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1   **Difference in fine specificity to polysaccharides of *C. albicans* mannoprotein**

2   **between mouse SIGNR1 and human DC-SIGN**

3

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6

7   **Running title:** *Recognition of C. albicans polysaccharides by SIGNR1*

8

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20

21　**Abbreviations used in this paper:** antibody, Ab; carbohydrate recognition domain, CRD;

22　fluorescein isothiocyanate, FITC; human DC-SIGN, hDC-SIGN; monoclonal antibody, mAb;

23　phycoerythrin, PE; phosphorylated mannose, PM; RAW264.7 cells expressing human DC-SIGN,

24　RAW-hDC-SIGN; RAW264.7 cells expressing hDC-SIGN of which CRD is replaced with

25　SIGNR1 CRD, RAW-chimera; RAW264.7 cells expressing SIGNR1, RAW-SIGNR1; soluble

26　lectin, sLectin; soluble form SIGNR1, sSIGNR1; soluble form human DC-SIGN, shDC-SIGN;

27　toll-like receptors, TLRs.

28

28 **Abstract**

29   C-type lectin SIGNR1 directly recognizes *Candida albicans* and zymosan, and has

30 been considered to share properties of polysaccharide recognition with human (h)DC-SIGN.

31 However, the precise specificity of SIGNR1 and difference from that of hDC-SIGN remains to

32 be elucidated. We prepared soluble forms of SIGNR1 and hDC-SIGN and conducted

33 experiments to examine their respective specificities.

34   Soluble SIGNR1 (sSIGNR1) bound several types of live clinical isolate *C. albicans*

35 strains in an EDTA-sensitive manner. Inhibition analyses of sSIGNR1 binding by glycans from

36 various yeast strains demonstrated that SIGNR1 preferentially recognizes N-glycan α-mannose

37 side chains in Candida mannoproteins, as reported in hDC-SIGN. Unlike shDC-SIGN, however,

38 sSIGNR1 recognized not only *S. cerevisiae* but also *C. albicans* J-1012 glycan even after

39 α-mannosidase treatment that leaves only β1,2-mannose capped α-mannose side chains. In

40 addition, the glyco-microarray analyses showed that sSIGNR1 binds mannans from *C. albicans*

41 and *S. cerevisiae*, but does not recognize Lewis[a/b/x/y] antigen polysaccharides as in shDC-SIGN.

42 Consistent with these results, RAW264.7 cells expressing hDC-SIGN of which the carbohydrate

43 recognition domain (CRD) was replaced with that of SIGNR1 (RAW-chimera) produced

44 comparable amounts of IL-10 in response to glycans from *C. albicans* and *S. cerevisiae*, but

45 those expressing hDC-SIGN produced less IL-10 to *S. cerevisiae* than *C. albicans*. Furthermore,

46 RAW-hDC-SIGN cells remarkably reduced IL-10 production after α-mannosidase treatment

47   compared with RAW-chimera.

48   These results indicate that SIGNR1 recognizes *C. albicans*/yeast through a partly

49   distinct specificity from its homologue hDC-SIGN.

50

50   **Introduction**

51   Numerous microbes are covered with the polysaccharides. Recognition of the

52   polysaccharides by pattern recognition receptors (PRRs), including lectins, is vital in order to

53   recognize pathogens, since recognition of the outermost components is the first interactive step

54   with immune cells during infection to evoke innate and adaptive immune responses.

55   *Candida albicans* is an opportunistic agent of infection in immune compromised

56   patients. In the host innate immune system, several types of receptors for sensing ligands on the

57   microbe have been defined, *e.g.*, C-type lectins and toll-like receptors (TLRs) (18). Ligands for

58   these receptors are present in the outer structure of microbes. However, some ligands are

59   sequestered by the outermost polysaccharides, which consist of mannoproteins, as reported in the

60   case of β-glucan, a ligand for Dectin-1 (6). Mannoproteins are rich in polysaccharides composed

61   of mostly α- and β-mannose and recognized by mannose/mannan type lectins.

62   C-type lectin human (h)DC-SIGN (CD209) has been shown to interact with a wide

63   range of pathogens, including microbes, viruses and protozoa (11) *via* mannose and fucose

64   moieties on the surface of the pathogens. Microbes such as *Mycobacterium tuberculosis* and *C.*

65   *albicans* are endocytosed and processed for antigen presentation to induce the subsequent T

66   cell-mediated immune responses. However, the recognition also induces immunosuppressive

67   responses in cooperation with TLRs (8).

68   Mice have eight hDC-SIGN homologues (19, 20). One of these homologues, SIGNR1,

69   is structurally related to hDC-SIGN based on its long neck domain. SIGNR1 is expressed on

70   particular subsets of macrophages (Mɸ)/dendritic cells (DC) in the marginal zones of the spleen,

71   resident peritoneal cavity, medulla of lymph nodes, skin and lamina propria (4, 10, 17, 30).

72   SIGNR1 on these cells plays a role as a sentinel in the recognition of pathogens through capsular

73   polysaccharides. In fact, SIGNR1 on marginal zone Mɸ recognizes *Streptococcus pneumoniae*

74   (10), leading to efficient activation of the complement system *in situ* (9).

75   Previously, we reported that SIGNR1 recognizes Gram-negative bacteria (*Salmonella*

76   *typhimurium* and *Escherichia coli*) and *C. albicans* (27). The former are recognized through their

77   non-reductive end of the lipopolysaccharide core sequence by SIGNR1 (17). This is also the case

78   of hDC-SIGN in recognition of *E. coli* (13). Recently, hDC-SIGN has been reported to strongly

79   recognize the α-mannose structure of N-glycan side chains of *C. albicans,* but weakly that of *S.*

80   *cerevisiae* (2). However, the recognition motif on *C. albicans* by SIGNR1 is not clear at present.

81   Therefore, we aimed to elucidate the properties of SIGNR1 in the recognition of

82   polysaccharide on *C. albicans*. To this end, we prepared soluble forms of SIGNR1 (sSIGNR1)

83   and hDC-SIGN (shDC-SIGN) and used structurally distinguished glycans purified from various

84   types of *C. albicans* and *S. cerevisiae* as well as respective microbes. The results indicate that

85   sSIGNR1 binds equally well to glycans from *S. cerevisiae* as to *C. albicans.* Furthermore,

86   sSIGNR1, but not shDC-SIGN, was shown to readily recognize *C. albicans* glycan treated with

87   α-mannosidase. In addition, a glyco-microarray based on an evanescent-field fluorescence

88   detection method clearly revealed that sSIGNR1 binds α-mannose monosaccharide and mannans

89   from *C. albicans* and *S. cerevisiae*, but dose not recognize Lewis[a/b/x/y] antigen polysaccharides as

90   in shDC-SIGN. Different properties in recognition of yeast glycans between SIGNR1 and

91   hDC-SIGN CRD were also observed in induction of IL-10 from RAW264.7 cells.

92

92   **Materials and methods**

93   **Cells and cultures.** Maintenance of human embryonic kidney (HEK) 293T cells and

94   macrophage-like RAW264.7 cells and preparation of RAW264.7 expressing SIGNR1

95   (RAW-SIGNR1) were as described previously (27). In order to prepare RAW-hDC-SIGN,

96   cDNA encoded hDC-SIGN (kindly provided by Dr. R.M. Steinman, Rockefeller University) was

97   cloned into pMX-IRES-puromycin (12). RAW264.7 cells were transfected by the plasmid with

98   Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's protocol.

