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Supplementary Information for

Glycosylation-dependent galectin-receptor interactions promote *Chlamydia trachomatis* infection

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Other supplementary materials for this manuscript include the following:

Video S1

SI Methods

Cell culture, antibodies, recombinant galectins and reagents

Primary antibodies used were: anti-β-actin (Abcam), anti-MOMP and anti-OmcB generously provided by Ted Hackstadt (National Institutes of Health, USA) and rabbit anti-Gal1 polyclonal IgG (1.5 μg/mL) generated as described (56). Neutralizing antibodies used were: anti-FGFR2 (R&D Systems), anti-PDGFRβ (R&D Systems), anti-β_1 integrin (R&D Systems) and anti-$\alpha_V\beta_3$ integrins (R&D Systems). Secondary antibodies used were: goat anti-rabbit Cy3-labeled IgG, goat anti-rabbit Alexa488-labeled IgG, goat anti-rabbit Alexa647-coupled IgG, goat anti-mouse HRP-conjugated IgG and goat anti-rabbit HRP-conjugated IgG (Jackson Immunoresearch Laboratories). Propidium Iodide (PI) and DAPI were purchased from Molecular Probes (Life Technologies). Mowiol 4-88 reagent was from Calbiochem (Merck).

Bacterial strains

For bacterial propagation, HeLa cells were infected at a multiplicity of infection (MOI) of 20 and incubated for 48 h. Then, infected cells were lysed and EBs were purified on a density gradient as previously described (1). The purified EBs were suspended in 0.2 M sucrose, L-glutamine and phosphate buffer (SPG) (pH=7.2) and titrated by quantification of inclusion forming units (IFUs) using confocal microscopy and flow cytometry.

Glycophenotype analysis

HeLa cells were incubated with biotinylated lectins including Con A (5 μg/mL), L-PHA (2 μg/mL), E-PHA (2 μg/mL), LEL (1 μg/mL), SNA (5 μg/mL), MAA (10 μg/mL), PNA (10 μg/mL), HPA (10 μg/mL), Gal1 (10 μg/mL), ECL (5 μg/mL), AAL (10 μg/mL) and UEA (10 μg/mL) (all purchased from Vector Labs). Phycoerythrin (PE)-coupled streptavidin was purchased from Sigma.

Gal1 binding assays

Chlamydial EBs ($1x10^6$) were incubated with 0.3 μM recombinant Gal1 for 30 min at 4ºC (step 1). Then, bacteria were centrifuged for 45 min at 35,000 rpm at 4ºC (step 2). Supernatants were discarded and pellets were washed with 100 mM lactose at 4ºC (step 3) and centrifuged as described above (step 4). Eluate was separated and new EB pellets (EB1) were further washed with lactose (step 5) and centrifuged (step 6) to generate second pellets (EB2). The experimental procedure is depicted in Fig. S1.

Transmission electron microscopy

After fixation, 4% low melting agarose was added to detach cells which were immediately centrifuged at 3,200 rpm for 10 min at 30°C. Cell pellets were kept at 4°C for 3 h for agarose solidification. Each sample was post-fixed in 1% OsO_4 for 1 h at room temperature, dehydrated in a graded acetone series, and embedded in low-viscosity epoxy resin (Pelco International). Polymerization was performed for 48 h at 70ºC. Ultrathin sections with interference color gray were cut by an ultramicrotome (Ultracut R Leica), mounted on grids, and stained with uranyl acetate and Reynold's lead citrate (Ted Pella Inc).

Inclusion forming unit assay

Plates were centrifuged for 15 min at 4ºC at 1,500 rpm (binding step), then cells were washed three times with 500 µL cold PBS, and finally incubated for 48 h in 5% CO_2 at 37ºC. At the end of the chlamydial developmental cycle (48 h pi), cells were collected and lysed in SPG medium. Cell lysates were centrifuged for 10 min at 500 x g to remove debris and progressive dilutions containing harvested EBs were inoculated onto new HeLa cells seeded on 24-well plates to titrate bacterial progeny. After 24 h, the number of inclusions generated by chlamydial progenies was assessed by flow cytometry or by confocal microscopy as described (2).

