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Predicting receptor for mannose-binding lectin on neutrophil surface

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ABSTRACT

Neutrophils play as major phagocytes that participate in the various effector phase of immunity. Mannose-binding lectin (MBL) assisted priming of neutrophils could trigger various processes including modulation of endocytosis rate, reactive oxygen production, chemotaxis, etc., through interactions with cell surface receptors. The physiological receptor for MBL on neutrophil's surface is still unreported. Macromolecular docking could be attempted to determine the protein-protein interactions which are important for understanding cellular function and organization. The study was performed to identify the interacting partner of MBL present on neutrophils surface which leads to the activation of various cell processes. Protein network analysis, homology modeling, and Rigid docking were performed to explore structural features and binding mechanism of MBL with its cellular receptors. The results indicates that CR1 interact with the MBL and may act as MBL receptor.

1. INTRODUCTION

Proteins are building blocks and molecular devices for the execution of biological functions. Function of all the proteins depends on their three-dimensional structure and could be affected by various physical and biochemical factors. The three-dimensional structure of proteins is a key component to understand their function and mechanism at the molecular level. The protein-protein interaction could elucidate a better understanding of processes including immune responses, metabolic control, signal transduction, and gene regulation [1].

The activity of neutrophils depends on the effective recognition and intracellular signal transduction pathways to remove the pathogens. The cells have innate immune receptors including Toll-like receptors (TLRs) and C-type lectins and activation of these receptors leads to complex cellular activation and processes like phagocytosis, release of neutrophil extracellular traps, chemotactic migration, and cytokine release [2]. Neutrophils play a major role in innate immunity and they also participate in the effector phase of adaptive immunity and mannose-binding lectin (MBL) assisted priming could assist or trigger the functions [3–7]. MBL function is mediated by cell surface receptors and information on their interaction may be utilized for therapeutic purposes in diseases with a neutrophil-mediated pathogenic component. In view of the above, in the present section, a study was performed using *in-silico* approach to validate and identify the receptor interacting with MBL on neutrophil's surface.

2. METHODS

2.1. Network Analysis & Homology Modeling

MBL interacting protein was mapped by searching the Search Tool for the Retrieval of Interacting Genes (STRING) database [8,9] version 10.5 at a confidence level of 0.15–0.5. The protein association network was represented with proteins as nodes, connected by lines. MBL amino acid sequence of *Capra hircus* (goat) was retrieved from the NCBI database (online resource: Table 1). Templates were identified by Position-Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST) Hits (against available structure). Each identified highest quality structure has then been selected as a template for model building. The protein models were built by "Homology modeling" approach

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Table 1: Affinity energy (KJ/mol) and number of complexes generated at RMSD 1Å for the receptor–ligand complex. *The proteins exhibiting MBL binding through trans-membrane region could not be considered as receptors since the trans-membrane site would be buried in the lipid bilayer and would be unavailable for binding. CR1 binds with the ligand with the highest affinity among receptors, thus considered as a receptor for MBL.

Candidate receptor	Affinity energy (KJ/mol)	No. of complexes	Binding at transmembrane region*
C3AR1	−1,140	31	Yes
C3a-anaphylaxis receptor	−1,102	31	Yes
C5AR1	−1,051	35	Yes
F2R or PAR1	−1,000	19	Yes
PAR4	−974	24	Yes
PAR3	−961	28	Yes
Epidermal growth factor-transmembrane (EGF-TM7) or CD97	−957	27	Yes
CR1	−795	52	No
IGTAX	−729	33	Yes
CR2 or CD31	−711	44	No
ITGAM or CR3	−701	29	Yes
CD40 M	−670	28	No
IgGFcRN	−669	89	No
Lymphotoxin-β	−662	102	Yes
TNFRSF27 or CD27	−661	22	No
ICAM1	−643	29	No
TLR2	−615	25	No
PRTPC	−600	45	No
TNF13	−588	61	Yes
CD40 ligand (monomer)	−587	68	No

and SWISS-Model [10,11] and Robetta [12] were used. Homo-oligomeric structure of the MBL is predicted through the galaxy server in the case of the Robetta model. Models were validated on PDBSum [13], RAMPAGE [14], and ProSA [15] servers for various parameters.