99   RAW264.7 cells expressing hDC-SIGN (RAW-hDC-SIGN) were maintained in the presence of

100   4 μg/ml puromycin (InvivoGen, San Diego, CA). To prepare RAW264.7 cells expressing

101   chimeric lectin consisting of hDC-SIGN and SIGNR1 (RAW-chimera), cDNA fragments

102   encoding amino acids 1 - 253 (corresponding to cytosolic-neck domain of hDC-SIGN) and 193 -

103   325 (corresponding to CRD of SIGNR1) were amplified using KOD polymerase (Toyobo,

104   Tokyo, Japan) using primer pairs (5'-ggtggtacgggaattcatgagtgactccaaggaaccaagac-3',

105   5'-ggcacaggcgttccac-3') and (5'-tggaacgcctgtgccgactctgcccctgggactggacattc-3',

106   5'-atttacgtagcggccgcctagccttcagtgcatggggttgc-3'), respectively. These were introduced into *Eco*

107   RI - *Not* I site of pMX-IRES-puromycin using the In-Fusion PCR Cloning System (Clontech,

108   Mountain View, CA). RAW264.7 cells were transfected by the plasmid as described above.

109

110   **Reagents and yeast strains.** Alexa647-coupled hamster anti-SIGNR1 mAb 22D1 and rabbit

111   anti-hDC-SIGN Ab (H-200) were purchased from eBioscience (San Diego, CA) and Santa Cruz

112   Biotechnology (Santa Cruz, CA), respectively. Anti-hDC-SIGN mAb (DCS-8C1; eBioscience)

113   was labeled with Alexa-555 (Invitrogen) in accordance with the manufacturer's protocol.

114   Glycogen of bovine liver type IX (G0885) and Jack bean (*Canavalia ensiformis*) α-mannosidase

115   (EC 3.2.1.24) were from Sigma-Aldrich (Irvine, CA). *C. albicans* (J-1012: serotype A,

116   NBRC1060 and NIH B-792: serotype B, NBRC10108), *C. lusitaniae* (NBRC1019) and *S.*

117   *cerevisiae* X2180-1A (WT) (BY21559) were obtained from the Biological Resource Center of

118   the National Institute of Technology and Evaluation (Tokyo, Japan). *C. albicans* (JCM1542) was

119   from the Japan Collection of Microorganisms, RIKEN (Saitama, Japan).

120

121   **Preparation of mannan from yeast strains.** Glycan was prepared from mannoprotein of the

122   blastospore (yeast) form using Fehling's solution, as previously described (24). Glycans used in

123   this study were purified from strains of *C. albicans* J-1012 (25), *C. albicans* NIH B-792 (22), *C.*

124   *stellatoidea* (24), *C. parapsilosis* (22), *C. lusitaniae* (23), *S. cerevisiae* (WT) (1), *S. cerevisiae*

125   (*mnn1/mnn4*) (1) and *S. cerevisiae* (*mnn2*) (21). The α-mannosidase treatment of *C. albicans*

126   J-1012 mannan was carried out in 50 mM sodium acetate buffer (pH 4.6) containing 20 units of

127   α-mannosidase at 37°C for 48 h.

128

129   **Preparation of sSIGNR1 and shDC-SIGN and binding analyses to microbes.** Soluble lectin

9

130   (sLectin) tetramers, such as sSIGNR1 and shDC-SIGN, were prepared as described (26). Briefly,

131   cDNA fragments encoding their extracellular domains were cloned into pEXPR-IBA44 (IBA,

132   Göttingen, Germany) to add N-terminal BM40 secretion signal and *Strep*-Tag II sequences,

133   followed by the transfer into pEF6/V5-His (Invitrogen). HEK293T cells were then transfected

134   with each plasmid using the calcium phosphate method (3) and cultured in serum-free medium

135   293 SFM II (Invitrogen) for the last 48 hr. sSIGNR1 and shDC-SIGN in the supernatant were

136   purified using *Strep*-Tactin Sepharose (IBA) in accordance with the manufacturer's protocol (>

137   95% of purity by SDS-PAGE).

138   Purified sLectins (2.5 μg/ml) were incubated with PE-labeled *Strep*-Tactin (7.5 μg/ml)

139   in 18 μl of Hanks' balanced salt solution (pH 8.3) (binding buffer) for 2 h at 4°C and for a further

140   10 min at 37°C. The tetramers thus formed were incubated with 5 x 10^6 live microbes for 4 h at

141   4°C in the presence of 1% BSA (total volume 25 μl). After washing with the binding buffer, the

142   amount of bound PE-*Strep*-Tactin was measured by Gemini EM (Molecular Devices, Sunnyvale,

143   CA). The direct binding of sSIGNR1 is shown as an arbitrary unit of fluorescence intensity.

144   The % inhibition was calculated using the following formula: [1 - (fluorescence intensity of *C.*

145   *albicans* by the staining with sLectin plus inhibitor - that without sLectin) / (that with sLectin

146   without inhibitor - that without sLectin)] x 100.

147

148   **Lectin ELISA.** sSIGNR1 and shDC-SIGN were formed by incubating sLectins (62.5 ng) with

149   HRP-*Strep*-Tactin (12.5 ng) in 20 μl of the solution as above. Microtiter plates were coated with

150   50 μl of mannan/glycan (5 mg/ml) in 50 mM sodium bicarbonate buffer (pH 9.6) for 12 h at 4°C,

151   followed by the incubation with 2.5% BSA at room temperature for 2 h after washing with 25

152   mM Tris-HCl pH8.3 + 150 mM NaCl. The plates were then incubated with sSIGNR1 or

153   shDC-SIGN in the presence of 1% BSA for 2 h at 4°C. For inhibition experiments, sLectin

154   tetramer was pre-incubated with inhibitors for 1 h at 4°C before adding to plates. After washing,

155   binding of sLectin tetramer was measured as the absorbance of TMB (eBioscience) at 450 nm by

156   VERSAmax (Molecular Devices). The % inhibition was calculated as above.

157

158   **Inhibition of FITC-dextran binding to RAW-SIGNR1 with glycans.** RAW-SIGNR1 cells (2

159   x 10^5 cells) were pre-incubated with various types of glycan and EDTA (25 mM) for 30 min at

160   4ºC and then mixed with 80 μg/ml of FITC-dextran (2,000 kDa; Sigma-Aldrich) for 4 h at 4ºC.

161   Binding of FITC-dextran was analyzed by a flow cytometer. The % inhibition was calculated

162   using the following formula: [1 - (mean fluorescence intensity (MFI) of RWA-SIGNR1 cells

163   with FITC-dextran plus inhibitor- that without FITC-dextran) / (that with FITC-dextran without

164   inhibitor - that without FITC-dextran)] x 100.

165

166   **Glyco-microarray analyses of sLectins by evanescent-field fluorescence detection.** The

167   glyco-microarray analysis was performed as described (28). To form immune complex,

168   sSIGNR1 and shDC-SIGN (10 μg/ml) were pre-incubated with Alexa647-anti-SIGNR1 (22D1)

169   and Alexa555–anti-hDC-SIGN (DCS-8C1; 1 μg/ml) for 15 min at room temperature in 25 mM

170   Tris-HCl buffer (pH 7.4) containing 0.8% NaCl, 1% (v/v) Triton-X100, and 2 mM $CaCl_2$ with or

171   without 10 mM EDTA. This complex was directly added to the array immobilized with

172   multivalent glycan ligands (Supplementary Fig. S1), followed by incubation overnight at 20°C.

173   Binding was then detected using an evanescent-field fluorescence-assisted scanner. Data were

174   analyzed with the Array Pro analyzer ver. 4.5 (Media Cybernetics, Bethesda, MD).

175

176   **IL-10 production of RAW264.7 transfectants by stimulation in microplates coated with**

177   **glycan.** Non-treated plates were pre-coated with 600 μg/ml glycan in PBS for 12 h. After

178   blocking with RPMI1640 containing 10% FCS for 30 min, RAW264.7 transfectants (5 x 10^4

179   cells) were cultured in the presence of 100 ng/ml ultra pure LPS (Invitrogen) for 24 h. IL-10 in

180   the supernatants was analyzed using the Cytometric Bead Array (CBA) for mouse inflammation

181   kit (BD Biosciences, Franklin Lakes, NJ).

182

183   **Statistical analysis.** Data are expressed as the means ± SD of triplicate assays. Statistical

184   significance was determined by the two-tailed Student's *t*-test or multiple comparisons with

185   Tukey's multiple range test. All experiments were performed two or more times and

186   representative results are shown.

187

187   **Results and Discussion**

188   **SIGNR1 recognizes various types of Candida strains.** Each Candida strain has a unique set of

189   oligomannose side chains, generating a great diversity of N-glycans (see Fig. 1A) compared with

190   that of O-glycans. Moreover, N-glycans account for more than 95% of glycans in the surface

191   mannoproteins.