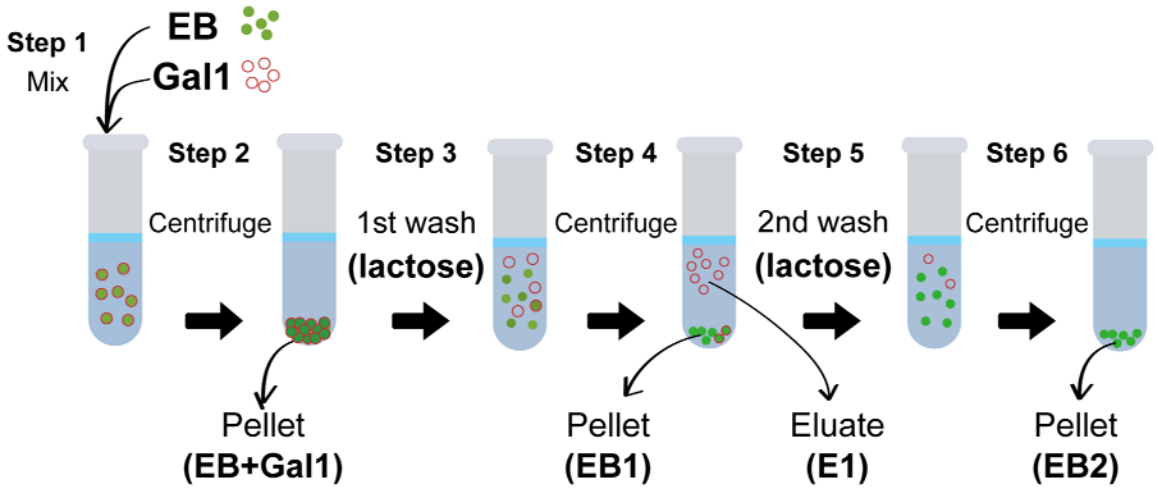

Fig. S1. Experimental design: Analysis of carbohydrate-dependent binding of Gal1 to EBs. Chlamydial EBs ($1x10^6$) were incubated with 0.3 µM recombinant Gal1 for 30 min at 4ºC (Step 1). Then, bacteria were centrifuged for 45 min at 35,000 rpm at 4ºC (Step 2). Supernatants were discarded and pellets (EBs) were washed with 100 mM lactose at 4ºC (Step 3) and centrifuged as described above (Step 4). Eluate (E1) was separated and new EB pellets (EB1) were further washed with lactose (Step 5) and centrifuged (Step 6) to generate new pellets (EB2). All bacterial pellets (EB, EB1 and EB2) and eluate (E1) were resolved on a 15% SDS-PAGE, transferred onto 0.45 µm nitrocellulose membranes (GE Healthcare) and incubated overnight with anti-Gal1 (1:1000) or anti-MOMP (1:500) antibodies followed by goat anti-rabbit HRP-conjugated IgG (1:5000, 1 h, 37°C). Protein bands were visualized using Pierce™ ECL Western blotting substrate in an ImageQuant LAS 4000.

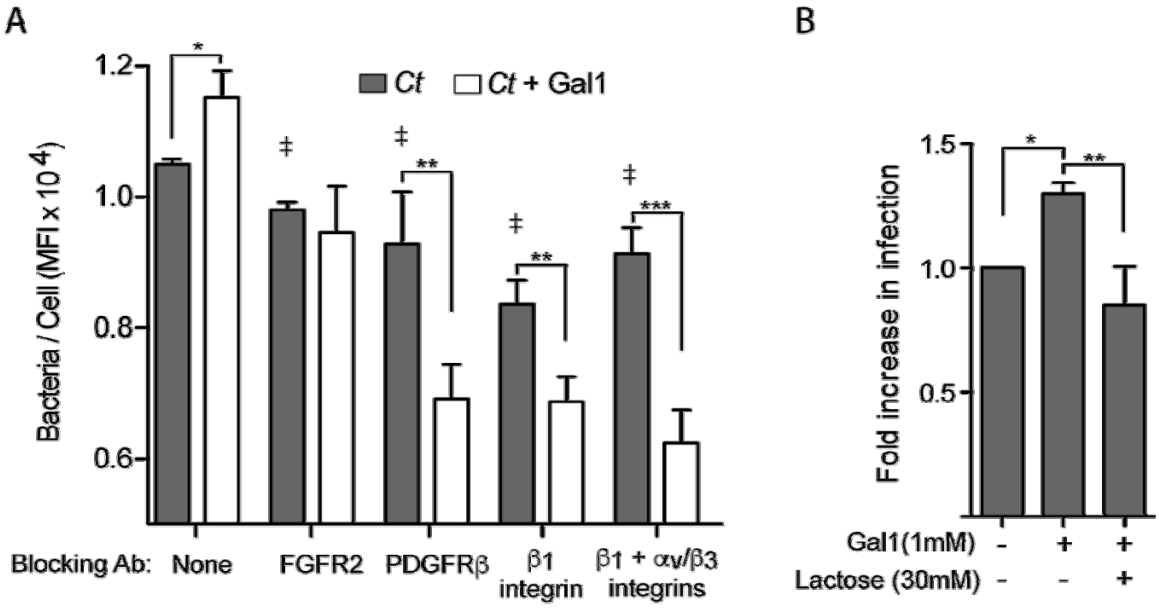

Fig. S2. (**A**) Contribution of FGFR2, PDGFRβ, β_1 integrin and $\alpha_v\beta_3$ integrins to *Ct* infection. HeLa cells were infected with *Ct* (MOI 3) in the absence (gray bar) or presence (white bar) of Gal1. Quantification of bacteria per cell measured by MFI at 24 h pi in *Ct*-infected HeLa cells incubated with or without 1 µM recombinant Gal1 in the absence (none) or presence of different neutralizing monoclonal antibodies. (**B**) Effect of lactose pre-treatment on Gal1-induced *Ct* binding to HeLa cells. (**A, B**) Data are the mean ± SEM of three independent experiments (*p<0.05, **p<0.01, ***p<0.001) (‡ p<0.01 vs. *Ct* none).

