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Human Macrophage Galactose-Type Lectin (MGL) Recognizes the Outer Core of *Escherichia coli* Lipooligosaccharide

Meriem Maalej,^[a, b] Rosa Ester Forgione,^[a] Roberta Marchetti,^{*[a]} François Bulteau,^[b] Michel Thépaut,^[b] Rosa Lanzetta,^[a] Cedric Laguri,^[b] Jean-Pierre Simorre,^[b] Franck Fieschi,^[b] Antonio Molinaro,^[a] and Alba Silipo^[a]

Carbohydrate–lectin interactions intervene in and mediate most biological processes, including a crucial modulation of immune responses to pathogens. Despite growing interest in investigating the association between host receptor lectins and exogenous glycan ligands, the molecular mechanisms underlying bacterial recognition by human lectins are still not fully understood. Herein, a novel molecular interaction between the human macrophage galactose-type lectin (MGL) and the lipooligosaccharide (LOS) of *Escherichia coli* strain R1 is described. Saturation transfer difference NMR spectroscopy analysis, supported by computational studies, demonstrated that MGL bound to the purified deacylated LOS_{R1} mainly through recognition of its outer core and established crucial interactions with the terminal Gal α (1,2)Gal epitope. These results assess the ability of MGL to recognise glycan moieties exposed on Gram-negative bacterial surfaces.

Bacterial cell surfaces are decorated with highly diverse glycoconjugates, in the form of capsular polysaccharides, peptidoglycans, lipopolysaccharides (LPSs) and other glycolipids,^[1] which perform several functions, ranging from structural to protective roles.^[2] Bacterial glycans take part in many key biological events, including pathogen recognition, receptor activation, cell adhesion and signal transduction. Additionally, these structures often serve as molecular patterns that are recognised by specific glycan-binding receptors of the host immune system; thus triggering a pathogen-specific immune response.

It is well known that LPSs, the major constituents of the outer membrane of Gram-negative bacteria,^[3] are one of the

main virulence factors of several feared bacterial strains, including enteropathogenic *Escherichia coli*, which is implicated in severe foodborne and urinary tract infections.^[4] From a structural point of view, LPS is composed of three structural motifs that can be distinguished because they are encoded by different gene clusters. Lipid A, which represents the glycolipid portion, is an acylated bis-phosphorylated glucosamine disaccharide that anchors the LPS to the outer membrane. Lipid A is covalently linked to a core oligosaccharide (OS) that can be further divided into two different portions: the more conserved inner region, which is characterised by the presence of peculiar sugar residues, such as 3-deoxy-D-manno-oct-2-ulopyranosonic acid (KDO), and the more variable outer core. Finally, the O-antigen, which is a polysaccharide composed of several OS repeating units, extends to the extracellular medium and acts as a hydrophilic coating surface.^[3,5] However, Gram-negative bacteria can also produce rough-type LPS, namely, lipooligosaccharide (LOS); a truncated version of LPS that lacks the O-antigen.

To date, the receptor complex formed by the toll-like receptor 4 and the small secreted MD2 protein is among the main species involved in bacterial LPS recognition by host immune cells.^[6] More recently, it has been shown that LPSs are also intracellularly detected by caspase.^[7] In both cases, the glycolipid part seems to be heavily involved in binding and in the follow-up of signalling.

Given their ability to selectively recognise carbohydrate structures, human lectins have emerged as potential LPS receptors specifically for their carbohydrate moiety.^[8] Lectins are ubiquitous oligomeric proteins that have multiple roles in cell–cell communication, cellular trafficking and regulation of immune cell functions; therefore, representing validated therapeutic targets.^[9,10] Within the many different families of lectins described in humans, to date, the C-type lectin (CTL) class is the biggest and most diverse, including both transmembrane and soluble receptors. Host immune cells, such as dendritic cells and macrophages, are known to express a wide range of CTLs that contain at least one carbohydrate recognition domain (CRD), which is responsible, in most cases, for the recognition of glycan structures in a Ca²⁺-dependent manner. In the last decade, the use of glycan arrays has provided valuable details on the ligand specificity of different human CTLs; thus highlighting their ability to recognise carbohydrate epitopes belonging to both mammalian and microbial glycans.^[11] However, although it is acknowledged that the recognition of

[a] M. Maalej,⁺ R. E. Forgione,⁺ Dr. R. Marchetti, Prof. R. Lanzetta, Prof. A. Molinaro, Prof. A. Silipo
Department of Chemical Sciences, University of Naples Federico II
via Cintia 4, ■■■post code?■■■Napoli (Italy)
E-mail: roberta.marchetti@unina.it

[b] M. Maalej,⁺ F. Bulteau, Dr. M. Thépaut, Dr. C. Laguri, Prof. J.-P. Simorre, Prof. F. Fieschi
CNRS, CEA, Institut de Biologie Structurale
Université Grenoble Alpes
■■■street?■■■, 38000 Grenoble (France)

[†] These authors contributed equally to this work.

