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Original Article

EFFECT OF TOLL-LIKE RECEPTOR INHIBITOR IMIQUIMOD ON IL1R1 INTERACTION WITH IL-1RA AND ITS SNP VARIANT-AN *IN SILICO* APPROACH

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ABSTRACT

Objective: Interleukin 1 receptor antagonist (IL1Ra) acts as an antagonist to Interleukin 1 beta (IL1β) signalling in maintaining homeostasis. A loss of function due to Single Nucleotide Polymorphism (SNP) occurrence in IL1Ra can lead to dysregulated state as seen in autoimmune disease pathogenesis. The current study aims at achieving conformational stability in the IL1R1-IL1Ra_SNP complex by introducing a ligand into the region apart from the active site of Interleukin 1 receptor type1 (IL1R1).

Methods: Protein-protein docking was performed using ClusPro, for IL1R1 with IL1β, IL1Ra and IL1Ra_SNP variant to study the difference in the interaction between the complexes. A known inhibitor, Imiquimod was docked using Glide, to the Il1R1-IL1Ra_SNP complex using flexible docking and the change in surface energy was calculated.

Results: Binding Interactions show that IL1Ra binds more avidly to IL1R1 than IL1β. Conformational instability was seen in the IL1R1-IL1Ra_SNP complex. The difference in the amino acid interactions between the IL1R1 with IL1Ra and IL1Ra_SNP variant further illustrated the change in binding residues and hydrogen bond formation. Upon docking of an appropriate ligand to the IL1R1-IL1Ra_SNP complex, the conformational stability of the IL1R1-IL1Ra_SNP complex enhanced considerably suggesting a possible mechanism for treating SNP-induced conformational instability.

Conclusion: Toll-like receptors like IL1R1 have many binding pockets apart from its active site. No strategies have yet been reported in targeting them for correcting conformational instability induced by SNP. Through our study, it was observed that the conformational instability of IL1R1-IL1Ra_SNP complex decreased upon introducing an appropriate small molecule.

Keywords: IL1β, IL1R1, IL1Ra, SNP

INTRODUCTION

Inflammation is the body's protective response to injury and infection. It is a complex process involving many cell types, as well as different components of blood interacting with each other to maintain homeostasis. Autoimmunity is a condition which leads to failure of our immune system to differentiate our cells with foreign agents. Among many cell types, T-helper cell (Th cell) differentiation has been chiefly associated with Systemic Autoimmune disease like Rheumatoid Arthritis (RA)[1]. Pro-inflammatory cytokines signalling plays a crucial role in autoimmune disease pathogenesis as they regulate T-helper cell differentiation.

More than any other pro-inflammatory cytokine family, the interleukin 1 (IL1) family is closely linked to the innate immune response. Its role along with IL1Ra in Th17 differentiation is critical in autoimmune disease pathogenesis. IL1Ra deficient mice develop autoimmune arthritis in which IL17A plays a crucial role [2]. The cytoplasmic domain of the IL1R1 is highly homologous to the cytoplasmic domains of all Toll-like receptors (TLR) [3]. IL1β is the most studied member of the IL1 family because of its role in mediating autoimmune diseases. Although the TLR and IL1 families evolved to assist in host defence against infection, unlike the TLR family, the IL1 family also includes members that suppress inflammation, both specifically within the IL1 family but also non-specifically for TLR ligands and the innate immune response [4].