192   Therefore, we first examined the direct binding of sSIGNR1 to several Candida strains

193   (Fig. 1B). We used sSIGNR1 tetramerized with *Strep*-Tactin, because the affinity of sSIGNR1

194   monomer is weak (20). This method not only helps to increase the affinity of sSIGNR1 but also

195   helps to avoid the formation of large complexes using Fc-fusion lectins polymerized with anti-Fc

196   polyclonal Ab. Before using this sSIGNR1-tetramer for experiments, we confirmed that

197   sSIGNR1-tetramer bound to mannan-agarose was eluted with EDTA (data not shown), although

198   the yield was less than half the amounts applied. sSIGNR1 bound clinical isolate live *C. albicans*

199   JCM1542 (serotype A) in an EDTA-sensitive manner, indicating that sSIGNR1 binding occurs

200   *via* the carbohydrate recognition domain (CRD). The other clinical isolate strains, *C. albicans*

201   J-1012 (serotype A) and NIH B-792 (serotype B), were also recognized by sSIGNR1, although

202   sSIGNR1 binding was much less to nosocomial strain *C. lusitaniae*.

203   It is of note that sSIGNR1 binds to *S. cerevisiae* comparably to clinical isolate *C.*

204   *albicans* (Fig. 1B), and its binding to *C. albicans* J-1012 microbes was equally inhibited by

205   N-glycans from *S. cerevisiae* (WT and *mnn1/mnn4*) and *C. albicans* NIH B-792 as *C. albicans*

206   J-1012 (Fig. 1C).

207

208   **SIGNR1 possibly recognizes α-mannose in side chains of *C. albicans* N-glycan.** To delineate

209   the polysaccharide structure recognized by SIGNR1, inhibition experiments with lectin ELISA

210   were conducted using N-glycans from the various yeast strains listed (Fig. 1A and Table I).

211   The direct binding of sSIGNR1 to *C. albicans* J-1012 N-glycan, which is composed of

212   complexed side chains, was inhibited by glycans from several types of microbes: *C. albicans*

213   NIH B-792 and *C. stellatoidea* glycans lacking β1,2-mannose, *C. parapsilosis* glycan

214   lacking β1,2-mannose and α1,6-branched mannose, and *S. cerevisiae* WT glycan composed of

215   short side chains lacking β1,2-mannose and α1,6-branched mannose (Fig. 2A). These glycans

216   share the α1,2-mannose side chain structure, suggesting that SIGNR1 recognizes similar moiety

217   in N-glycan as hDC-SIGN (2). Moreover, *S. cerevisiae* 4484-24D-1 (*mnn1/mnn4*) glycan, which

218   lacks phosphorylated mannose (PM), β1,2-mannose, α1,3-mannose, and α1,6-branched mannose

219   but possesses the short (mono- or di-) mannose side chain structure, appeared to be recognized

220   by SIGNR1. However, *S. cerevisiae* X2180-1A-5 (*mnn2*) glycan, which lacks all side chains,

221   wasn't effective (Figs. 2A and B), suggesting the crucial involvement of the side chain structure.

222   In addition, *C. lusitaniae* glycan, the side chain of which is composed of more than 75% of

223   β1,2-mannose (mono- ~ tri-β-mannose)-capped side chain (23), was also ineffective in inhibiting

224   the sSIGNR1 binding (Figs. 2A and B), implying that these β1,2- mannoses disturb the access of

14

225 SIGNR1 to the α-di-mannose to some extent. Glycogen itself had no effect. As in the case of

226 sSIGNR1 binding to microbes, that to *C. albicans* J-1012–derived N-glycan was EDTA-sensitive

227 (Fig. 2A).

228   Interestingly, treatment of *C. albicans* J-1012 mannan with α-mannosidase, which

229 removed the α-mannose side chains other than the β1,2-mannose-capped side chains (see Fig.

230 1B) (14), did not affect the inhibitory activity (Fig. 2A). This result also indicates that

231 β1,2-mannose capped α-mannose side chains in the N-glycan are sufficient to be recognized by

232 SIGNR1. We also obtained similar results when mannan from *S. cerevisiae* (M7504;

233 Sigma-Aldrich) was employed to coat plastic plates in lectin ELISA (data not shown). Therefore,

234 it is feasible that SIGNR1 recognizes long internal α-mannose (tri- or more-mannose) capped

235 with β1,2-mannose in addition to α-mannose side chains.

236   Since RAW-SIGNR1 cells effectively bind and endocytose high molecular weight

237 FITC-dextran in an EDTA-sensitive manner (27), we carried out the inhibition analysis using

238 RAW-SIGNR1 cells to bind FITC-dextran instead of lectin ELISA (Fig. 2C). The inhibition

239 activities of glycans from *C. albicans* J-1012 and NIH B-792, and *S. cerevisiae* and its mutant

240 (*mnn1/mnn4*) were again comparably effective. The treatment of *C. albicans* J-1012 glycan with

241 α-mannosidase was also ineffective in reducing the inhibitory activity. In addition, low

242 efficiencies of glycans from *S. cerevisiae* (*mnn2*) and *C. lusitaniae* were also confirmed in this

243 experimental system. When resident peritoneal Mφ that express SIGNR1 (27) were used, similar

244 results were obtained (data not shown). Together with the results using N-glycan only consisting

245 of α-mannose in side chain, these results strengthen the possibility that SIGNR1 recognizes both

246 α-mannose side chain and β1,2-mannose capped α-mannose side chains composed of more than

247 tri-mannoses in N-glycan.

248

249 **Specificity of N-glycan recognition by hDC-SIGN.** Cambi *et al*. previously reported that

250 hDC-SIGN recognizes glycans of *S. cerevisiae* strains less efficiently than those of Candida

251 strains (2). In contrast, SIGNR1 equally recognized glycans from both wild type and *mnn1/mnn4*

252 mutant of *S. cerevisiae* as *C. albicans*, suggesting that the specificities of hDC-SIGN and

253 SIGNR1 are somehow different from each other. In order to examine this possibility, we

254 prepared shDC-SIGN and compared the sugar specificity with that of sSIGNR1.

255 In lectin ELISA, the binding of shDC-SIGN to *C. albicans* J-1012 glycan was more

256 sensitive to fucose than mannose, and less sensitive to glucose and GlcNAc (Fig. 3A), as

257 reported (16). Results using this probe showed that shDC-SGIN bound comparably well to *C.*

258 *albicans* J-1012 and NIH B-792 (Fig. 3B). Unlike sSIGNR1, shDC-SIGN bound less to *S.*

259 *cerevisiae* microbes, as reported (2).

260 In addition, shDC-SIGN binding to *C. albicans* J-1012 glycan was not efficiently

261 blocked by glycans from *S. cerevisiae* and *mnn1/mnn4* mutants (Fig. 3C), indicating different

262 specificity in N-glycan recognition between hDC-SIGN and SIGNR1. It is worth noting that

16

263 treating *C. albicans* J-1012 glycan with α-mannosidase dramatically reduced its inhibitory

264 activity (Fig. 3C). We further confirmed the abrogation of inhibitory activity of N-glycan from *C.*

265 *albicans* J-1012 by α-mannosidase treatment by titrating its dose (Fig. 3D), showing that the

266 reduced activity is nearly comparable to that of *S. cerevisiae*. These results suggested that

267 hDC-SIGN only recognizes the α1,2-mannose at the non-reductive end of α-mannose side

268 chains, but not the internal α1,2-mannose capped with β1,2-mannose and short α-mannose

269 chains that are recognized by SIGNR1, of the N-glycan side chains in mannoprotein.

270   It has been reported that another SIGNR lectin, SIGNR3, also recognizes *C. albicans*

271 (27) and that its saccharide specificity resembles that of hDC-SIGN (20). Based on the inhibition

272 assay using lectin ELISA, SINGR3 was shown to bind a little more efficiently to wild type and

273 the *mnn1/mnn4* mutant of *S. cerevisiae* than hDC-SIGN but less so than SIGNR1

274 (Supplementary Fig. S2).

