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. Templates were identified by Position-Specific Iterative Local Alignment Search Tool (PSI-BLAST) Hits (against available structure). Each identified highest quality structure was retrieved from the NCBI database (online resource: Table 1). Templates were identified by Position-Specific Iterative 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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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 PROCHECK [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. Lidgand–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 region is buried in the lipid bilayer, the ligand–receptor interactions are non-feasible in vivo conditions. Hence, these candidates could not be considered as putative receptors.

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.
Several groups of receptors facilitate neutrophils recognition of pathogens and activation or priming of phagocytosis. Receptors on the neutrophil surface included FcγRI 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 > IgGFcRn > T N F SF27 > ICAM1 > TLR2 > PTPRC. 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 Lymphotxin-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 chemotaactrant-mediated signaling, only few reports showed 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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