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A new surface plasmon resonance assay for *in vitro* screening of mannose-binding lectin inhibitors

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Abstract:	Mannose-binding lectin (MBL) is a circulating protein that acts as a soluble pattern recognition molecule of the innate immunity. It binds to carbohydrate patterns on the surface of pathogens or of "altered cells", with activation of the lectin pathway of the complement system. Recent evidence indicates that MBL contributes to the pathophysiology of ischemia-reperfusion injury and other conditions. Thus, MBL inhibitors offer promising therapeutic strategies, since they prevent preventing the interaction of MBL with its target sugar arrays. We developed and characterized a novel assay based on surface plasmon resonance for <i>in vitro</i> screening of these compounds, which may be useful before the more expensive and time-consuming <i>in vivo</i> studies. The assay measures the inhibitor's ability to interfere with the binding of murine MBL-A or MBL-C, or of human recombinant MBL, to mannose residues immobilized on the sensor chip surface. We have applied the assay to measure the IC ₅₀ of synthetic glycodendrimers, two of them with neuroprotective properties in animal models of MBL-mediated injuries.

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A new surface plasmon resonance assay for *in vitro* screening of mannose-binding lectin inhibitors

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Keywords: surface plasmon resonance; immunoassay; mannose binding lectin; inhibitor's
screening; glycodendrimers.

ABSTRACT

Mannose-binding lectin (MBL) is a circulating protein that acts as a soluble pattern recognition molecule of the innate immunity. It binds to carbohydrate patterns on the surface of pathogens or of “altered cells”, with activation of the lectin pathway of the complement system. Recent evidence indicates that MBL contributes to the pathophysiology of ischemia-reperfusion injury and other conditions. Thus, MBL inhibitors offer promising therapeutic strategies, since they prevent preventing the interaction of MBL with its target sugar arrays. We developed and characterized a novel assay based on surface plasmon resonance for *in vitro* screening of these compounds, which may be useful before the more expensive and time-consuming *in vivo* studies. The assay measures the inhibitor’s ability to interfere with the binding of murine MBL-A or MBL-C, or of human recombinant MBL, to mannose residues immobilized on the sensor chip surface. We have applied the assay to measure the IC₅₀ of synthetic glycodendrimers, two of them with neuroprotective properties in animal models of MBL-mediated injuries.

Introduction

Mannose binding lectin (MBL) is a calcium-dependent (C-type) lectin, belonging to the collectin family, with an important role in innate immunity¹. MBL circulates in blood complexed with MBL-serine proteases (MASPs) and acts as a soluble pattern recognition receptor, binding specific carbohydrate patterns exposed on the surface of pathogens or of “altered-self” cells through its carbohydrate-recognition domain (CRD). On binding the target cells, MASPs are activated, initiating the lectin pathway (LP) of the complement system.

MBL has been implicated in experimental models of myocardial², renal³ and gastrointestinal injury⁴, cerebral ischemia/reperfusion injury^{5,6} and traumatic brain injury^{7,8} as well as pre-eclampsia⁹. In all these conditions, MBL appears to contribute to tissue injury and its deletion or inhibition is protective. MBL inhibitors might therefore be as promising therapeutic strategies, and reliable *in vitro* assays are needed to identify them, before more expensive and time-consuming *in vivo* studies.

MBL-binding properties of compounds can be investigated by a direct surface plasmon resonance (SPR) assay in which unlabeled putative MBL ligands are flowed onto recombinant MBL immobilized on the sensor chip⁵. Binding is revealed in real time as a change of mass at the surface, and the interaction can be characterized in terms of on and off rates (kinetics) and binding strength¹⁰. Although this approach is fairly straightforward and useful for initial screening purposes, it suffers some limitations: MBL can be bound on a site different from CRD, so it may not result in inhibitory activity; binding affinities can be overestimated due to multivalent interactions; immobilization procedures can alter the binding properties of MBL. We experienced difficulties in immobilizing recombinant murine MBL-C (mMBL-C, one of the two murine isoforms, together with mMBL-A¹¹). Then, last

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4 but not least, immobilization of MBL on a surface makes this configuration different from the
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6 physiological condition.
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8 We have now developed and characterized a novel SPR-based assay that allows *in vitro*
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10 identification of MBL inhibitors, with a design that closely mimics real conditions, since the
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12 compound's effects are measured by their ability to inhibit MBL binding to mannose residues
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14 exposed on the (sensor) surface. Thus, this approach can assess the inhibition of a property
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16 of MBL which is relevant to its functional activity. The assay is suited to study the inhibition
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18 of native plasmatic mMBL-A and mMBLC, and of recombinant human MBL (rhMBL). We
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20 used it to measure the inhibitory effects of synthetic polymannosylated glycodendrimers, two
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22 of them – Polyman2 and Polyman9 – with protective properties in animal models of MBL-
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24 mediated injuries^{5,8,9}.
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30 **Materials and Methods**

31 *Animals*

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33 Plasma was obtained from eight-week-old C57Bl/6 mice (wild-type, WT, 20-25g; Harlan
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35 Laboratories) and from C57Bl/6 mice with a target mutation of both MBL-A and MBL-C
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37 genes (MBL^{-/-}, 20-25g, purchased from Jackson Laboratories-USA and colonized at the
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39 Mario Negri Institute). Briefly, blood samples were collected from the vena cava in 10 mM
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41 ethylenediaminetetraacetic acid and 0.125% polybrene (Sigma-Aldrich). Plasma was separated
42
43 by centrifugation at 2000 g at 4°C for 15 min, immediately stored and kept at -80°C until use.
44
45 The IRCCS-Istituto di Ricerche Farmacologiche Mario Negri adheres to the principles set out
46
47 in the following laws, regulations, and policies governing the care and use of laboratory
48
49 animals: Italian Governing Law (D.lgs 26/2014; Authorisation n.19/2008-A issued March 6,
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51 2008 by Ministry of Health); Mario Negri Institutional Regulations and Policies providing
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4 internal authorisation for those conducting animal experiments (Quality Management System
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6 Certificate – UNI EN ISO 9001:2008 – Reg. No. 6121); the NIH Guide for the Care and Use
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8 of Laboratory Animals (2011 edition) and EU directives and guidelines (EEC Council
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10 Directive 2010/63/UE). The Statement of Compliance (Assurance) with the Public Health
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12 Service (PHS) Policy on Human Care and Use of Laboratory Animals has been recently
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14 reviewed (9/9/2014) and will expire on September 30, 2019 (Animal Welfare Assurance
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16 #A5023-01). The studies reported here were within a project specifically authorized by the
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18 Italian Ministry of Health (Decree n° 161/2014B).
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24 25 *SPR-based assay*