IL1 type cytokines are major mediators of innate immune reactions, Interleukin 1 alpha (IL1α) and IL1β promote an inflammatory process which is regulated by IL1Ra antagonist activity [5]. IL1α and IL1β rapidly increase mRNA expression of hundreds of genes in different cell types [6]. The potent pro-inflammatory activities of IL1β and IL1α are restricted at three major levels: (i) synthesis and release of IL1β and IL1α (ii) membrane receptors IL1R1 and IL1R2 and (iii) intracellular signal transduction. In response to IL1β binding to IL1R1, the cell undergoes a complex sequence of combinatorial phosphorylation and ubiquitination of second messengers resulting in activation of Nuclear Factor kappa B signalling, Janus Kinase, p38 and Mitogen-Activated Protein Kinase (MAPK) pathway proteins, which cooperatively induce the expression of canonical IL1 target genes (such as IL6, IL8, Monocyte Chemoattractant Protein-1, Cyclooxygenase-2, IL1α, IL1β, Map Kinase Phosphatase-1) by transcriptional and posttranscriptional mechanisms [7]. Dysregulated, prolonged synthesis and release of IL-1 cytokines in chronic inflammatory situations contribute to Systemic autoimmune diseases like rheumatoid arthritis [8]. IL1Ra as an antagonist to IL1β, binds to IL1R1 and inhibits IL1β mediated cellular proliferation [9]. The IL1R1 shares same binding site for IL1β and IL1Ra [10]. IL1Ra is an important natural anti-inflammatory protein, and any loss of its function could lead to an autoimmune condition like rheumatoid arthritis [11]. Many SNP's have been associated to IL1Ra, among these, the non-synonymous SNPs could potentially induce conformational instability in IL1R1-IL1Ra complex, thereby leading to dysregulation of the Th cell signalling mechanism and leading to loss of homeostasis which contributes to autoimmune disease pathogenesis [12]. The comparative conformational analysis of the underlying mechanisms involved in IL1R1 complex formation with IL1β, IL1Ra and the effect of non-synonymous SNP variant (IL1Ra_SNP) can provide insights into conformational stability [13]. A ligand-induced conformational stability for the IL1R1-IL1Ra_SNP complex has been explored.

MATERIALS AND METHODS

PDB IDs: 3O4O, 31B1 and 1ILR for IL1R1, IL1β and IL1Ra respectively were retrieved from Protein Data Bank (PDB) with a resolution ranging from 2Å to 3Å [14]. Homology modelling was performed using Easy Modeller for IL1Ra_SNP variant and IL1R1

using IL1Ra and IL1R1 PDB structures respectively as the template [15]. Protein-protein docking for IL1R1 with IL1β, IL1Ra and IL1Ra_SNP variant was performed using ClusPro, an online server to get the respective complex structure and their binding energies [16]. Chrion, a rapid protein energy minimization server was used to minimize the docked complexes IL1R1-IL1β, IL1R1-IL1Ra, and IL1R1-IL1Ra_SNP [17]. The docking and minimization structures were visualised using Pymol and the structure was coloured based on chains. The SNP's associated with IL1Ra were identified from dbSNP [18]. Among 2525 SNPs, a non-synonymous SNP, rs45507693 was predicted to induce conformational instability resulting from Alanine to Threonine substitution in IL1Ra.

LIGPLOT+,a program that generates a schematic representation of protein-ligand interaction was used to find the interaction surface to elaborate the difference in the binding of IL1R1 with IL1β and IL1Ra [19]. SCFbio, an online supercomputing facility was used to find out all the possible binding pockets in the receptor apart from the active site, which could act as potential regions for ligand binding [20]. Glide 6.7 (Schrödinger Inc.) was used to perform docking studies of IL1R1-IL1Ra_SNP complex with Imiquimod, a known inhibitor of Toll-like Receptor [21]. Surface area changes for the complexes were studied using Inter Pro Surf, which is used to find the total surface area of protein in its monomeric and complex forms [22].

RESULTS

The 3D models of IL1R1 and IL1Ra_SNP variant was predicted by Homology modelling, and the structural validation was performed using Ramachandran plot. The maximum amino acids distribution was found in the allowed region. Root Mean Square deviation (RMSD) was predicted between the IL1Ra and IL1Ra_SNP variant to be 0.890. The RMSD clearly indicates the conformational change induced by the SNP.

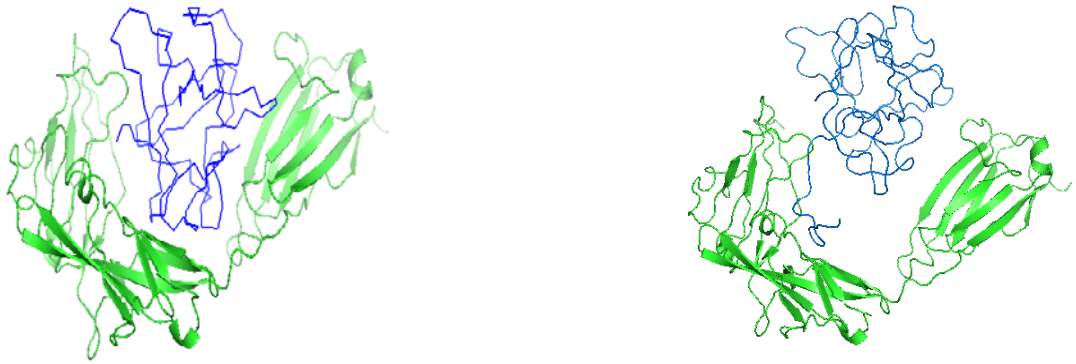
Protein-protein docking studies performed for IL1R1 with IL1β and IL1Ra suggested that IL1Ra binds more avidly to IL1R1 than IL1β. Binding free energy scores in table 1 shows that IL1Ra antagonist binding with IL1R1 plays a crucial role in controlling inflammatory mechanism and any conformational disturbance in IL1R1-IL1Ra complex can lead to dysregulation in homeostasis as found in autoimmune diseases.