Supporting information and the ORCID identification numbers for the authors of this article can be found under <https://doi.org/10.1002/cbic.201900087>: experimental procedures and additional data, including detailed NMR spectroscopy analysis of the core oligosaccharide structure.

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1 pathogen-associated glycostructures by human CTLs represents a key point of host immune defence,^[9,12,13] more insights into the molecular basis of the binding mechanisms between these pattern recognition receptors and their bacterial ligands, including the LPS, are still needed.

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6 Within this framework, we have set up a proof of concept study and investigated the ability of the macrophage galactose-type lectin (MGL; also referred to as CLEC10 A or CD301),^[14] to recognise the carbohydrate moiety of bacterial LPS. In contrast to several other CTLs, including dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and the macrophage mannose (MR) receptors, which exhibit a preference for mannose-type sugars, human MGL is characterised by an exquisite specificity for terminal GalNAc/Gal residues; thus making it potentially an attractive target for the galactose-containing LOS structure of *E. coli* R1.^[15]

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18 Core OS recognition by MGL has been investigated by means of saturation transfer difference (STD) NMR spectroscopy analysis combined with computational studies.

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Herein, we used the sequence of isoform 2 of human MGL, which is shorter than that of MGL isoform-1 (also called DC-ASGPR), for cloning and recombinant production. Initially, two groups cloned these two MGL isoforms, firstly from macrophages (this CTL was therefore named macrophage galactose lectin) and then from DCs, leading to the name DC-ASGPR due to similarity to liver ASGPR with regard to selectivity.^[14,16] DC-ASGPR is a longer variant of MGL that results from alternative splicing with an insertion of 27 amino acids and a deletion of 3 amino acids in the neck domain. Both MGL and DC-ASGPR possess identical CRDs, which suggests that there is no difference in their carbohydrate recognition specificities, mainly characterised on MGL up to now. The MGL extracellular domain (MGL-ECD; Figure 1 A, B) has been cloned and overexpressed at high levels in *E. coli* as inclusion bodies (Figure 1 C). Inclusion bodies were isolated and refolded as described herein. Folded functional MGLs have an oligomeric state that allows tight binding, through an avidity-based mechanism, on a GalNAc-agarose column.^[17] Elution is performed thanks to the use of ethylenediaminetetraacetic acid (EDTA); thus dem-

Roberta Marchetti studied chemistry at the University of Naples "Federico II". In 2013, she received her PhD on NMR studies of molecular recognition processes. Since 2016, she has been a researcher at the Department of Chemical Sciences, University of Naples "Federico II". She is specialised in organic chemistry, with a special focus on the isolation, purification and characterisation of bacterial cell wall glycoconjugates. Her research interests are mainly focused on the comprehension of glycoconjugate-protein interactions through the application of NMR spectroscopy methods.