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27 SPR is a powerful label-free method widely used to study binding between two
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29 macromolecules¹². Typically, one of the interacting partners is immobilized on a sensor chip
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31 surface and the other is flowed through a microfluidic system in contact with the chip surface.
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33 Binding is revealed in real time as a change of mass at the surface. Although the most
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35 common application of SPR instruments is to determine affinity parameters for biomolecular
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37 interactions¹⁰, its versatility permits many other uses, including label-free immunoassays¹³⁻
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The novel assay described here involves several steps. Fig. 1 summarizes the four steps required for studies with murine plasma (two mMBL isoforms); studies with rhMBL are limited to the first two steps. The first step is the covalent immobilization of mannosylated bovine serum albumin (Man-BSA, Dextra Laboratories, Reading, UK) on the sensor chip (GLC, Bio-Rad) using amine coupling chemistry¹⁶. Thus, Man-BSA molecules are bound to the sensor surface through the protein moieties leaving the mannose residues exposed. The ProteOn XPR36 Protein Interaction Array system (Bio-Rad Laboratories, Hercules, CA) used

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4 for these SPR studies, has six parallel flow channels that can immobilize up to six ligands on
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6 the same sensor chip. In parallel to Man-BSA we always immobilized BSA (Sigma) as
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8 reference. Immobilization levels were about 3000 and 4000 resonance units (RU, 1 RU 1 pg
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10 protein/mm²). After the first immobilization step, the ProteOn XPR36 fluidic system is
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12 rotated 90°¹⁷ so that up to six different solutions can be injected simultaneously over the
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14 immobilized ligands (Fig. 1). In this second step, we injected MBL-containing solutions,
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16 preincubated with or without the inhibitors (see below). We used mouse and human plasma,
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18 diluted (as indicated) in 10 mM running buffer (TRIS buffer containing 150 mM NaCl and
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20 0.005% Tween 20, pH 7.4 , plus 1.2 mM CaCl₂); or running buffer with rhMBL added (R&D
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22 Systems, Minneapolis, MN). This second step aims to capture MBL (mMBL isoforms or
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24 rhMBL) on the Man-BSA surface. The mannose-dependent SPR binding signal is obtained
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26 by subtracting the mannose-independent nonspecific response on the reference surface (BSA
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28 only). The following steps are carried out after further rotation of the fluidic system (Fig. 1)
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30 and regards the sequential injections of anti-MBL antibodies. After injecting murine plasma
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32 we injected 10 µg/mL anti-mMBL-A and 10 µg/mL anti-mMBL-C antibodies (Hycult
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34 Biotech, The Netherlands) to specifically recognize and quantify the isoform captured during
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36 the second step (Fig. 1). Two anti-hMBL antibodies were tested after injection of human
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38 plasma: 10 µg/mL anti-hMBL antibody 3E7 (Hycult Biotech, The Netherlands) and 10 µg/ml
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40 anti-hMBL antibody 3B6 (Abcam, Cambridge, UK). All SPR assays were run at 25°C. The
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42 sensorgrams (time course of the SPR signal in RU) were normalized to a base-line of 0.
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44 For the analysis of putative MBL inhibitors, diluted plasma or running buffer spiked with
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46 rhMBL were preincubated for 30 min at 25°C, with or without glycodendrimers at different
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48 concentrations and then flowed onto immobilized Man-BSA and BSA (Fig 1, step 2). The
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50 six parallel flow channels meant we could analyze the effect of up to four concentrations
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4 simultaneously, leaving one for mouse plasma preincubated with vehicle alone (total binding,
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6 TB) and one channel for MBL^{-/-} mouse plasma (non-specific binding, NSB). The SPR
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8 binding signal at t=100 s, in the presence of each drug concentration (BD), was used to
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10 calculate the percentage inhibition of specific binding (%INH-SB) as follows:

$$\%INH-SB = ((TB - BD) / (TB-NSB)) * 100$$

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15 The aim of these studies was to identify compounds that prevent the MBL interaction with
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17 mannose residues and to determine their potency, i.e. the concentration inhibiting MBL
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19 binding by 50% (IC₅₀).
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24 *Synthetic polymannosylated glycodendrimers*

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27 The glycodendrimers we tested as MBL inhibitors are shown in Fig. 2. They are Polyman2
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29 (also referred to as Dendron 12¹⁸ which is a tetravalent glycodendron carrying a pseudo-
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31 trimannoside headgroup; Polyman9, a hexavalent glycodendrimer with a flexible core,
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33 carrying a pseudo-dimannoside headgroup (also referred to as glycodendrimer 13.3¹⁹) and
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35 Polyman31, a hexavalent glycodendron carrying the same pseudo-dimannoside headgroup on
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37 a rigid-core architecture²⁰. The synthesis and characterization of the compounds we used has
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39 been described previously¹⁸⁻²⁰.
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48 **Results**

49 *Characterization of the SPR assay for mMBL-A and mMBL-C*

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52 Diluted plasma from WT mice injected over a sensor surface coated with Man-BSA resulted
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54 in a marked SPR signal (2550 and 1500 RU, on average, at plasma dilutions of 1:50 and
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4 1:100 respectively), much higher than in a parallel reference surface coated with BSA only
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6 (800 RU and 530 RU) (representative sensorgrams in Fig. S1A). This indicated the presence
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8 of a mannose-dependent binding signal, highlighted in sensorgrams obtained by subtracting
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10 the signal in the reference surface (Fig. S1B and Fig. 3A). Most of this specific signal was
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12 due to mMBL, since plasma from MBL^{-/-} mice gave a much lower specific signal, only
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14 20±11 % of the WT plasma value (mean±SD, n=18) (Fig. 3A, Fig. S1B).
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18 The binding of plasmatic mMBL to immobilized mannose residues was very stable as
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20 shown by the low dissociation rates after the injections (Fig. 3A) thus allowing sequential
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22 injection of the antibodies against mMBL-A and mMBL-C. These injections resulted in clear
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24 SPR binding signals (Fig. 3B-C) indicating that both murine isoforms were actually captured
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26 during the plasma injection; much lower signals were observed in the surfaces where plasma
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28 from MBL^{-/-} had been flowed (23±16 % and 11±13 % with antibodies against mMBL-A and
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30 mMBL-C, mean±SD, n=18), thus confirming the specificity of the measured signal. The SPR
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32 signal with the anti-mMBL-A antibody was linear during the injection period whereas a
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34 plateau was reached during the injection of the anti-mMBL-C antibody. This indicates a
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36 higher association rate constant of the anti-mMBL-C than the anti-mMBL-A antibody, and
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38 that less mMBL-C than mMBL-A was captured by mannose.
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42 As expected, the binding signals due to both mMBL-A and mMBL-C correlated with
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44 plasma dilution (Fig. S2A-B), although there was a tendency to saturation with the highest
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46 concentrations (1:50 and 1:25). The IC₅₀ was determined using plasma diluted at either 1:50
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48 or 1:100, with no significant differences in the results.
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51 Preincubation of plasma with different concentrations of mannose resulted in
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53 concentration-dependent inhibition of both mMBL-A and mMBL-C binding to immobilized
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mannose, as indicated by the subsequent injections of the corresponding antibodies. The IC_{50} was similar for the two isoforms, respectively 5.4 and 5.2 mM for mMBL-A and mMBL-C.