275

276 **Glyco-microarray analyses of SIGNR1 and hDC-SIGN.** Previously, Powlesland *et al.*

277 reported that sSIGNR1 did not bind any ligand on array using the regular method (20),

278 suggesting a weak affinity of SIGNR1. However, a sensitive glyco-microarray, based on an

279 evanescent-field fluorescence-assisted detection, has recently been developed (28). This method,

280 in which analysis was performed in the presence of lectin probe without washing, enabled us to

281 detect weak glycan-lectin interactions in the equilibrium state (15, 29), possibly representing

282   genuine interactions of ligand and cellular lectin.

283   An array plate immobilized with the glycans indicated (Fig. 4A and Supplementary Fig.

284   S1) was visualized by staining with Alexa647-coupled sSIGNR1/anti-SIGNR1 mAb complex

285   (Fig. 4B). The results clearly show that SIGNR1 binds to α-mannose and yeast mannans from *C.*

286   *albicans* and *S. cerevisiae*, but not β-mannose, in an EDTA-sensitive manner (Figs. 4B and C).

287   Of note, SIGNR1 did not recognize fucose-containing moieties, such as Fucα2Gal and

288   Lewis[a/b/x/y] antigen. The binding of sSIGNR1 to heparin is likely to be false positive, because of

289   the insensitivity to EDTA.

290   We previously performed array analyses using shDC-SIGN-Fc fusion as a probe (28).

291   However, structural forms between SIGNR1 and shDC-SIGN-Fc were different and this made it

292   difficult to compare the precise sugar specificities. Therefore, we again performed glyco-array

293   analysis using shDC-SIGN dimer as in sSIGNR1. The results clearly showed the recognition of

294   fucose-containing glycans and *C. albicans*, but not *S. cerevisiae*, mannan by shDC-SIGN (Fig.

295   4D), being consistent with our results in lectin ELISA and direct binding to microbes.

296   However, there are some discrepancies in our current observation and previous array

297   results. It has been shown that SIGNR1 can recognize fucose-containing moiety using

298   glyco-array and solid phase competition binding assays (5). One of our previous microarray

299   analyses also demonstrated that hDC-SIGN-Fc fusion was able to bind to glycans from *C.*

300   *albicans* and *S. cerevisiae* (28). In both reports, lectin CRD was fused with the Fc portion of IgG

301   and polymerized with anti-Fc polyclonal Ab, giving rise to a very large and multi-valent

302   complexed probe. In the current glyco-microarray analyses, we utilized dimerized lectin probes,

303   which possibly have lower avidity than those used in previous studies, enabling us to uncover the

304   difference in binding activity of shDC-SIGN to *S. cerevisiae* and *C. albicans* glycan.

305

306   **Biological significance of the different sugar specificity between SIGNR1 and hDC-SIGN in**

307   **IL-10 production using RAW264.7 transfectants.** To examine the biological significance of

308   distinct saccharide recognition between SIGNR1 and hDC-SIGN, we measured IL-10 production

309   by RAW-SIGNR1 and RAW-hDC-SIGN cells, because hDC-SIGN on DCs is known to induce

310   IL-10 production by recognizing *C. albicans* (7), and lamina propria DCs expressing SIGNR1

311   are also capable of producing IL-10 (30). After stimulation with *C. albicans* J-1012 microbe and

312   glycan coated on plastic plate, RAW-hDC-SIGN, but not RAW-SIGNR1, produced IL-10 (data

313   not shown). Therefore, we prepared RAW264.7 cells expressing the chimeric hDC-SIGN

314   molecule (RAW-chimera), of which CRD was replaced with SIGNR1 CRD, to compare glycan

315   recognition specificity in terms of the induction of IL-10 production. The RAW-chimera cells

316   expressed comparable levels of lectin to that of RAW-hDC-SIGN cells (Fig. 5A). Upon

317   stimulation with *S. cerevisiae* X2180-1A (WT) glycan, RAW-hDC-SIGN and RAW-chimera

318   cells produced equivalent amounts of IL-10 (Fig. 5B, *upper panel*). RAW-hDC-SIGN produced

319   much less IL-10 in response to *S. cerevisiae* than *C. albicans* glycan in comparison with

320   RAW-chimera (Fig. 5B, *lower panel*). Interestingly, in the case of *C. albicans* J-1012 glycan,

321   RAW-hDC-SIGN produced more IL-10 than RAW-chimera (Fig. 5B, *upper panel*). However,

322   the former significantly reduced IL-10 production after α-mannosidase treatment compared with

323   the latter (Fig. 5C), being consistent with the results showing that SIGNR1, but not hDC-SING,

324   recognizes β-mannose capped α-mannose side chains in *C. albicans* glycan.

325   It is feasible that *C. albicans* induces higher IL-10 production than *S. cerevisiae via*

326   hDC-SIGN on DCs in humans, leading to the immunosuppressive milieu at the site of infection.

327   This may explain why *C. albicans* is more virulent than *S. cerevisiae*. Regarding the sugar

328   specificity of SIGNR1, there were no significant differences in the binding specificity to

329   mannose moieties between *C. albicans* and *S. cerevisiae* at the molecular level. However, IL-10

330   production was slightly but significantly higher in response to *C. albicans* than *S. cerevisiae*,

331   implying that cellular responses by the recognition through lectin receptors is affected by some

332   other unknown factors. In this study, we used glycans prepared from the blastospore (yeast) form

333   of each yeast strain. However, it should also be kept in mind that the difference in the growth

334   form between *C. albicans* and *S. cerevisiae* might modulate cellular activity against microbes *in*

335   *situ*. hDC-SIGN in the mouse cells prepared in this study properly transduce signals for IL-10

336   production. Although it is unknown how hDC-SIGN activates the mouse Src family and

337   subsequent Raf-1 kinase in this situation, hDC-SIGN likely has a certain motif that is lacking in

338   SIGNR1, to work in both human and mouse cells for IL-10 production.

339

340   Collectively, SIGNR1 and hDC-SIGN bind polysaccharides in surface mannoprotein

341   on live *C. albicans*. However, SIGNR1 recognizes both α-mannose and β1,2-mannose capped

342   α-mannose side chains composed of more than tri-mannoses, whereas hDC-SIGN recognizes

343   only α-mannose at the non-reductive end of the side chains, but not internal α-mannose capped

344   with β1,2-mannose and short α-mannose chains, of N-glycan side chains in mannoprotein.

345   Differential recognition of yeast strains by SIGNR1 and hDC-SIGN may be relevant to the

346   differences in cellular responsiveness.

347

347 **Acknowledgment**

352

352   **References**

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451

452

452   **Figure legends**

453   Fig. 1. sSIGNR1 binds various types of yeast strains.

454   (A) Structural diagrams of N-glycan used in this study. Structures of N-glycan of *C. albicans*

455   J-1012 (25), *C. albicans* NIH B-792 (22), *C. stellatoidea* (24), *C. parapsilosis* (22) and *C.*

456   *lusitaniae* (23) are adopted from our structural analyses using NMR. Structure of *S. cerevisiae*

457   wild type, *S. cerevisiae* 4484-24D-1 (*mnn1/mnn4*) and *S. cerevisiae* X2180-1-A-5 (*mnn2*) are

458   based on previous reports (1, 21). Side chains that are digested by treatment with α-mannosidase

459   in *C. albicans* J-1012 N-glycan are *shaded*. The side chain sequence is not specified. (B) Binding

460   of sSIGNR1 to Candida and *S. cerevisiae* strains. PE-*Strep*-Tactin alone (-) or PE-sSIGNR1 (+)

461   were incubated with the indicated live yeast strains with or without EDTA (25 mM). (C)

462   Inhibition of sSIGNR1 binding to *C. albicans* J-1012 by glycans purified from the *C. albicans*

463   and *S. cerevisiae* strains indicated. sSIGNR1 was pre-incubated with 50 μg/ml of glycans before

464   mixing with microbes. Glycogen was used as a negative control. Inhibition is indicated as the

465   percent decrease of fluorescence intensity in experimental groups compared with the control

466   without inhibitor. The results are shown as the mean ± SD of triplicate assays. *$p < 0.05$ on solid

467   line by Tukey's multiple range test. Grey lines show no significant differences.