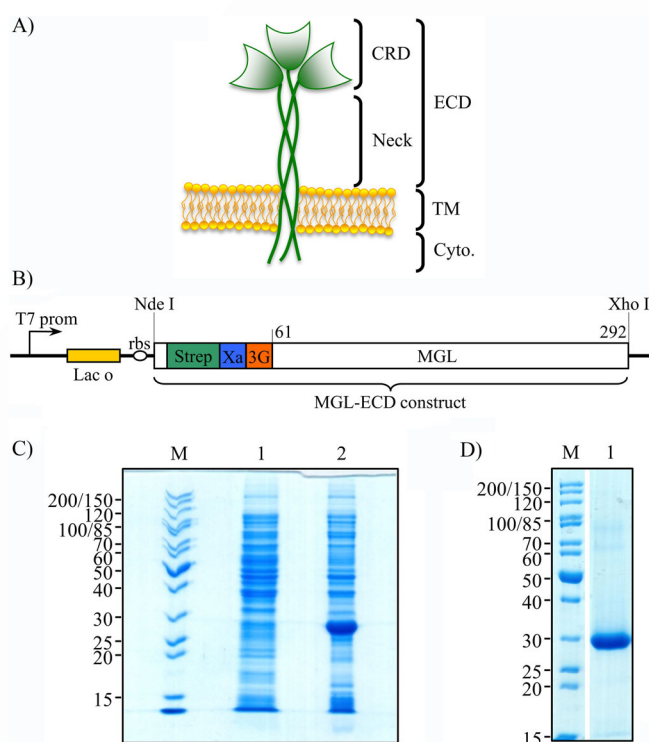


Figure 1. Cloning and purification of MGL-ECD. A) Domain organisation of MGL. TM: transmembrane domain. Cyto.: cytoplasmic domain. B) Construct for the overproduction of MGL-ECD. Strep: StrepTag II. Xa: factor Xa protease cleavage site. 3G: tri-glycine linker. C) SDS-PAGE analysis of MGL-ECD overexpression. Lane M: PageRuler unstained protein ladder (Fermentas); lane 1: total proteins before induction; lane 2: total proteins after induction. A band at about 28 kDa corresponding to MGL-ECD MW is overexpressed. D) SDS-PAGE analysis of purified MGL-ECD. Lane 1: A unique band at about 28 kDa corresponding to pure MGL-ECD is observed.

onstrating that binding is Ca^{2+} dependent. This first affinity purification step ensures the correct sorting of functionally folded MGL-ECDs, which are reloaded with Ca^{2+} in a second purification step of size-exclusion chromatography. MGL-ECD oligomers, reported as trimers,^[17] are then pure and ready to use for biophysical studies (Figure 1 D).

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Firstly, the LOS of *E. coli* R1 (OS_{R1}) was extracted, purified and de-acylated to derive a soluble OS, which was, in turn, further characterised by means of NMR spectroscopy (Figure 2 A,B and see the Supporting Information for more details). Once the NMR resonances were assigned, the ability of the MGL-ECD to recognise and bind the core OS was assessed by means of STD NMR; a method that allows the measurement of the transfer of saturation from the receptor to the ligand, and thus, provides a reliable mapping of the ligand epitope in the bound state.^[18,19]

The observation of STD enhancements in the STD spectrum of the mixture of MGL-ECD/ OS_{R1} clearly revealed the protein-ligand interaction (Figure 2 B,C). The great overlap between the NMR resonances of sugar protons hampered an extensive and quantitative study of the STD effects. However, analysis of signals resonating in isolated regions of the spectrum, together with the strong differences in multiplicity and relative intensities of STD signals with respect to the corresponding refer-

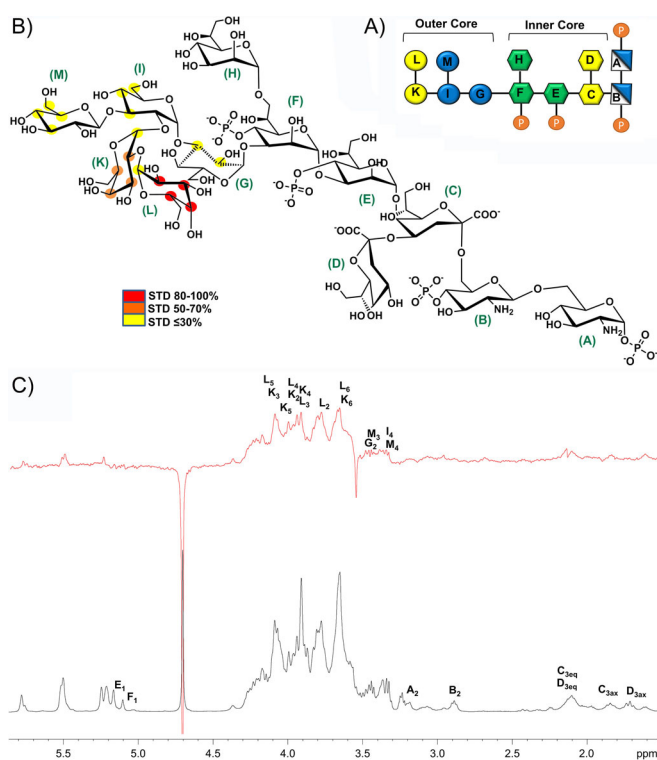


Figure 2. STD NMR spectroscopy analysis of the MGL-OS_{R1} mixture. A) Schematic structure of OS_{R1} derived from the LOS of *E. coli* R1. Symbol nomenclature for glycans has been used.^[22] Phosphate groups are depicted as white letter P in orange circles. B) STD-derived epitope mapping of the MGL:OS_{R1} interaction, with colour coding from the highest (red) to lowest (yellow) observed STD effects. C) ¹H NMR reference spectrum (bottom) and 1D STD NMR spectrum (up) of the 1:100 mixture of MGL-OS_{R1}. Some key proton resonances are labelled. STD NMR spectroscopy analysis indicated that the inner core pointed farther from the surface of the MGL protein, and was mainly involved in the interaction with the terminal region of the OS.