Characterization of the assay for hMBL

Injection of diluted human plasma over immobilized Man-BSA resulted in a marked SPR signal (400 RU on average, at plasma dilutions of 1:50), much higher than that observed in a parallel reference surface coated with BSA only (50 RU) (representative sensorgrams in Fig. S1C). This indicated the presence of a mannose-dependent binding signal (i.e. obtained by subtraction of the signal in the reference surface), although this was much lower than with murine plasma (Fig. S1D vs Fig. S1B).

Despite the presence of a mannose-dependent binding signal, we were not able to confirm the capture of hMBL with anti-hMBL antibodies. Results were negative with two different anti-hMBL antibodies, at concentrations up to 10 $\mu\text{g/mL}$, even when plasma was only diluted 1:10 (data not shown). To overcome this problem, we developed a different approach testing the compounds for their ability to inhibit the binding of rhMBL to immobilized mannose. Different concentrations of rhMBL (Fig S2C), diluted in running buffer, resulted in a concentration-dependent binding signal, linear in the 0.5-8 nM range (Fig. S2D). Global fitting of these sensorgrams according to the simplest 1:1 interaction model (Langmuir model) gave an estimated K_D of 1 nM.

Inhibitory effects of polymannosylated glycodendrimers

Polyman2 inhibited the binding of mMBL-A and mMBL-C to immobilized mannose with similar IC_{50} values (93 and 84 μM , Fig. 4A-B) whereas Polyman31 was highly selective for

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4 the two isoforms, inhibiting mMBL-C with a very low IC_{50} (0.5 μ M) but being much less
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6 active on mMBL-A ($IC_{50}>200$ μ M) (Fig. 4A-B). Polyman9 also had a lower IC_{50} on mMBL-
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8 C (65 μ M) than on mMBL-A ($IC_{50}>200$ μ M) (Fig. 4A-B⁸).
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11 As an example of raw data, Fig. S3A shows representative sensorgrams obtained
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13 injecting of plasma preincubated or not with 3 μ M Polyman31, in parallel with plasma from
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15 MBL-/- mice. At this concentration, Polyman31 had no effect on MBL-A binding (Fig.
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17 S3B), but it reduced MBL-C binding by 85% (Fig. S3C). The 20% decrease during the
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19 plasma injection (Fig. S3A) indicated that most (~ 77%) of the MBL captured by mannose
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21 was accounted for by MBL-A.
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25 Preincubation of rhMBL (8 nM) with different concentrations of dendrimers resulted in
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27 dose-dependent inhibition of rhMBL binding to immobilized mannose (Fig. 4C). In this
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29 assay Polyman31 was the most potent inhibitor (IC_{50} 62 μ M), followed by Polyman9 (~ 136
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31 μ M) and Polyman2 (~ 270 μ M).
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36 Discussion