Table S1. Names, abbreviations and specificities of lectins used for glycophenotyping bacterial and host cell glycoproteins.

	Abbreviation	Specificity
Concanavalin A	Con A	Oligomannose type N-glycans
Erythrina cristagalli	ECL	Non sialylated LacNAc structures; Gal, GalNAc
Peanut Agglutinin	PNA	Galβ1-3GalNAc (asialo core 1 O-glycans)
Maackia amurensis	MAA	α2-3-sialylated Gal
Sambucus nigra	SNA	High affinity for α2-6 sialic acid and low affinity for α2-3 sialic acid; Gal
Ulex europaeus	UEA	Fucα1-2Gal
Aleuria aurantia	AAL	Fuc α1-3/ Fuc α1-6-GlcNAc ; Fuc α1-2 Gal
Helix pomatia	HPA	GalNAc
Lycopersicon esculentum	LEL	$(Gal\ \beta1\text{-}4GlcNAc)_3$
L-phytohemagglutinin (L-Phaseolus vulgaris)	L-PHA	β 1-6 branched complex N-glycans Gal(β1-4)GlcNAc(β1-2); [Gal(β1-4)GlcNAc(β1-6)]Man
E-phytohemagglutinin (E-Phaseolus vulgaris)	E-PHA	Di-galactosylated and bisected N-glycans

Table S2. Lectin reactivity with *Chlamydia trachomatis* infectious form. Lectin binding is indicated by (+) and the absence of binding by (-). Molecular weight (MW) of bacterial glycoproteins is indicated at the left side.

Lectin:	Con A	L-PHA	ECL	SNA	HPA	PNA	Gal1
MW(kDa)							
170	+	-	-	-	-	-	-
130	+	-	-	-	-	-	-
	+	-	-	-	-	-	+
100	+	-	-	-	-	-	-
70	-	-	-	+	-	-	-
	-	-	-	+	-	-	-
55	+	+	+	+	+	-	+
	+	-	+	-	-	-	+
40	+	+	+	+	+	-	+
	-	-	-	-	-	-	+
35	-	-	-	-	-	-	-
	-	-	-	-	-	-	+
25	-	-	-	+	-	-	-
15	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-

Table S3. Glycosylated receptors involved in *Chlamydia trachomatis* recognition and entry.

Gene	Protein	N-linked (GlcNAc) asparagine position	Neutralizing antibodies
ITGB1	**Integrin β_1**	50, 94, 97, 212, 269, 363, 403, 406, 411, 417, 481, 520, 584, 669.	Human integrin β_1/CD29 antibody. Monoclonal IgG_1 Clone#P5D2. Catalog number: MAB17781(R&D)
ITGB3	**Integrin β_3**	125, 346, 397, 478, 585, 680.	Human integrin $\alpha_V\beta_3$ antibody. Monoclonal IgG_1 Clone#23C6. Catalog number: MAB3050 (R&D)
ITGAV	**Integrin α_V**	74, 290, 296, 488, 554, 615, 704, 835, 851, 874, 945, 973, 980.	
ITGA5	**Integrin α_5**	84, 182, 297, 307, 316, 525, 530, 593, 609, 675, 712, 724, 771, 773, 868.	Human integrin α_5/CD49e antibody. Polyclonal IgG. Catalog number: AF1864 (R&D)
FGFR2	**Fibroblast growth factor receptor 2**	83, 123, 228, 241, 265, 297, 318, 331.	Human FGFR2 antibody. Monoclonal IgG_1 Clone#98739. Catalog number: MAB6843 (R&D).
PDGFRB	**Platelet-derived growth factor receptor-β**	45, 89, 103, 215, 230, 292, 307, 354, 371, 468, 479.	Human PDGFRβ antibody. Polyclonal goat IgG. Catalog number: AF385 (R&D)

Video S1. Confocal *z* planes of a HeLa cell infected with *Ct* for 18 h (MOI 1). Gal1 was detected using a rabbit anti-Gal1 antibody followed by a goat anti-rabbit Alexa488-labeled IgG (green). DNA was stained with DAPI (blue).

SI References

1. Scidmore MA (2005) Cultivation and laboratory maintenance of *Chlamydia trachomatis*. *Curr Prot Microbiol* (John Wiley & Sons, Inc., Hoboken, NJ, USA), Chapter 11:Unit 11A.1.

2. Capmany A, Damiani MT (2010) *Chlamydia trachomatis* intercepts Golgi-derived sphingolipids through a Rab14-mediated transport required for bacterial development and replication. *PLos ONE* 5(11):e14084