468

469   Fig. 2. Recognition of α-mannose side chains in N-glycan by sSIGNR1.

470   (A) Inhibition analysis by lectin ELISA. Binding of sSIGNR1 to *C. albicans* J-1012 glycan

471   coated on microtiter plates was analyzed in the presence of glycans (25 µg/ml) purified from

472   various types of yeast strains. Blocking activities of inhibitors are shown as the % inhibition of

473   sSIGNR1 binding. (B) Titration of inhibitory activity of glycans from the indicated yeast strains

474   for sSIGNR1 binding by lectin ELISA. Half of maximal inhibition activity was indicated by

475   dashed line. (C) Inhibition of FITC-binding to RAW-SIGNR1 cells by glycans. Transfectants

476   were incubated with the graded dose of glycans as in (B) prior to FITC-dextran. The results are

477   shown as % inhibition. Results are shown as the mean ± SD of triplicate assays. *p* < 0.05 on

478   solid line by Tukey's multiple range test. Grey lines show no significant differences.

479

480   Fig. 3. Binding of shDC-SIGN to microbes and inhibition of shDC-SIGN binding by glycans.

481   (A) Inhibition analysis of shDC-SIGN binding by monosaccharides (50 mM) and glycan from *C.*

482   *albicans* J-1012 using lectin ELISA. (B) Binding of shDC-SIGN to yeast strains was analyzed as

483   in Fig. 1B. (C) Inhibition analysis using glycans from various yeast strains as in Fig. 2A. (D)

484   Inhibition assay was performed in the presence of graded doses of glycans from the indicated

485   yeast strains. Half of maximal inhibition activity was indicated by dashed line. The results are

486   shown as the mean ± SD of triplicate assays. *p* < 0.05 on solid line by Tukey's multiple range

487   test. Grey lines show no significant differences.

488

489   Fig. 4. Glyco-microarray analysis of sSIGNR1.

490   (A) The layout of glyco-microarray. (B) Results of the glyco-microarray analyses. Binding of

491   soluble SIGNR1/Alexa647-anti-SIGNR1 mAb immune complex to the array was performed in

492   the absence (*left panel*) or presence (*right panel*) of 10 mM EDTA and detected by an

493   evanescent-field fluorescence-assisted scanner. (C) Data analyzed with the Array Pro analyzer

494   ver. 4.5. (D) Glyco-array analysis was performed using immune complex of soluble

495   hDC-SIGN/Alexa555-anti-hDC-SIGN mAb as in sSIGNR1.

496

497   Fig. 5. IL-10 production of RAW264.7 transfectants upon stimulation with glycan coated on

498   plastic plate.

499   (A) RAW-control, RAW-hDC-SIGN and RAW-chimera cells were analyzed by flowcytometry

500   using polyclonal anti-hDC-SIGN Ab and anti-SIGNR1 mAb (22D1) specific to SIGNR1 CRD.

501   (B) The transfectants (5×10^4 cells) were cultured on plates pre-coated with *C. albicans* J-1012

502   and *S. cerevisiae* X2180-1A (WT) glycan in the presence or absence of LPS (100 ng/ml). After

503   24 h, IL-10 in the supernatants was analyzed (*upper panel*). IL-10 production against *S.*

504   *cerevisiae* glycan is shown as a percentage to that against *C. albicans* glycan (*lower panel*). (C)

505   IL-10 production after stimulation with native and α-mannosidase-treated *C. albicans* J-1012

506   glycan was analyzed as in B. IL-10 production against the treated glycan is shown as a

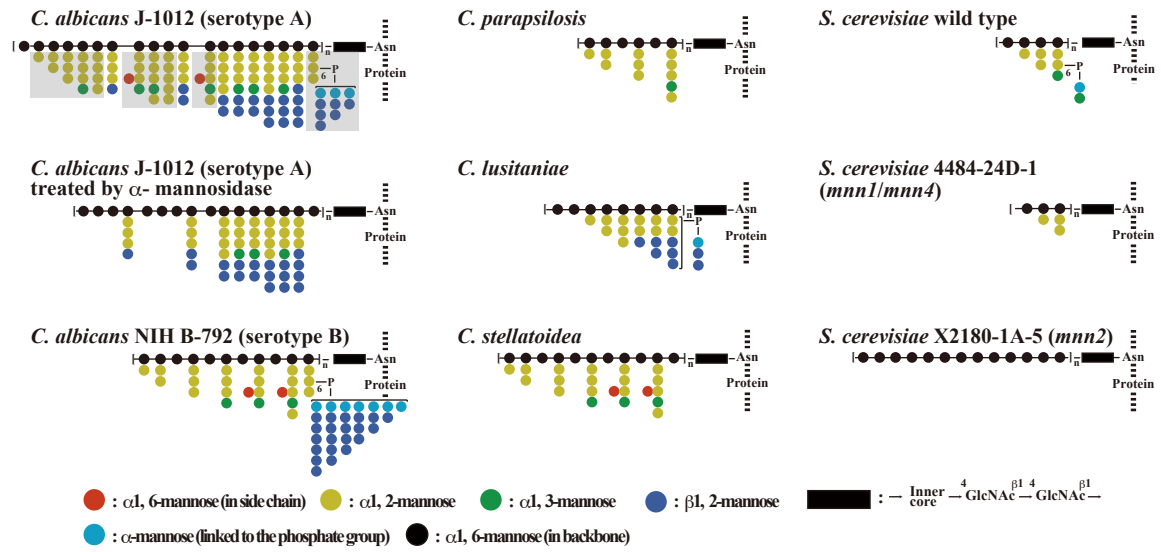507   percentage to that against the native glycan. $**p = 0.0067$, $***p = 0.0001$ by Student's *t*-test.

1   TABLE 1. Composition of side chains and properties of N-glycan used in this study.

2   _____

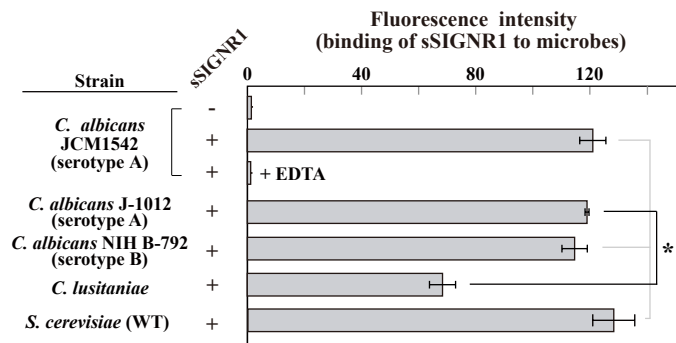| 3 N-glycan	Presence of side chain[a]				
6	α1,3-mannose	α1,2-mannose	α1,6-mannose[b]	β1,2-mannose	PM
8 *C. albicans* J-1012 (serotype A)	+	+	+	+	+
9 *C. albicans* NIH B-792 (serotype B)	+	+	+	+	+
10 *S. cerevisiae* (WT)	-	+	-	-	+
11 *S. cerevisiae* 4484-24D-1 (*mnn1/mnn4*)	-	+	-	-	-
12 *S. cerevisiae* X2180-1A-5 (*mnn2*)	-	-	-	-	-
13 *C. stellatoidea*	+	+	+	-	-
14 *C. parapsilosis*	+	+	-	-	-
15 *C. lusitaniae*	-	+	-	+	+
16 *C. albicans* J-1012 (α-mannosidase treated)	+	+	-	+	-