2.2. Rigid Docking Studies

ClusPro 2.0, a fully automated web server for the computational docking of protein structures was used to study the receptor–ligand interactions [16]. The coordinate files of ligand (MBL) and receptors in Protein Data Bank (PDB) format were uploaded through the web interface. Within this server are enclosed three computational steps that include: rigid body docking using the fast Fourier transform (FFT) correlation approach, root mean square deviation (RMSD) based clustering of the structures generated to find the largest cluster that will represent the likely models of the complex, and refinement of selected structures [16]. By default server settings, ClusPro 2.0 simultaneously generates four types of models using the scoring algorithms called designated as balanced, electrostatic-favored, hydrophobic-favored, and van der Waals + electrostatic. We selected the first ten docking structures with the relatively low energies that were scored by the server. HexDock 8.0 and Z-dock were also used to perform the rigid docking (results not shown).

3. RESULTS AND DISCUSSION

MBL interacting protein was mapped by searching the STRING database version 10.5 at a confidence level of 0.15–0.5, Where MBL, MBL-associated serine protease (MASP), Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, Mono-nitrogen oxides synthase (NOX1-4), and Neutrophils cytosolic factor (NCFs) were used as input proteins. A number of membranous and cytosolic protein were found to be associated with the MBL and could initiate various cascades. However, on careful screening of associated known functions and increasing the search confidence level, the number of associated proteins was reduced. To identify the putative receptors, the proteins of the network were manually screened for the presence of trans-membrane protein between initial and terminal input proteins. Reactive oxygen species (ROS) production and phagocytosis related protein were considered as terminal protein of signaling/association which start with MBL (initial protein). To assign potential signaling pathways followed by MBL interaction, the identified putative receptors were searched in the associated KEGG pathway (online resource).

The BLAST-P search of retrieved amino acid sequences of putative receptors and ligand, against PDB sequence entries, was performed to find the highest scoring matches. Average four models per input amino acid sequence were generated using different templates and the best fit was used for further studies after validations. The models were validated using PROCHEK [17] to determine the stereochemical quality of dihedral φ–ψ angles of amino acid residues and sterically allowed regions for these angles. The Ramachandran plot shows φ–ψ torsion angles for amino acid residues in the structure, except chain termini amino acids. The final models indicate that an average of more than 97% of residue φ–ψ angles are in the favored or additional allowed regions of Ramachandran plot. Most of the residues of the modeled proteins (80%–96%) are within the most favored regions,

whereas 2%–6% residues were lying within the additional allowed regions. About 1.5%–3% residues of modeled proteins are falling within the beige, generously allowed regions followed by 0.0%–2% residues in the disallowed white region only (online resource). The analysis showed that the overall stereochemical properties of the generated models were highly reliable and could be used for further molecular docking studies. In summary, homology models were built and validated and found suitable for the rigid docking studies.