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38 We report the characterization of a novel SPR assay for *in vitro* screening of MBL inhibitors,
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40 analyzed for their ability to interfere with MBL binding to carbohydrate patterns. This was
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42 achieved by flowing MBL-containing solutions, with or without inhibitors, over a sensor chip
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44 exposing mannose residues.
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48 The mannose-dependent SPR signal with diluted murine plasma is mostly (80% on
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50 average) due to mMBL, as indicated by the results with plasma of MBL^{-/-} mice. This binding
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52 has a very low dissociation rate, as expected for multivalent binding, so permitting
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54 subsequent injections of antibodies against mMBL-A and mMBL-C to specifically recognize
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56 and quantify the isoform captured by mannose. In our conditions 70-80% of the mMBL-
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4 dependent SPR signal is accounted for by mMBL-A, although its serum levels in C57Bl/6
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6 mice are 4-5 times lower than those of mMBL-C^{21,22}. Since the molecular mass of the two
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8 isoforms is comparable²¹, this suggests that mMBL-A has greater affinity than mMBL-C for
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10 immobilized mannose. However, the smaller proportion of mMBL-C captured by
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12 immobilized mannose is compensated by the higher affinity of the corresponding antibody.
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14 Thus, the SPR signal 100 sec after injection of the anti-mMBL-C antibody was similar, on
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16 average, to that seen 100 sec after injection of the anti-mMBL-A antibody.
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20 The SPR data obtained injecting diluted human plasma indicated a mannose-dependent
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22 binding signal which, however could not be definitely associated to the capture of hMBL. In
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24 fact, there was no SPR signal on subsequent injection of anti-hMBL antibodies. Possibly the
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26 “mannose-dependent” binding signal after injection of human plasma is either: i) not due to
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28 hMBL capture or ii) actually due to hMBL, but the two anti-hMBL antibodies tested did not
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30 bind it efficiently enough for detection in our set-up.
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34 The mannose-dependent binding signal with human plasma was much lower (20%) than
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36 the signal with murine plasma, in agreement with the lower hMBL concentration (1-1.5
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38 $\mu\text{g/mL}$)^{23,24} than mMBL-A (8-20 $\mu\text{g/mL}$) or mMBL-C (30-100 $\mu\text{g/mL}$)^{21,22}. Insufficient
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40 capture of hMBL on the sensor chip might thus contribute to the lack of measurable binding
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42 with anti-hMBL antibodies, although results were also negative using plasma or serum ten-
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44 fold only diluted. Other factors required for an efficient SPR assay, and possibly not met with
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46 native hMBL, are: i) adequate affinity of hMBL for the immobilized mannose residues,
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48 particularly an association rate constant permitting the capture of enough hMBL during the
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50 short association phase (few minutes), and/or ii) suitable affinity of the anti-hMBL antibodies
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52 employed for these studies. In this regard, successful detection of plasmatic mMBL-A is very
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54 likely due to its high affinity for immobilized mannose (higher than that of mMBL-C),
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4 whereas successful detection of mMBL-C is probably likely due to the high serum
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6 concentrations and the high affinity of the anti-mMBL-C antibody (higher than that of anti-
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8 mMBL-A antibody).
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11 To evaluate the effects of potential inhibitors on hMBL, we developed an SPR assay
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13 using rhMBL. A suitable SPR binding signal was obtained by flowing 8 nM rhMBL,
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15 dissolved in buffer, over immobilized mannose residues (about 7 $\mu\text{g}/\text{mL}$, i.e. much higher
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17 than the concentration present in diluted human plasma), while lower concentrations gave
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19 proportionally lower SPR signals.
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22 We used these assays to study the inhibitory effects of synthetic polymannosylated
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24 glycodendrimers. Native MBL is found as oligomers of a trimeric building block, with
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26 multiple CRD domains²⁵ which efficiently bind to carbohydrate patterns through multivalent
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28 protein-sugar interactions. Thus, to interfere with these, multivalent inhibitors such as
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30 glycodendrimers have been designed and studied over the past ten years as potential
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32 antagonists^{26, 27}. Polyman2¹⁸ is a tetravalent pseudo-trimannoside dendron which showed
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34 potent protective activity in mouse models of cerebral ischemia⁵ or pre-eclampsia⁹ where
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36 MBL significantly contributed to the injury. Polyman9 is a hexavalent pseudo-dimannoside
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38 dendrimer based on a flexible scaffold¹⁹ which showed neuroprotective effects in a murine
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40 model of traumatic brain injury⁸. Polyman31 is a new hexavalent pseudo-dimannoside
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42 dendrimer based on a rigid-core architecture²⁰. In our assay, Polyman2 showed no
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44 selectivity between mMBL-A and mMBL-C (IC_{50} 93 and 84 μM) whereas Polyman-31 had a
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46 completely different interaction profile, being very potent on MBL-C (IC_{50} 0.5 μM) but
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48 almost inactive on mMBL-A up to 200 μM . Polyman9 had a similar profile, inhibiting
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50 mMBL-C (IC_{50} 65 μM) but not MBL-A (IC_{50} $\gg 200$ μM)⁸. These molecules also inhibited
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52 rhMBL binding of to mannose residues with IC_{50} from 62-270 μM .
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4 The inhibitor concentrations we used (μM range) greatly exceed those of MBL (nM
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6 range, in diluted murine plasma and rhMBL). Thus, the percentage inhibition of MBL
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8 binding to mannose is indicative of the occupancy of MBL proteins and the IC_{50} is a measure
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10 of the inhibitor's affinity for MBL. The affinities of Polyman2 for mMBL-A and rhMBL
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12 (IC_{50} 93 and $\sim 270 \mu\text{M}$) are higher than those measured by direct SPR assay, i.e. directly
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14 flowing the dendrimer over immobilized recombinant mMBL-A (K_D 4.7 μM , data not
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16 shown), or rhMBL (K_D 6 μM ,⁵). A similar difference was reported for polymeric inhibitors
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18 of the lectin DC-SIGN using analogous SPR approaches¹⁵ and is possibly due to a lower
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20 impact of avidity effects when interactions occur in solution.
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24 A mannan immunoassay has been described in which murine plasma was incubated on
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26 mannan-coated microtiter wells and murine MBL isoforms were then recognized by
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28 biotinylated anti-MBL antibodies and europium-labeled streptavidin²¹. This method was
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30 used to quantify mMBL-A and mMBL-C concentrations in mouse sera, but to the best of our
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32 knowledge it has never been used to screen MBL inhibitors. In comparison with our SPR
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34 assay, immunoassays in microtiter wells are probably more sensitive, due to signal
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36 amplification. However, the SPR assay has other worthwhile advantages: i) it does not need
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38 any labeled reagent and takes less of the antibodies than for microtiter wells; ii) the amount
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40 of bound anti-MBL antibody - and thus MBL - is immediately determined by SPR whereas
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42 of bound anti-MBL antibody - and thus MBL - is immediately determined by SPR whereas
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44 further steps are required when using microtiter wells, e.g. incubation with europium-labeled
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46 streptavidin for immunofluorometric assay²¹; iii) the long incubation of plasma in mannan-
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48 coated microtiter wells may reduce the binding between the inhibitor and plasmatic MBL,
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50 due to the equilibrium shift induced by the high-affinity binding of free MBL to immobilized
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52 sugars; this potential artifact, leading to underestimation of the compound's inhibitory effect,
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4 is probably absent during the very short flow of plasma over the mannose-coated SPR
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6 surface.
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9 In conclusion, we have extensively characterized a novel assay, convenient for *in vitro*
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11 screening of putative MBL inhibitors, to select the appropriate compounds for the more
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13 expensive and time-consuming *in vivo* studies. This approach has been successfully used to
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15 identify a selective mMBL-C inhibitor, Polyman9, which conferred neurobehavioral
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17 protection when given shortly after traumatic brain injury ⁸. We also found another
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19 glycodendrimer, Polyman31, with even higher affinity and selectivity for mMBL-C.
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31 **Declaration of Conflicting Interests**

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34 The authors declared no potential conflicts of interest with respect to the research, authorship,
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36 and/or publication of this article.
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Figure Legends

Figure 1. Schematic summary of the surface plasmon resonance assay for inhibitors of murine MBL isoforms.

Figure 2. Structures of the glycodendrons used in this study: Polyman2, Polyman9 and Polyman31.

Figure 3. Capture of plasmatic mMBL-A and mMBL-C by immobilized mannose residues.

A: representative sensorgrams (i.e. time course of SPR signal in resonance units, RU) with plasma (1:50) from wild-type (WT) and MBL-/- (KO) mice injected over chip surfaces coated with mannosylated-BSA (Man-BSA). These sensorgrams were obtained after subtraction of the non-specific signal measured on a parallel surface coated with BSA (see also Fig S1). Plasma was injected for 10 min (bars). B-C: representative sensorgrams with sequential injections of anti-MBL-A or anti-MBL-C antibodies shortly after the injection of plasma.

Figure 4. Inhibitory effect of dendrimeric compounds on mMBL-A (A), mMBL-C (B) or rhMBL (C) binding to mannose residues. Polyman31 is indicated in blu, Polyman2 in red, Polyman9 in green (data of Polyman9 from ref. 8). Each value is the mean \pm SD of 2-3 independent SPR sessions.