17   [a] PM, phosphorylated mannose. Side chain structure presence and absence in the N-glycan are indicated by + and -, respectively.
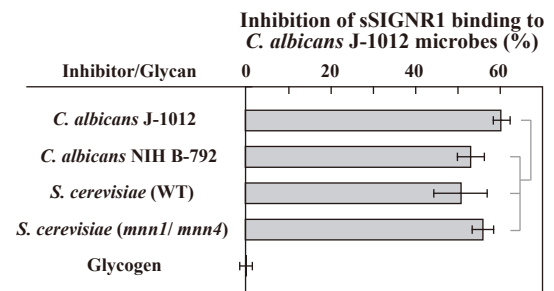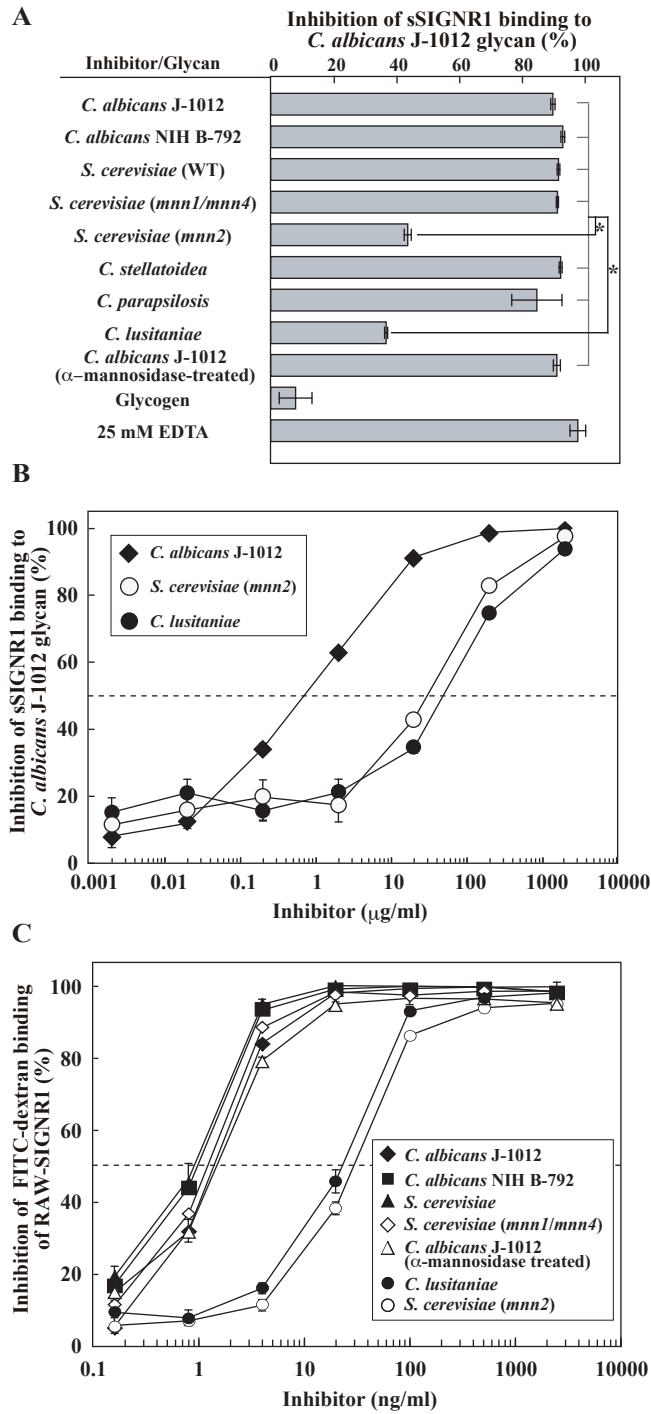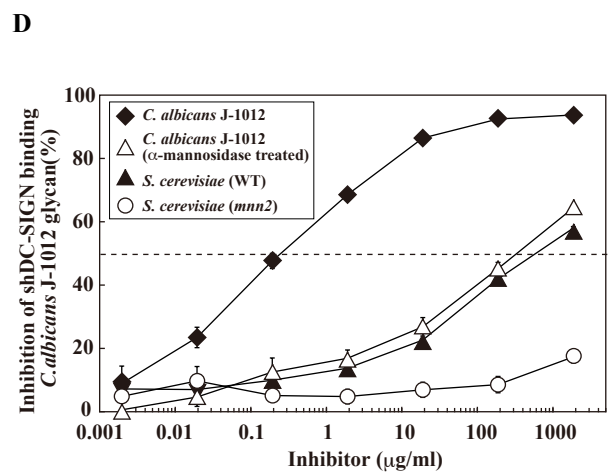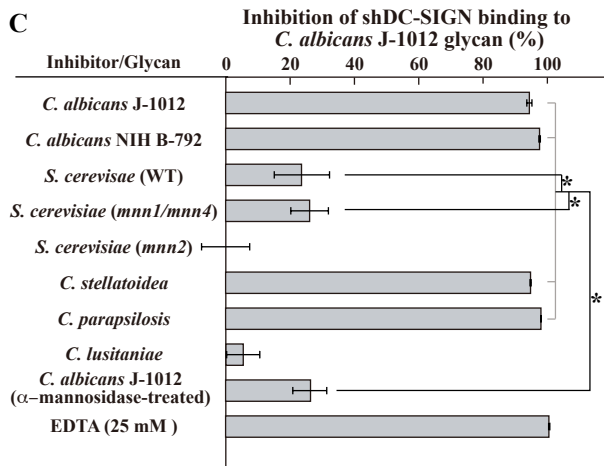
18   [b] Branching in side chain.

A

C. albicans J-1012 (serotype A)

C. parapsilosis

S. cerevisiae wild type

C. albicans J-1012 (serotype A) treated by α-mannosidase

C. lusitaniae

S. cerevisiae 4484-24D-1 (*mnn1/mnn4*)

C. albicans NIH B-792 (serotype B)

C. stellatoidea

S. cerevisiae X2180-1A-5 (*mnn2*)

: α1, 6-mannose (in side chain)   : α1, 2-mannose   : α1, 3-mannose   : β1, 2-mannose   ■ : — Inner core — GlcNAc —β1 4— GlcNAc —β1

: α-mannose (linked to the phosphate group)   : α1, 6-mannose (in backbone)

B

Fluorescence intensity (binding of sSIGNR1 to microbes)

Strain	sSIGNR1	
C. albicans JCM1542 (serotype A)	−	
	+	
	+	+ EDTA
C. albicans J-1012 (serotype A)	+	
C. albicans NIH B-792 (serotype B)	+	
C. lusitaniae	+	
S. cerevisiae (WT)	+	

0   40   80   120

*

C

Inhibition of sSIGNR1 binding to *C. albicans* J-1012 microbes (%)

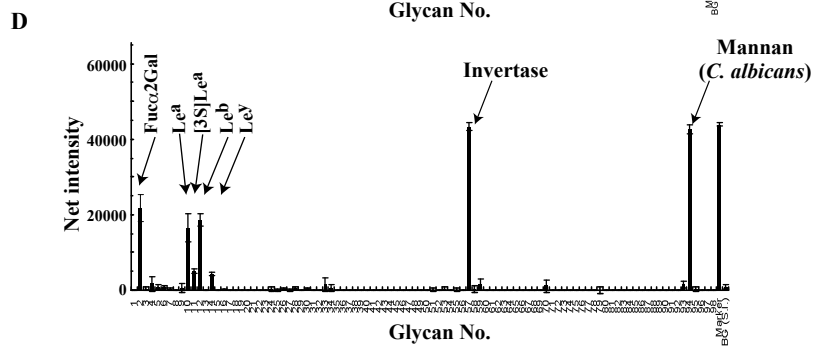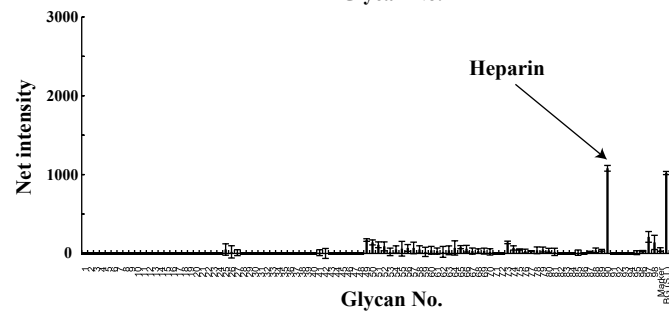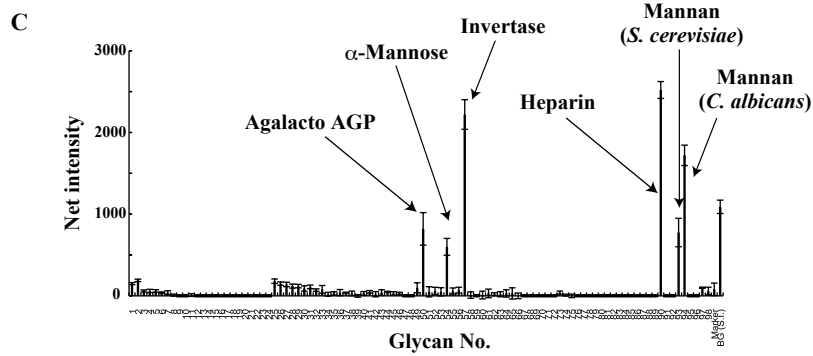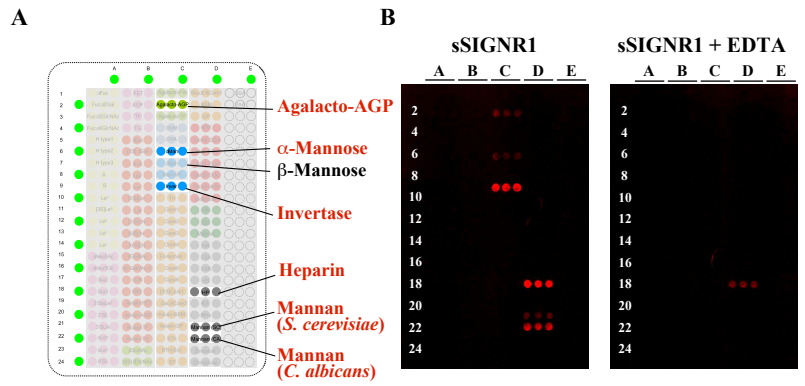| Inhibitor/Glycan	
C. albicans J-1012	
C. albicans NIH B-792	
S. cerevisiae (WT)	
S. cerevisiae (*mnn1/ mnn4*)	
Glycogen	