MBL trimeric subunit and identified putative receptors were used as input for the rigid docking at ClusPro server 2.0. Ten models were generated for each ligand–receptor pair input on the ClusPro server after performing rigid docking. The N-terminal cysteine-rich region of MBL trimer and assembled larger oligomers are responsible for the effector activity of protein [18]. Thus, interactions of MBL (ligand) with receptor through its N-terminal cysteine-rich region were used as primary criteria for interaction analysis and any interactions through Carbohydrate recognition domain (CRD) were excluded. Affinity energy (KJ/mol) and number of complexes generated in the 5 Å were secondary criteria [16] for the receptor–ligand complex selection and are summarized in Table 1. The third important criterion taken into account during analysis was the exclusion of transmembrane span of the receptor protein, i.e., some ligand–receptor complexes may interact but the site of bonding of receptor falls in transmembrane span, thus the interactions would not be feasible in *in-vivo* conditions. Ligand–receptor interactions were individually visualized and analyzed. The proteins exhibiting MBL binding through the transmembrane region could not be considered as receptors since the trans-membrane site would be buried in the lipid bilayer and would be unavailable for binding. The transmembrane span of the model structure was analyzed with the Transmembrane Helices Hidden Markov Models (TMHMM) server. For instance, C3a anaphylaxis receptor interacts with ligand (MBL) via Ser31, Cys140, Leu125, Lys144 but all of the amino acid residues were predicted to be lying in membrane buried region when analyzed with TMHMM server [19,20]. Likewise, C3AR1 receptor: Leu25, Val28, Cys381, Val377, Ser349; C5AR1 receptor:Leu119, Leu166, Ala123, Val114, Leu116 were predicted to be lying in membrane region and could not be accessed directly for ligand interactions. The results were similar for calcium-receptor, Cluster of differentiation (CD40) ligand, lymphotoxin-β, Integrin subunit alpha M (IGTAM), and Integrin subunit alpha X (IGTAX). Whereas Protease-activated receptors (PAR1), PAR3, PAR4, Epidermal growth factor (EGF)-TM27 and IGTAM potentially interact with hydrophobic forces with MBL. Since the trans-membrane region is buried in the lipid bilayer, the ligand–receptor interactions are non-feasible. Hence, these candidates could not be considered as putative receptors. Tumor necrosis actor receptor superfamily (TNFSF27) amino acids Ser52, Trp53, Gly54, His55 form a β-turn and also interact with the N-terminal of MBL subunit. TLR-2 interacts with MBL on two sites at N-terminal amino acid of MBL via Leu528, Asn433, and Leu542 by 5.9, 6.8, and 6.6 Å distance, respectively. TLR interacts with the CRD site of MBL Thr200–Pro32 and Gly201–Gly34 with a distance of 4.3 and 5.4 Å, respectively. CD40M was found to interact with the three N-terminal Ala of MBL via Ser114, Leu84, and Pro85, where the distance between interacting amino acids was calculated to be 5.7, 6.0, and 6.9 Å, respectively. They were found to interact with the CRD site but the interacting amino acid was predicted to lie in a cytosolic part of the transmembrane structure. IgGFcRN interactions with the MBL domain are potentially hydrophobic in nature, amino acid Ala73, Trp74, Val75, Trp76, Glu77 form a helix which got buried in the small cavity formed at the n-terminal of the trimer. Protein tyrosine phosphatase, receptor type C (PRTPC) was found to interact with domain whose few amino acid were predicted to lie in the transmembrane or cytosolic region. Thus, accessibility for interactions with MBL is rare. ICAM1 was found to interact with the MBL subunit via Lys426 and hydrophobic forces with the N-terminal cysteine-rich region. CR1 amino acids Thr81, Cys93, and Pro94 were in close proximity to one of the polypeptide chain at the CRD site (Glu193, Lys137). On the N-terminal, Ala109, Ala107, and Ala109 (b chain) interact

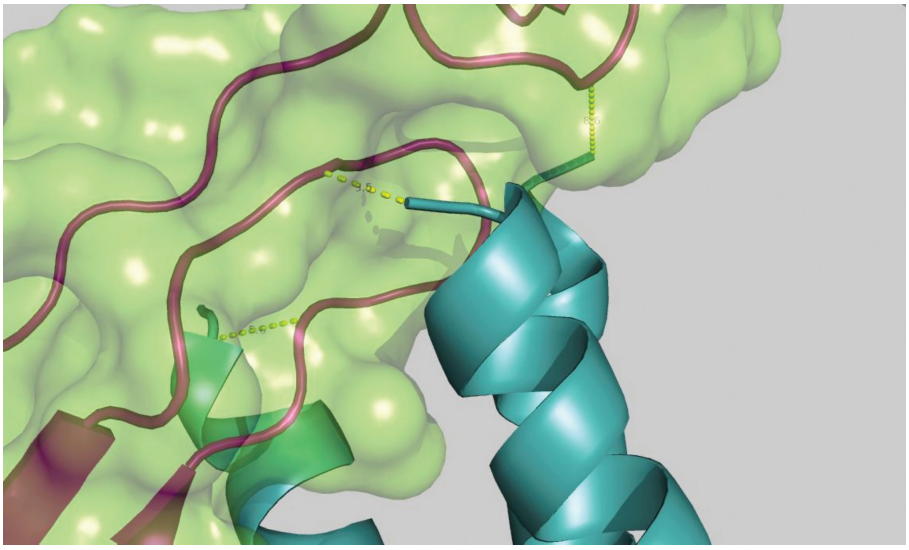

Figure 1: The interactions of N-terminal of MBL and CR1 receptor. The CR1 is represented as surfaced cartoon model and cartoon mode helices are representing MBL trimeric subunit. Dotted lines represent the possible interactions between them.

with Thr113, Thr110, and Val109 with a measured distance of 5.2, 5.4, and 3.5 Å, respectively (Fig. 1). In view of its binding energy (795.2 KJ/mol) and the part that binding is not lying in the trans-membrane region CR1 is the most competent receptor for MBL.