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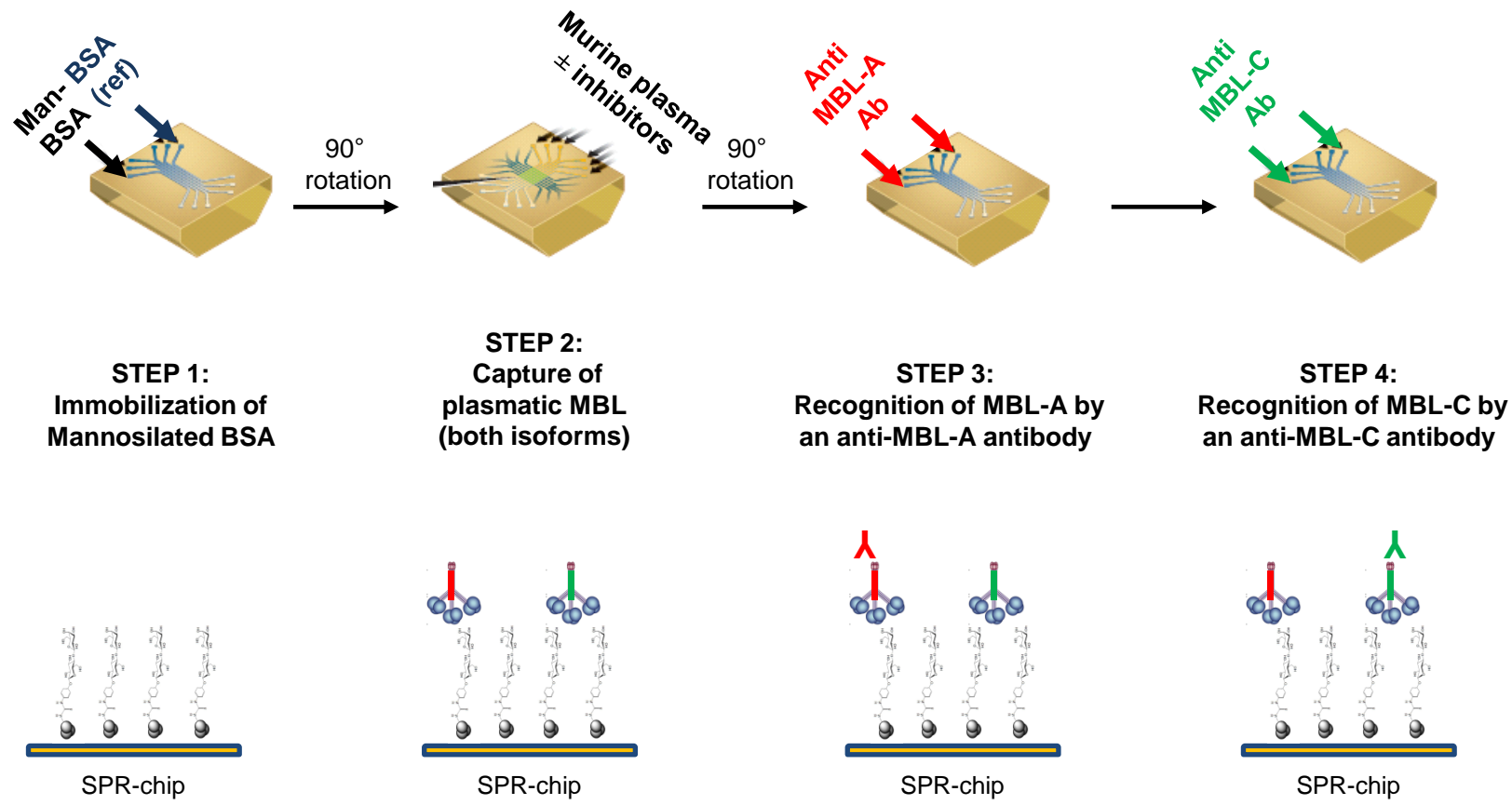
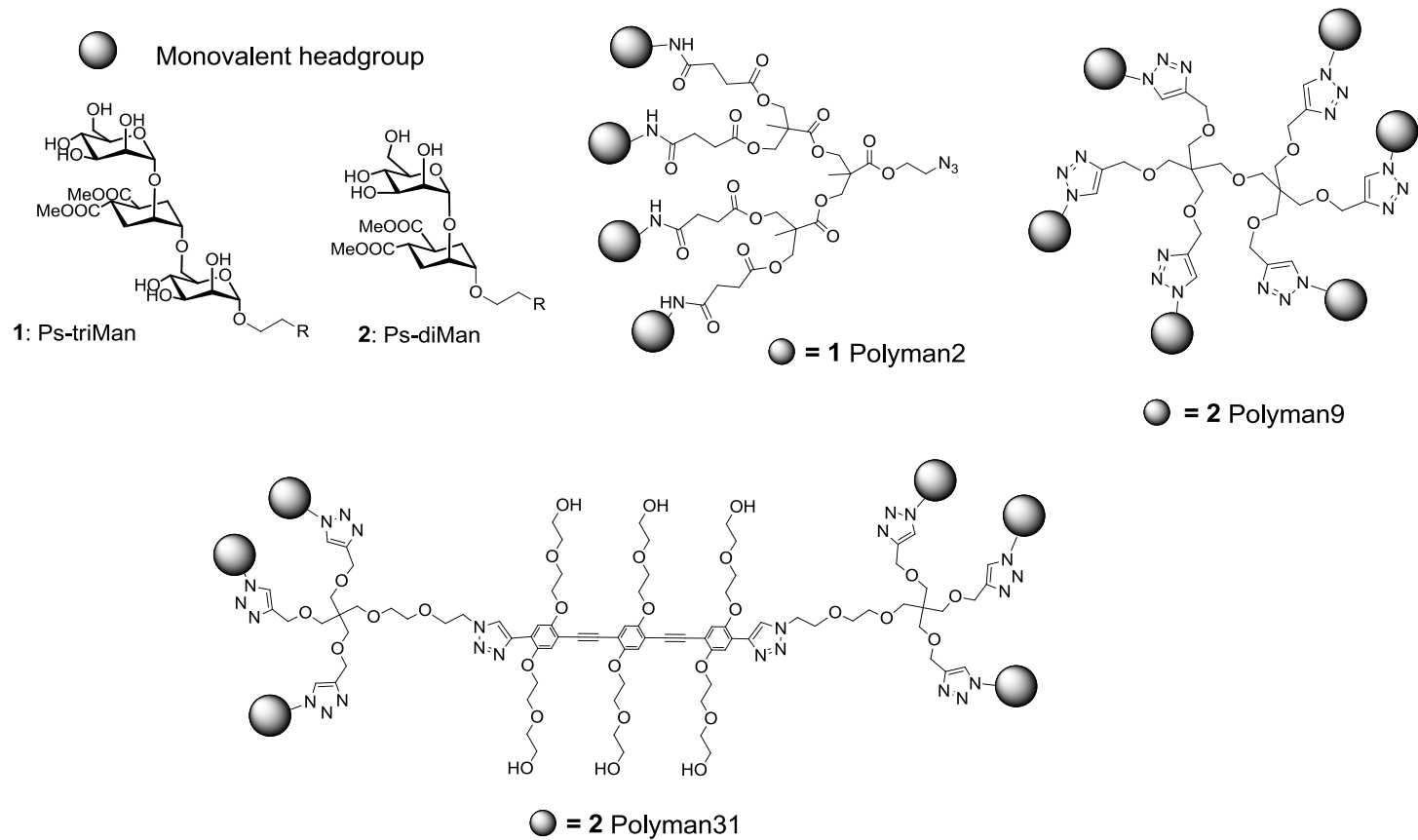