0   20   40   60

Takahara *et al.* Fig. 1.

A

Inhibition of sSIGNR1 binding to
C. albicans J-1012 glycan (%)

Inhibitor/Glycan	0 20 40 60 80 100

C. albicans J-1012

C. albicans NIH B-792

S. cerevisiae (WT)

S. cerevisiae (*mnn1/mnn4*)

S. cerevisiae (*mnn2*)

C. stellatoidea

C. parapsilosis

C. lusitaniae

C. albicans J-1012
(α−mannosidase-treated)

Glycogen

25 mM EDTA

B

C

Takahara *et al*. **Fig. 2.**

A Inhibition of shDC-SIGN binding to *C. albicans* J-1012 glycan (%)

B Fluorescence intensity (binding of shDC-SIGN to microbes)
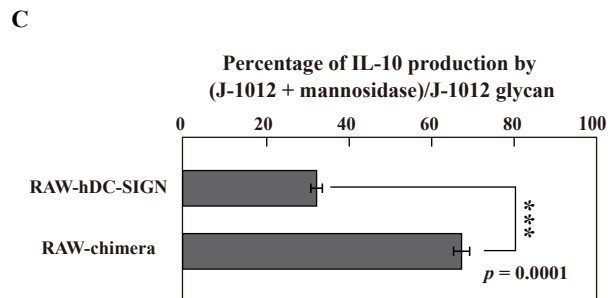
C Inhibition of shDC-SIGN binding to *C. albicans* J-1012 glycan (%)

D

Takahara *et al*. Fig. 3.

A

Agalacto-AGP
α-Mannose
β-Mannose
Invertase
Heparin
Mannan (*S. cerevisiae*)
Mannan (*C. albicans*)

B

sSIGNR1   sSIGNR1 + EDTA

A B C D E   A B C D E

C

Agalacto AGP
α-Mannose
Invertase
Heparin
Mannan (*S. cerevisiae*)
Mannan (*C. albicans*)

Net intensity
Glycan No.

Heparin

Net intensity
Glycan No.

D

Fucα2Gal
Le^a
[3S]Le^a
Le^b
Le^y
Invertase
Mannan (*C. albicans*)

Net intensity
Glycan No.

Takahara *et al*. Fig. 4.

A

B

IL-10 (pg/ml)

Percentage of IL-10 production
by A2180-1A/J-1012 glycan

p = 0.0067

C

Percentage of IL-10 production by
(J-1012 + mannosidase)/J-1012 glycan

p = 0.0001

Takahara *et al*. **Fig. 5.**

Trivial name	Presentation	Glycans	Co.	Cat#
αFuc	PAA	Fucα1-PAA	Glycotech	01-007
Fucα2Gal	PAA	Fucα1-2Galβ1-PAA	Glycotech	01-019
Fucα3GlcNAc	PAA	Fucα1-3GlcNAcβ1-PAA	Glycotech	01-024
Fucα4GlcNAc	PAA	Fucα1-4GlcNAcβ1-PAA	Glycotech	01-025
H type1	PAA	Fucα1-2Galβ1-3GlcNAcβ1-PAA	Glycotech	01-037
H type2	PAA	Fucα1-2Galβ1-4GlcNAcβ1-PAA	Glycotech	08-034
H type3	PAA	Fucα1-2Galβ1-3GalNAcα1-PAA	Glycotech	08-060
A	PAA	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-PAA	Glycotech	08-091
B	PAA	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-PAA	Glycotech	08-092
Le^a	PAA	Galβ1-3(Fucα1-4)GlcNAcβ1-PAA	Glycotech	01-035
[3S]Le^a	PAA	(3OSO₃)Galβ1-3(Fucα1-4)GlcNAcβ1-PAA	Glycotech	01-040
Le^b	PAA	Fucα1-2Galβ1-3(Fucα1-3)GlcNAcβ1-PAA	Glycotech	08-042
Le^x	PAA	Galβ1-4(Fucα1-3)GlcNAcβ1-PAA	Glycotech	01-036
Le^y	PAA	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-PAA	Glycotech	08-043
αNeu5Ac	PAA	Neu5Acα2-PAA	Glycotech	01-012
αNeu5Gc	PAA	Neu5Gcα2-PAA	Glycotech	01-051
Sia2	PAA	Neu5Acα2-8Neu5Acα2-PAA	Glycotech	08-064
Sia3	PAA	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-PAA	Glycotech	01-081
3'SiaLe^c	PAA	Neu5Acα2-3Galβ1-3GlcNAcβ1-PAA	Glycotech	01-078
3'SL	PAA	Neu5Acα2-3Galβ1-PAA	Glycotech	01-038
3'SLN	PAA	Neu5Acα2-3Galβ1-4GlcNAcβ1-PAA	Glycotech	01-077
sLe^a	PAA	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ1-PAA	Glycotech	08-044
sLe^x	PAA	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-PAA	Glycotech	01-045
6'SL	PAA	Neu5Acα2-6Galβ1-4GlcNAcβ1-PAA	Glycotech	01-039
FET	Glycoprotein	Fetuin (Complex-type N-glycans and O-glycans)	Sigma	F3004
AGP	Glycoprotein	α1-acid glycoprotein (Complex-type N-glycans-)	Sigma	G9885
TF	Glycoprotein	Transferrin (Complex-type N-glycans)	Sigma	T3309
TG	Glycoprotein	Porcine thyroglobulin (Complex and high-mannnose-type N-glycans)	Sigma	T1126
βGal	PAA	Galβ1-PAA	Glycotech	01-004
[3S]βGal	PAA	(3OSO₃)Galβ1-PAA	Glycotech	01-015
A-di	PAA	GalNAcαβ-3Galβ1-PAA	Glycotech	01-017
Lac	PAA	Galβ1-4Glcβ1-PAA	Glycotech	01-021
Le^c	PAA	Galβ1-3GlcNAcβ1-PAA	Glycotech	01-020
[3'S]Le^c	PAA	(3OSO₃)Galβ1-3GlcNAcβ1-PAA	Glycotech	01-062
LN	PAA	Galβ1-4GlcNAcβ1-PAA	Glycotech	01-022
[3'S]LN	PAA	(3OSO₃)Galβ1-4GlcNAcβ1-PAA	Glycotech	01-061
[6S]LN	PAA	Galβ1-4(6OSO₃)GlcNAcβ1-PAA	Glycotech	01-066
[6'S]LN	PAA	(6OSO₃)Galβ1-4GlcNAcβ1-PAA	Glycotech	01-068
βGlcNAc	PAA	GlcNAcβ1-PAA	Glycotech	01-011
di-GalNAcβ	PAA	GalNAcβ1-3GalNAcβ1-PAA	Glycotech	01-070
LDN	PAA	GalNAcβ1-4GlcNAcβ1-PAA	Glycotech	01-057
GA2	PAA	GalNAcβ1-4Galβ1-4Glcβ1-PAA	Glycotech	08-074
Asialo-FET	Glycoprotein	Asialo fetuin (Desialylated complex-type N- and O-glycans)	Sigma	F3004 (Acid-treated)
Asialo-AGP	Glycoprotein	Asialo α1-acid glycoprotein (Desialylated complex-type N-glycans)	Sigma	G9885 (Acid-treated)
Asialo-TF	Glycoprotein	Asialo transferrin (Desialylated complex-type N-glycans)	Sigma	T3309 (Acid-treated)
Asialo-TG	Glycoprotein	Asialo porcine thyroglobulin (Desialylated complex-type N-glycans, high-mannose-type N-glycans)	Sigma	T1126 (Acid-treated)