ence, suggested that the outer core was the moiety more engaged in binding with the lectin. Notably, several STD signals belonged to the outer core region. In detail, the strongest STD effects derived from the terminal galactose units (L and K) and, in particular, protons H-2–H-5 of residue L (see the discussion on the computational analysis below). This observation was consistent with the previously reported ability of the human MGL to accommodate not only the *N*-acetylgalactosamine, but also the galactose moiety, in its binding site.^[20] Saturation transfer to the glucose residues, I, G and M, adjacent to the terminal galactose disaccharide, was also detected, although to a lesser extent; thus suggesting lesser involvement in the interaction with MGL. On the contrary, the residues of the inner core region do not seem to be involved in the binding interface, as suggested, for instance, by the absence of STD effects for protons at position 3 of KDO moieties and those of protons at position 2 of the two glucosamine units of lipid A. To further demonstrate the pivotal role of the terminal galactose moiety in the recognition and interaction process, we investigated the binding of MGL with the deacylated core OS from *E. coli* R3, the structure of which contains the same inner core as that of OS_{R1}, but differs in the composition of the outer core by the presence of glucose instead of galactose residues

in the terminal region (Figure S4 A). In particular, OS_{R3} is composed of an α -Glc-(1-2)- α -Glc-(1-2)-[α -GlcNAc-(1-3)]- α -Gal-(1-3)- α -Glc branched pentasaccharide connected to the inner core. Interestingly, the lack of terminal galactose units totally destroyed the binding with MGL, which resulted in the absence of STD NMR signals (Figure S4 B).

The interaction of MGL-ECD with LOS_{R1} was further characterised by using molecular modelling and computational studies to gain a 3D perspective of the molecular recognition mechanism. Because the crystal structure of the protein has not yet been elucidated, a homology model of the MGL CRD (Cys157–Leu284) was built by using the protein structure characterised by the highest sequence identity (74%), namely, the CRD of the mammalian asialoglycoprotein receptor (PDB ID: 1DV8; see also Figure S4) as a template.^[21] Then, docking calculations of OS_{R1} within the modelled structure were performed.

As a first step toward understanding the binding mode of the MGL in the interaction with the bacterial OS, the terminal disaccharide Gal α (1,2)Gal was docked into the primary binding site of the MGL; thus allowing us to confirm the binding specificity of the protein for the galactose. In accordance with data previously reported by Marcelo et al. on the interaction of MGL with a single galactose unit,^[22] **■ ■ [22] not by Marcelo, change to [22]? ■ ■** our results revealed that the terminal galactose residue was the major determinant of the binding process. Indeed, although the second galactose moiety was predicted to be close to the binding site of the protein, it seemed to play a secondary role in the interaction with the MGL (see the Supporting Information).

Considering that, from the results of the STD NMR spectroscopy analysis, the outer core of LOS was the moiety in closest proximity to the MGL binding surface, the galactose-containing branched pentasaccharide, which composed the terminal part of the LOS, was then used for docking calculations (Figure 3). The resulting 3D model of the complex revealed that the terminal Gal moiety was located inside the Ca²⁺-containing sugar binding site of the MGL CRD. Crucial contacts between OH-3 and OH-4 of the galactoside ring and the Ca²⁺ ion were indeed observed and confirmed the specificity of the receptor toward galactoside residues (Figure 2). From the analysis of the receptor/ligand complex, relevant polar interactions were found between the terminal Gal OH-3 and OH-4 and the side chains of Asp245, Asn268, Asp269 and Glu256 residues. In addition, hydrogen-bond interactions with the carboxylate group of Glu242 were observed, namely, with OH-2 of the glucose residue (G) and OH-6 of the branched Glc moiety (M; Figure 2 A). Interestingly, in contrast with the results obtained on the disaccharide Gal α (1,2)Gal (see the Supporting Information), only one possible binding mode has been predicted for the pentasaccharide docked in the protein binding pocket (Figure S7 B,C). The selection of this binding mode, in which the polar contacts were markedly reinforced by stacking interactions between the aromatic system of the conserved Trp247 and protons H-1 and H-2 of the galactose moiety, could be ascribable to steric hindrance of the pentasaccharide branched structure, which permitted only one orientation of the galactose residue (Figure S7 B,C).