Comparison of IL1R1-IL1Ra complex with IL1R1-IL1Ra_SNP complex

IL1R1 was docked with IL1Ra_SNP variant. Upon comparing binding free energies of three complexes as shown in table 1, it was observed that the binding free energies of the IL1R1-IL1Ra_SNP complex decreased considerably than the IL1R1-IL1Ra complex. The structure of the docked complexes IL1R1-IL1Ra and IL1R1-IL1Ra_SNP clearly shows the conformational change between the two complexes as shown in fig. 1.

Table 1: Comparison of binding free energy for IL1R1-IL1β, IL1R1-IL1Ra, and IL1R1-IL1Ra_SNP complexes

Protein-protein complex	Binding free energy (kJ/mol)
IL1R1-IL1β	-759.7
IL1R1-IL1Ra	-945.3
IL1R1-IL1Ra-SNPVariant	-891.3



(a) **(b)**

Fig. 1: (a) Interaction of IL1R1 with IL1Ra (b) Interaction of IL1R1 with IL1Ra_SNP variant. IL1Ra is represented in blue colour wireframe and its receptor IL1R1 in green colour ribbon format

To elaborate the difference in binding between, the interacting surface of the IL1R1-IL1Ra complex and the IL1R1-IL1Ra_SNP complex was studied. The amino acids participating in the Hydrogen bond interactions between ligand and receptor for both complexes are shown in table 2. The change in bond formation was the contributing factor to the conformational change. Among the interactions, IL1R1-IL1Ra complex has more hydrophobic amino acids participating in stabilising the conformation which was observed to be reduced in IL1R1-IL1Ra_SNP complex. Further, the set of amino acids involved in the both complexes are different.

Table 2: Residues forming Hydrogen Bonds in IL1R1-IL1Ra and IL1R1-IL1Ra_SNP complexes

a. IL1R1 with IL-Ra

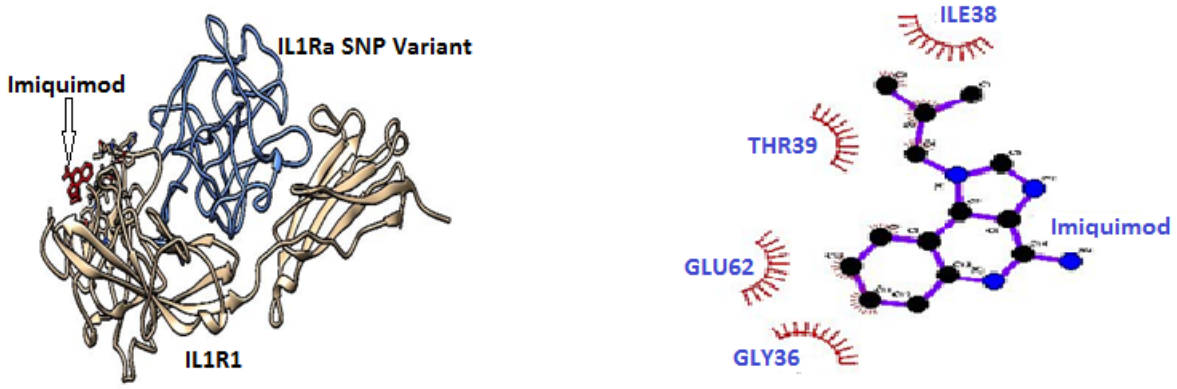
IL1R1	IL-1 Ra
Ile11	Asn39
Lys9	Leu25
Thr297	His54
Glu8	Arg26
Glu126	Ala127
Tyr124	Tyr34
Ala106	Gln36
Val13	Gln36
lIe107	Gly37
Arg160	Val18
Lys111	Gln20
Lys111	Trp16

b. IL1R1 with IL1Ra-SNP variant

IL1R1	IL1Ra_SNP
Arg5	Glu129
Arg8	Ile110
Arg8	Ala109
Ser9	Tyr127
His10	Val24
Leu14	Arg9
Cys4	Asp162
Glu2	Lys161
Arg117	Asp260
Arg117	Tyr261