Figure 1



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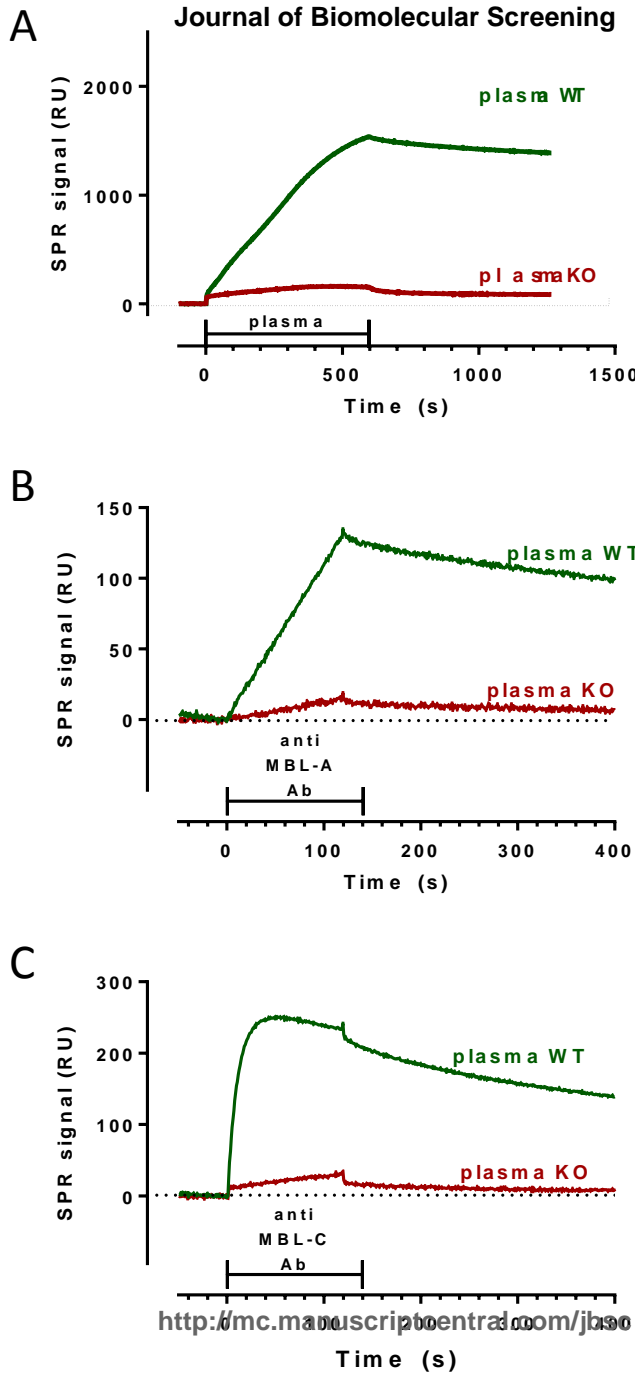


Figure 3

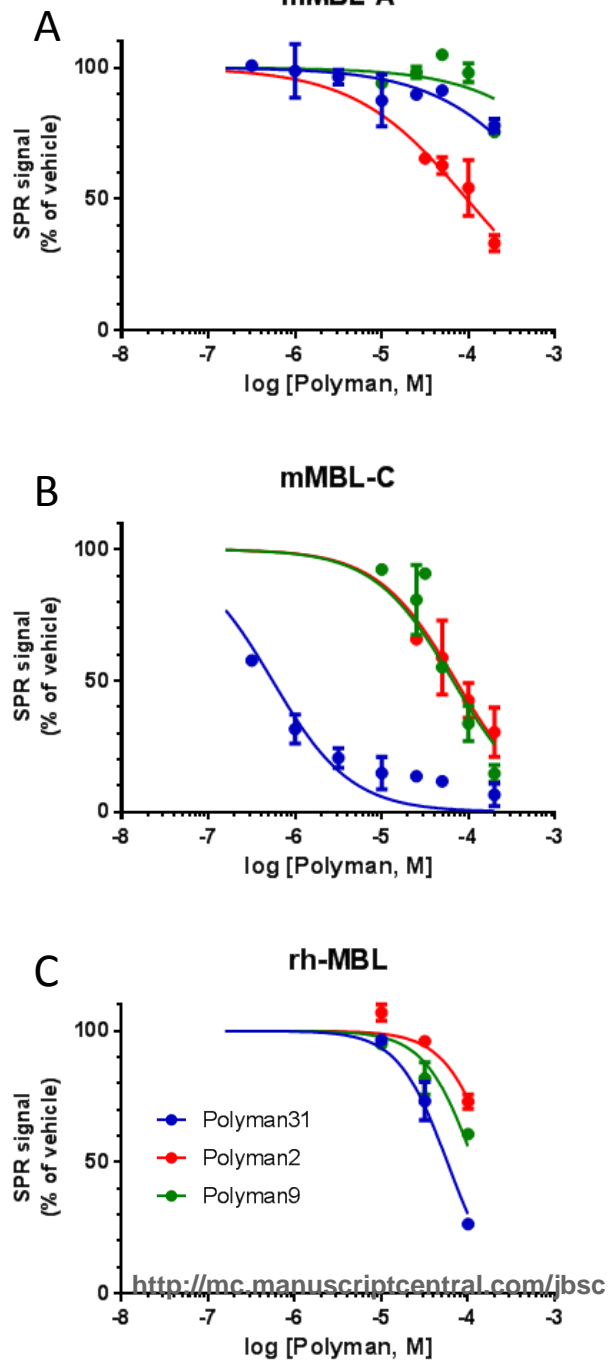


Figure 4

Supporting Information

A new surface plasmon resonance assay for *in vitro* screening of
mannose-binding lectin inhibitors

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Goti, Anna Bernardi, Maria-Grazia De Simoni, and Marco Gobbi