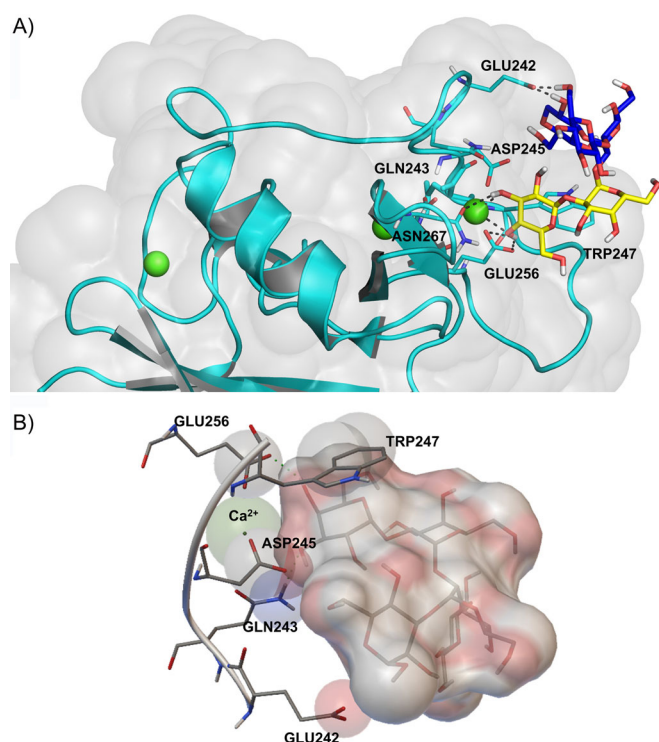


Figure 3. A 3D model of the MGL–pentasaccharide complex. A) Pentasaccharide binding at the MGL binding site. The main amino acid residues involved in binding are labelled. Galactose and glucose residues are depicted in yellow and blue, respectively. B) The binding pocket of MGL in the presence of the pentasaccharide in the bound form, as derived from the AutoDock program. Only the amino acid residues involved in the binding process and the Ca^{2+} ion are depicted. Green dotted lines represent intermolecular hydrogen bonds.

The described MGL/pentasaccharide complex was used as a starting point to manually dock the whole OS_{R1} structure onto the CRD of MGL (Figure 4). The resulting 3D complex clearly indicated that the inner core region of the LOS was not implicated in the interaction with MGL because it pointed far away from the binding site of the receptor model.

Overall, these observations were in good accordance with the previously discussed STD data; thus further suggesting that significant interactions were mainly established between the protein and sugar residues of the outer core region, whereas the inner core did not seem to play a key role in the interaction with the protein.

In conclusion, we have demonstrated that human CTLs were potential binders of bacterial lipopolysaccharide in solution and that, as expected, they bound the LPS carbohydrate moiety. In particular, herein we have provided evidence that the human MGL was able to detect not only tumour-related carbohydrate structures present in mucin-like glycopeptides, but also glycan structures exposed on the bacterial surface. Whereas a wide range of infectious microorganisms were identified as pathogenic ligands of CTLs, such as DC-SIGN and MR,^[24,25] glycan microarray profiling on MGL revealed its interaction only with filoviruses and the helminth parasite *Schistosoma mansoni*.^[26] Moreover, only a few papers have been pub-

