlycoNZ, New Zealand) or glycan-free PAA (as a background control), 10 µg/ml (100 µl/well), in
31 the 50 mM sodium carbonate buffer (pH 9.6) for one hour at 37°C. The plates were blocked with
32 PBS/1% BSA, 150 µl/well, for one hour at 37°C. 200 µl of a serum with and without 5 mM EDTA
33 were added in the wells of the first line of the plate, all other wells were filled with 100 µl PBS/0.3%
34 BSA. The samples were two-fold diluted and incubated for one hour at 37°C. The plates were
35 washed with PBS-0.1% Tween-20 four times. HRP-labeled anti-human (IgM+IgG+IgA) secondary
36 antibodies (Southern Biotech, USA), diluted 1:4000 with PBS/0.3% BSA, was added to the plates,
37 100 µl per well, and the plates were incubated for one hour at 37°C. The plates were washed with
38 PBS-0.1% Tween-20 four times. Color was developed by a 15 min incubation at room temperature
39 with 0.1 M sodium phosphate / 0.1 M citrate buffer containing 0.04% of o-phenylene diamine
40 (Sigma-Aldrich, USA) and 0.03% of H₂O₂, 100 µl per well. The color reaction was stopped by the
41 addition of 30 µl 1 M H₂SO₄. The absorbance was read at 490 nm (0.1 s) with a plate reader

42 Wallac1420 Multilabel Counter, Victor2 (Perkin Elmer Life Sciences, Finland). All the tests were
43 performed in duplicate; the differences between readings (intra-assay) did not exceed 5%.

44 1.2 Result

45 AGA profiling is performed on blood serum samples that contain complement proteins, which can
46 directly interact with the array's glycoligands, or bind to AGA in the process of recognition of
47 glycans (what we really observe, see the results above and below). In the last case, complement
48 bound to the AGA distorts the pattern of standard AGA profiling via influence on interaction of the
49 secondary (labelled) antibodies. To find out whether such effects take place, we investigated the
50 interaction of blood serum in the presence and absence of EDTA, the reagent abolishing the binding
51 of complement proteins, using the example of the disaccharide Le^C (included in group №3) in the
52 ELISA format. It turned out that EDTA does not affect the binding of secondary antibodies to either
53 IgG or IgM of anti-Le^C specificity (Supplementary Figure 1).



54 (A) (B)
55 Supplementary Figure 1. Interaction of IgG (A) and IgM (B) of the donor's serum and blood plasma
56 with Le^C (as a polyacrylamide conjugate, PAA) using secondary antibodies. Data are provided
57 minus background (signal from wells with glycan-free PAA).

58 2 Activity of the lectin pathway of complement.

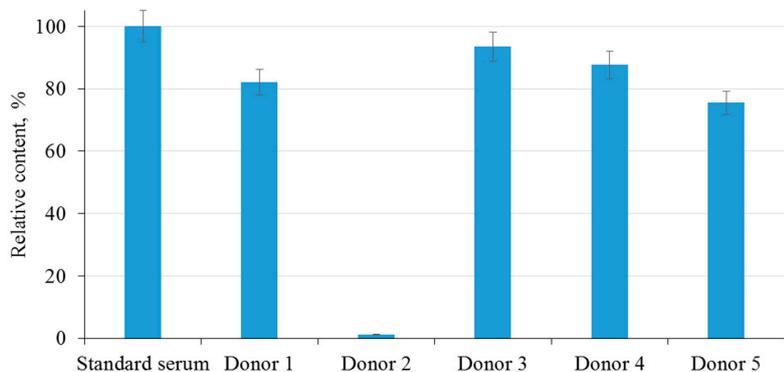
59 2.1 Method

60 Activation of the lectin pathway of complement was performed by using the commercial kit
61 Complement System MBL (Euro Diagnostica, Sweden) according to the manufacturer's instructions.
62 This assay based on measuring of activity of monoclonal antibodies specific for a neoepitope of the
63 terminal complement complex, C5b-9, produced as a result of MBL complement activation. Serum
64 with known MBL-activity was use as a standard (included to the kit).

65 2.2 Result

66 It is necessary to explain the presence of glycoligands, for which an interaction with the C3b/C3d
67 complement system proteins was observed, despite the absence of any AGA binding to the array. A
68 possible reason is the activation of the lectin pathway, primarily through the mannose-binding lectin
69 (MBL), which is able to independently interact with both array glycans and oligosaccharide residues
70 of bound anti-glycan IgA (see <https://doi.org/10.4049/jimmunol.167.5.2861>), which we did not detect
71 in these experiments.

72 To determine the possible involvement of MBL, an enzyme immunoassay was performed to estimate
73 the presence of this lectin in the donor's sera. It was found that only one donor had no MBL
74 (Supplementary Figure 2), while its content in serum of the rest donors was comparable to that in
75 standard serum, which is consistent with the assumption about the contribution of lectin pathway to
76 the observed results.



77

78 Supplementary Figure 2. The content of mannose-binding lectin (relative to standard serum with
79 known lectin pathway activity, %) in the studied donors (see the Table 1 in the main text for the
80 description). Averaged data for three experiments are given with standard deviations.