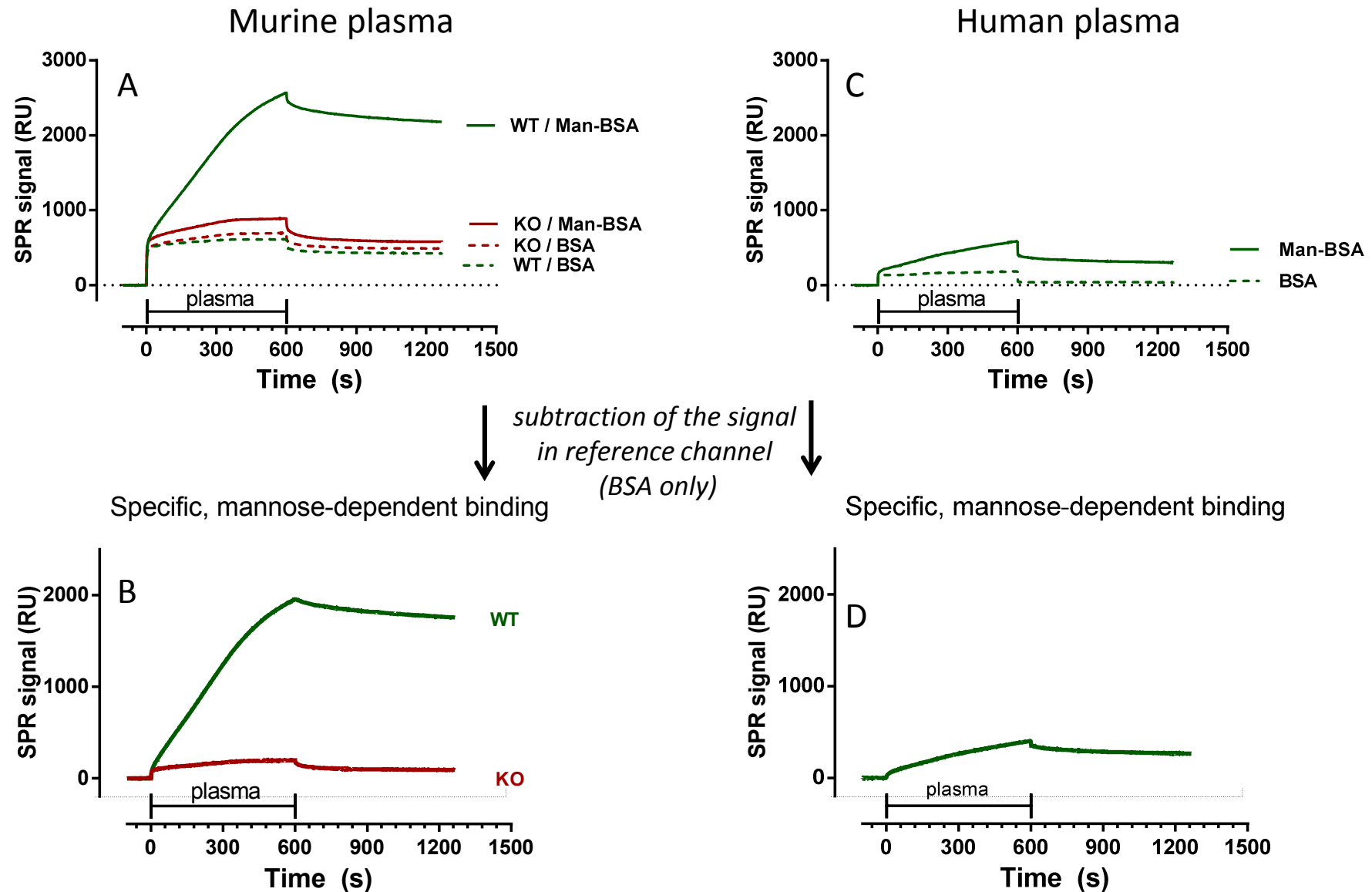


Figure S1

Raw sensorgrams after injection of murine and human plasma, and determination of the mannose-dependent binding signal.

A,C : representative sensorgrams after injection of plasma from wild-type (WT) and MBL^{-/-} (KO) mice, or human plasma over chip surfaces coated with mannosylated-BSA (Man-BSA) or BSA. Plasma (1:50) was injected for 10 min (bars). B,D: normalized sensorgrams obtained after subtraction of the signal observed on the BSA-coated surfaces only. This gives a mannose-dependent SPR binding signal