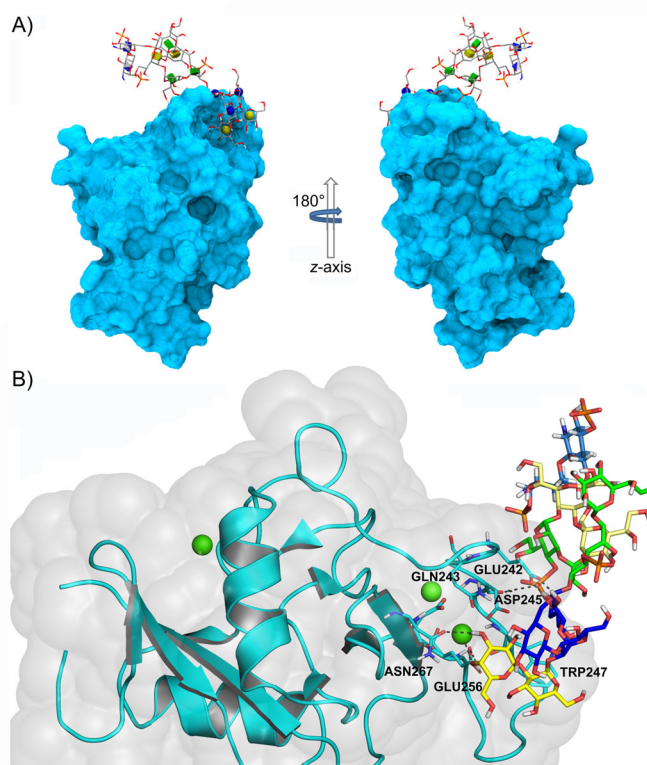


Figure 4. A 3D model of the MGL– OS_{R1} complex. A) Binding pose of the OS from *E. coli* R1, OS_{R1} , docked into the binding pocket of the MGL starting from the 3D model of the MGL–pentasaccharide complex and based on NMR spectroscopy data. The 3D symbol nomenclature for glycans has been used.^[23] B) Specific interactions between binding-site residues and the OS are depicted. Sugar residues are coloured according to the symbol nomenclature for glycans. Yellow dotted lines represent polar contacts.

lished, so far, attempting to prove the potential role of the MGL in detecting LPS structures derived from *Bordetella pertussis* and *Campylobacter jejunii*.^[27,28] Indeed, we provided molecular insights into the structure of the MGL–LOS complex. We proved, by means of STD NMR spectroscopy, that the MGL was able to recognise and bind the sugar part of the LOS of *E. coli* R1, the major glycoform of which exhibited a terminal Gal- $\alpha(1,2)$ Gal epitope. Further details of the binding mode of MGL to LOS were obtained by homology modelling and docking calculations. Our results improve, at a molecular level, knowledge of the interaction between human CTLs and bacterial glycans, and may help to better understand the role of MGL in shaping immunity upon bacterial recognition. MGL has already been reported as a good candidate receptor for DC-based cancer immunotherapy, given its well-known ability to endocytose specific tumour-related antigens.^[29] The discovery of the ability of MGL to detect an endotoxin component, LOS, of the outer membrane of the Gram-negative bacterium *E. coli* might offer great potential for the future development of therapeutics for bacterial disease intervention. Further studies will be necessary to investigate if and how structural variations between different LOSs could affect recognition and binding process; thus tailoring the outcome of the host–pathogen inter-

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: carbohydrates · lipopolysaccharides · molecular recognition · NMR spectroscopy · proteins

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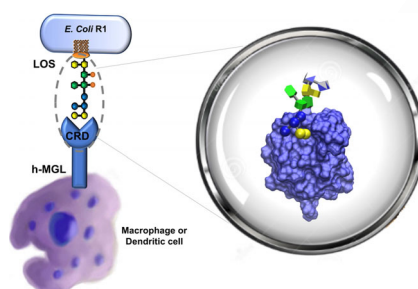
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M. Maalej, R. E. Forgione, R. Marchetti,*
F. Bulteau, M. Thépaut, R. Lanzetta,
C. Laguri, J.-P. Simorre, F. Fieschi,
A. Molinaro, A. Silipo



Human Macrophage Galactose-Type Lectin (MGL) Recognizes the Outer Core of *Escherichia coli* Lipooligosaccharide



Distinguishing features: Carbohydrate-lectin interactions intervene in and mediate most biological processes. From results of saturation transfer difference NMR spectroscopy and computational studies, the ability of human macrophage galactose-type lectin (h-MGL) to recognise glycan moieties exposed on the lipooligosaccharide (LOS) of *E. coli* strain R1 is revealed.



Investigation into human macrophage galactose-type lectin recognition of glycan moieties on exposed lipooligosaccharides by STD NMR spectroscopy (Marchetti @uninaIT) #ChemBioTalents **SPACE RESERVED FOR IMAGE AND LINK**

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Meriem Maalej
Rosa Ester Forgione
Roberta Marchetti <http://orcid.org/0000-0002-7173-7099>
François Bulteau
Michel Thépaut
Rosa Lanzetta
Cedric Laguri
Jean-Pierre Simorre
Franck Fieschi
Antonio Molinaro
Alba Silipo