Trivial name	Presentation	Glycans	Co.	Cat#
βGlcNAc	PAA	GlcNAc β1-PAA	Glycotech	01-009
[6S]βGlcNAc	PAA	(6OSO₃)GlcNAc β1-PAA	Glycotech	01-016
Agalacto-Fet	Glycoprotein	Agalacto fetuin (Agalactosylated complex-type N- and O-glycans)	Sigma	F3004 (Galactosidase-treated)
Agalacto-AGP	Glycoprotein	Agalacto α1-acid glycoprotein (Agalactosylated complex-type N-glycans)	Sigma	G9885 (Galactosidase-treated)
Agalacto-TF	Glycoprotein	Agalacto transferrin (Agalactosylated complex-type N-glycans, high-mannose-type N-glycans)	Sigma	T3309 (Galactosidase-treated)
OVM	Glycoprotein	Ovomucoid (Complex-type N-glycans)	Sigma	T2011
OVA	Glycoprotein	Ovalbumin (Hybrid-type N-glycans)	Sigma	A2512
αMan	PAA	Manα1-PAA	Glycotech	01-005
βMan	PAA	Manβ1-PAA	Glycotech	01-050
[6P]Man	PAA	(6OPO₄)Manα1-PAA	Glycotech	01-006
INV	Glycoprotein	Yeast invertase (High mannose-type N-glycans)	Sigma	I4504
Tn	PAA	GalNAcα1-PAA	Glycotech	01-010
Core1	PAA	Galβ1-3GalNAcα1-PAA	Glycotech	08-023
Core2	PAA	Galβ1-3(GlcNAcβ1-6)GalNAcα1-PAA	Glycotech	01-083
Core3	PAA	GlcNAcβ1-3GalNAcα1-PAA	Glycotech	01-071
Core4	PAA	GlcNAcβ1-3(GlcNAcβ1-6)GalNAcα1-PAA	Glycotech	01-089
Forssman	PAA	GalNAcα1-3GalNAcβ1-PAA	Glycotech	01-026
Core6	PAA	GlcNAcβ1-6GalNAcα1-PAA	Glycotech	01-072
Core8	PAA	Galα1-3GalNAcα1-PAA	Glycotech	01-028
[3'S]Core1	PAA	(3OSO₃)Galβ1-3GalNAcα1-PAA	Glycotech	08-069
Galβ-Core3	PAA	Galβ1-4GlcNAcβ1-3GalNAcα1-PAA	Glycotech	01-116
Asialo-BSM	Glycoprotein	Asialo bovine submaxillary mucin (Tn)	Sigma	M3895 (Acid-treated)
Asialo-GP	Glycoprotein	Asialo human glycophorin MN (T)	Sigma	A9791 (Acid-treated)
STn	PAA	Neu5Acα2-6GalNAcα1-PAA	Glycotech	01-059
STn (Gc)	PAA	Neu5Gcα2-6GalNAcα1-PAA	Glycotech	01-107
ST	PAA	Neu5Acα2-3Galβ1-3GalNAcα1-PAA	Glycotech	01-088
Sia α2-6Core 1	PAA	Galβ1-3(Neu5Acα2-6)GalNAcα1-PAA	Glycotech	01-113
BSM	Glycoprotein	Bovine submaxillary mucin (Sialyl Tn)	Sigma	M3895
GP	Glycoprotein	Human glycophorin (Disialyl T and sialyl Tn)	Sigma	G5017
αGal	PAA	Galα1-PAA	Glycotech	01-003
Galα1-2Gal	PAA	Galα1-2Galβ1-PAA	Glycotech	01-056
Galα1-3Gal	PAA	Galα1-3Galβ1-PAA	Glycotech	01-018
Galα1-3Lac	PAA	Galα1-3Galβ1-4Glcβ1-PAA	Glycotech	01-075
Galα1-3LN	PAA	Galα1-3Galβ1-4GlcNAcβ1-PAA	Glycotech	01-079
Galα1-4LN	PAA	Galα1-4Galβ1-4GlcNAcβ1-PAA	Glycotech	01-110
Melibiose	PAA	Galα1-6Glcβ1-PAA	Glycotech	01-063
αGlc	PAA	Glcα1-PAA	Glycotech	01-001
βGlc	PAA	Glcβ1-PAA	Glycotech	01-002
Maltose	PAA	Glcα1-4Glcβ1-PAA	Glycotech	01-054
HA	BSA	Hyaluronic acid-BSA	Seikagaku	400720
CSA	BSA	Chondroitin Sulfate A-BSA	Seikagaku	400655
CSB	BSA	Chondroitin Sulfate B-BSA	Seikagaku	400660
HS	BSA	Heparan Sulfate-BSA	Seikagaku	400700
HP	BSA	Heparin-BSA	Calbiochem	375095
KS	BSA	Keratan Sulfate-BSA	Seikagaku	400760
αRha	PAA	Rhamnoseα1-PAA	Glycotech	01-008
Mannan (SC)	Glycoprotein	S. cerevisiae mannan	Sigma	M7504
Mannan (CA)	Glycoprotein	C.albicans mannan	Takara	MG001
Zymosan	Glycoprotein	Zymosan	Sigma	Z4250
Chitobiose	PAA	GlcNAcβ1-4GlcNAcβ1-PAA	Glycotech	08-057
BSA	Glycoprotein	-	Sigma	A7638
Negative PAA	PAA	-	Glycotech	01-000

Takahara *et al*. Fig. S1.

Precise information of ligands on the glyco-microarray.

Inhibition of sSIGNR3 binding to
C. albicans **J-1012 glycan (%)**

Inhibitor/Glycan	0	20	40	60	80	100

C. albicans **J-1012**

C. albicans **NIH B-792**

S. cerevisiae **(WT)**

S. cerevisiae (*mnn1/mnn4*)

S. cerevisiae (*mnn2*)

C. stellatoidea

C. parapsilosis

C. lusitaniae

C. albicans **J-1012**
(α−mannosidase-treated)

Glycogen
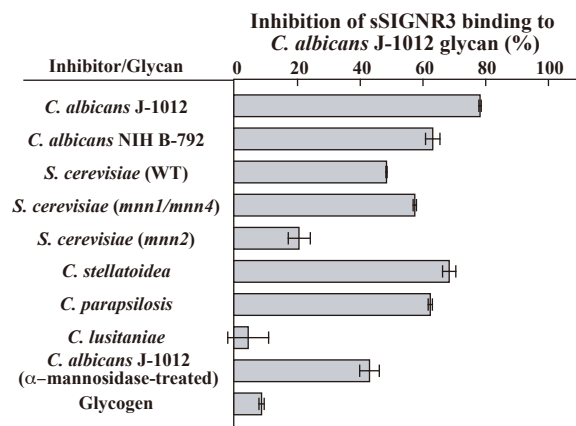
Takahara *et al.* Fig. S2.

Inhibition analysis by lectin ELISA. Binding of sSIGNR3 to *C. albicans* J-1012 glycan coated on microtiter plates was analyzed as in Fig. 2A.