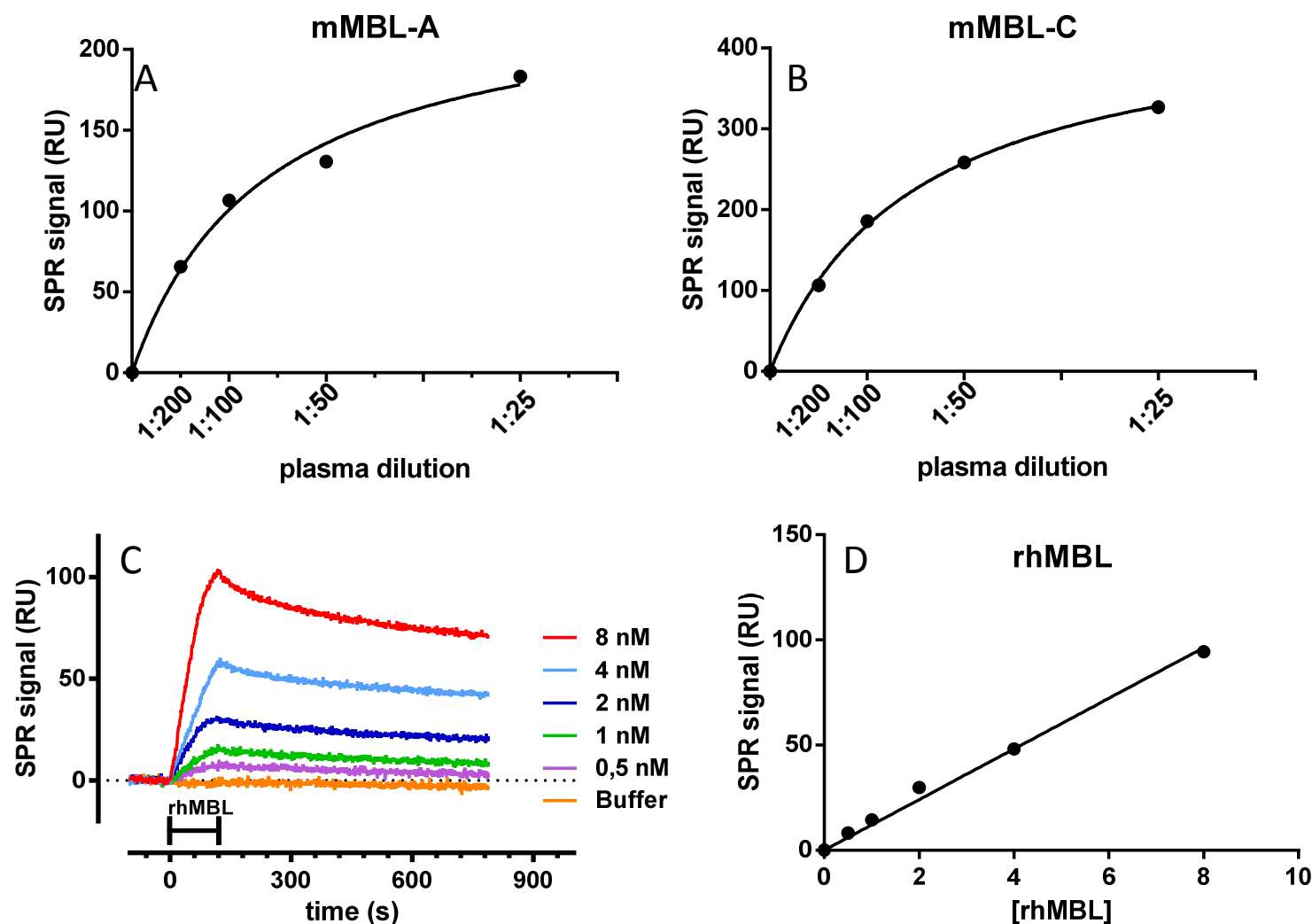
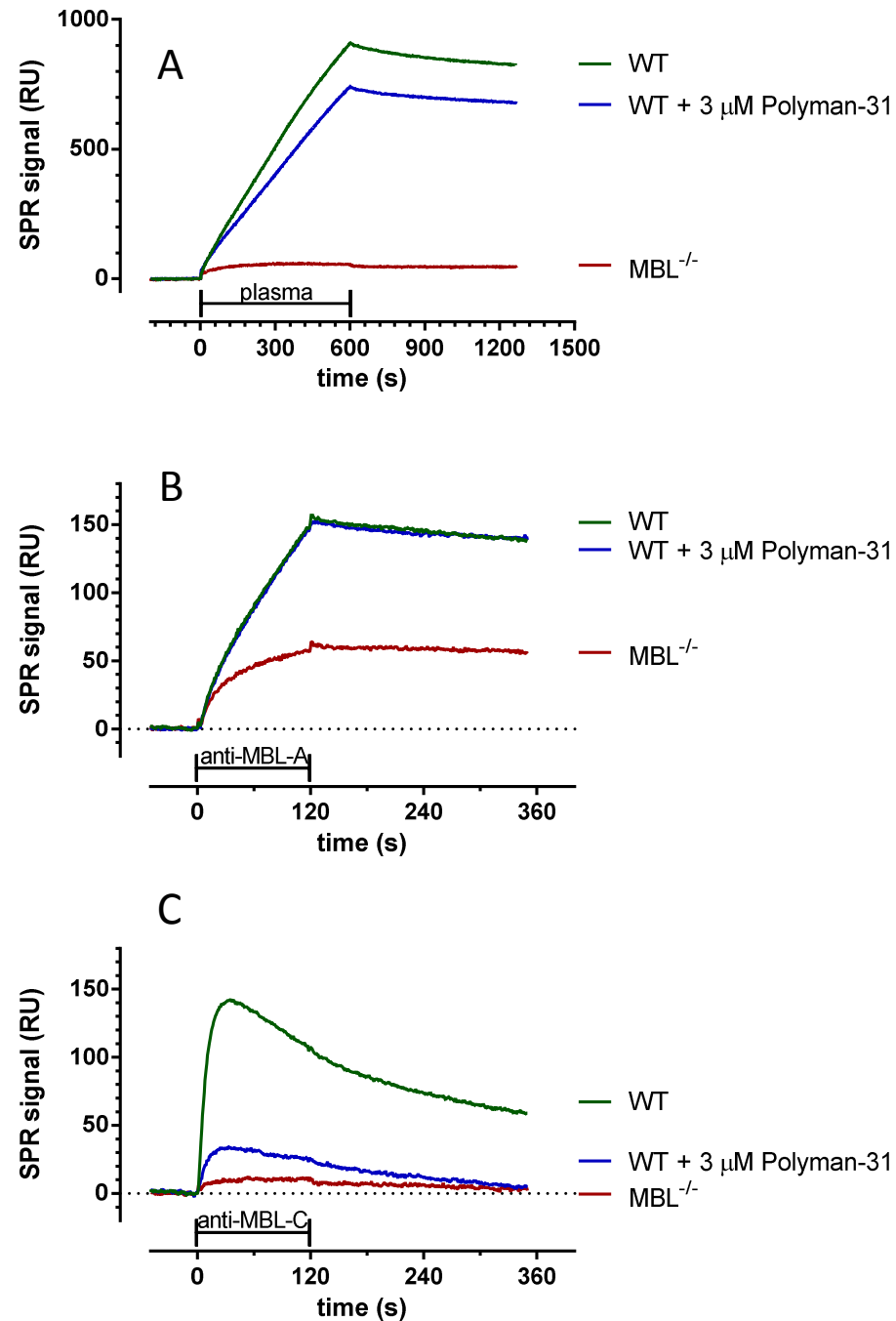


Figure S2

Correlation between MBL concentration and SPR signal.

A-B: the SPR signal due to mMBL-A and mMBL-C correlates with plasma dilution. SPR values (in resonance units, RU) were measured after injection of anti-mMBL-A or anti-mMBL-C antibodies. Data were fitted with a hyperbola equation. C: Sensorgrams obtained injecting different concentrations of recombinant human MBL (rhMBL) over chip surfaces coated with mannosylated-BSA. rhMBL was injected for 2 min (bar). These sensorgrams were obtained after subtraction of the non-specific signal (even if negligible) measured on a parallel surface coated with BSA. D: linearity between rhMBL concentration and SPR binding signal, measured 100 secs after injection of rhMBL-containing solutions.

**Figure S3****Raw sensorgrams showing the inhibitory effects of 3 μ M Polyman31.**

A: representative sensorgrams with injection of plasma from wild-type (WT) mice, incubated or not with 3 μ M Polyman31, and from MBL^{-/-} (KO) mice, over chip surfaces coated with mannosylated-BSA (Man-BSA). Sensorgrams were obtained after subtraction of the non-specific signal measured on a parallel surface coated with BSA (see also Fig S1). Plasma (1:100) was injected for 10 min (bars).

B-C: representative sensorgrams with sequential 2-min injections of anti-MBL-A or anti-MBL-C antibodies shortly after the injection of plasma.