Several groups of receptors facilitate neutrophils recognition of pathogens and activation or priming of phagocytosis. Receptors on the neutrophil surface included FcγRII and FcγRIII, CR1, CR3, C3aR, C5aR, CXCR1, and TNFR [21]. As indicated in results, TNFSF27, TLR2, CD40m, CR1, PTPRC, ICAM1, and IgGFcRN show a specific interaction with the N-terminal collagen region of MBL. Additionally, the affinity of receptor toward ligand is found to be CR1 > CR2 > CD40M > IgGF cRN > T N F SF27 >ICAM1 > TLR2 > PRTPC. This interaction might result in the induction of potent microbicidal substances in the macrophage, including reactive oxygen species and nitric oxide, leading to the destruction of ingested microbe. TNFSF27 receptor is required for the generation and long-term maintenance of T cell immunity and plays a key role in regulating B-cell activation. This receptor transduces signals that lead to the activation of NF-kappaB and Microtubule associated protein kinase (MAPK8)/ c-Jun N-terminal kinase (JNK), which are responsible for the regulation of cell stress and cellular processes like proliferation and differentiation respectively [22]. TLRs are not phagocytic receptors but participate in the link between phagocytosis and inflammatory responses by triggering the production of cytokines [21]. MBL binds to Lymphotoxin-alpha (LTA) and subsequent complexing with TLR2 to increase ligand delivery is explained to enhance TLR2 responses, as was measured by cytokine release by murine macrophages [18]. But this TLR2-mediated response was only effective when pathogens were delivered into the phagosome. The CD40 interactions are essential for T-cell-dependent B cell proliferation and differentiation. CD40 is mostly expressed on B-lymphocytes and monocytes, macrophages, dendritic cells, and fibroblasts. But the expression on the neutrophils is not reported [23] (NCBI Gene ID:958 accessed on 30-Mar-2019). PTPRC is rarely considered in chemoattractant-mediated signaling, only few reports shown to share redundant roles in positively regulating Src Family Kinases in immunoreceptor signaling pathways [24]. The protein is present in all differentiated hematopoietic cells and essential regulator of T- and B-cell antigen receptor signaling [25]. MBL deficiency reduces ICAM1 expression level [26]. A report [27] states that neutrophils with high ICAM1 are associated with enhanced phagocytosis of zymosan particles and ROS generation. However, in their study, pre-incubation with stimulants was done with whole blood. In whole blood, the possibility of various other proteins acting as opsonin could not be ruled out. Additionally, it is well-known that ICAM1 promotes junctional and non-junctional transendothelial migration in vascular endothelium [26].

IgGFcRn is known to enhance the rate of phagocytosis in Polymorphonuclear leukocyte (PMN) through IgG opsonization. The use of mutated IgG (H435A)/FcRn knock outs severely impaired phagocytosis under experimental conditions while retaining normal binding to classical leukocyte receptors [28]. CR1 facilitates both complement regulation and immune complex processing, whereas CR2 binds only to C3b derived ligands of complement pathway [6]. Additionally, the CR1 and 2 are products of alternative splicing, so the major receptor on the neutrophil cell surface becomes CR1. One of the reports earlier also showed the interaction of CR1 and MBL [29], confirm the CR1 candidature as the MBL receptor.

4. CONCLUSION

In the present work, protein network analysis, homology modeling, and rigid docking were performed, to explore structural features and binding mechanism of MBL with its cellular receptors. The protein network analysis, homology modeling, and rigid docking to explore structural features and binding mechanism of MBL with its cellular receptors confirm CR1 as a receptor on neutrophils.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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