Increasing conformational stability in IL1R1-IL1Ra_SNP using a known inhibitor, Imiquimod

To induce conformational stability in the IL1R1-IL1Ra_SNP complex, a protein-ligand interaction was performed. IL1R1 was analysed using SCFBio for finding binding pockets other than IL1Ra binding site. Out of 46 cavities generated in IL1R1, only 21 sites not used by IL1Ra binding were considered for docking studies. The residues listed in table 2 were also eliminated prior to docking. The final selected region for docking ranged from Proline26 to Arginine 89. A known inhibitor, Imiquimod was docked with the IL1R1-IL1Ra_SNP complex using Glide. Imiquimod is a common drug for psoriasis and targets Toll-like receptors. It has 2 H-bond donors and 4 H-bond acceptors. It has an xlogP value of 2.66 and has a net charge of 0 [23]. To study the surface area changes, the total surface area of protein in its monomeric and complex form were predicted. The docked complex consisting of IL1R1-IL1Ra_SNP with Imiquimod is shown in fig. 2.



(a) **(b)**

Fig. 2: (a) Docking image of Imiquimod bound to IL1R1-IL1Ra_SNP complex. (b) Non-bonded interactions between IL1R1 and Imiquimod

The presence of the ligand changed the surface area which is evident that it can alter the conformational effect induced by interaction with IL1Ra_SNP variant. The changes in surface area of IL1R1-IL1β, IL1R1-IL1Ra, IL1R1-IL1Ra_SNP complexes, and IL1R1-IL1Ra_SNP with Imiquimod complex are shown in table 3. A comparison was drawn between total surface energy and polar area energy for generated complexes as shown in table 3, the total surface energy for Il1R1-IL1Ra and IL1R1-IL1Ra_SNP complex was 21387.10 and 17694.89 respectively. On a known ligand imiquimod, the total surface energy, and polar area energy improved for the IL1R1-IL1Ra_SNP complex increased to 17870.66. The change in surface energy suggests a definite shift in conformational stability of the IL1R1-IL1Ra_SNP-Imiquimod complex.

Table 3: Total surface energy and polar energy difference between IL1R1-IL1β, IL1R1-IL1Ra, IL1R1-IL1Ra_SNP, and IL1R1-IL1Ra_SNP+imiquimod

Protein-protein complex	Polar area/energy	Total area/energy	Number of surface atom	Number of buried atom
IL1R1+IL1β	7170.28	22116.48	2114	1633
IL1R1+IL1Ra	7095.70	21387.10	2011	1730
IL1R1+IL1Ra_SNP	5844.79	17694.89	1494	1053
IL1R1+IL1Ra_SNP+imiquimod	6203.02	17870.66	1477	1070

DISCUSSION

IL1β downstream signalling plays a significant role in the IL-23/IL-17 proliferation. Studies show that the effect of gene polymorphism in the IL1 system has an effect on the inflammatory pathway, suggesting that any change in the IL1 pathway could contribute to autoimmune disease pathogenesis [24]. IL1R1 binds more avidly to IL1Ra than IL1β [25]. Protein-protein interaction studied between IL1R1-IL1β and IL1R1-IL1Ra predicted minimal free energy associated with the complexes. Minimum free energy implies that lesser the energy maximum the stability of the complex [26].

Docking studies performed suggest that the binding free energy of IL1Ra is minimum compared to IL1β, and establishes a more stable complex than IL1β, this factor could be critical in establishing the antagonistic role of IL1Ra and in maintaining the homeostasis. When comparing the binding free energy of IL1Ra_SNP to IL1Ra with IL1R1, it was observed that the binding affinity of IL1Ra_SNP

reduced considerably than IL1Ra but remained higher than that of IL1β. This provides insight that IL1Ra_SNP has more binding affinity than IL1β but fails to establish the same conformational stability as IL1Ra. This could be a possible factor in contributing to the sustained signalling proliferation as seen in autoimmune disease pathogenesis.

The conformational stability of IL1R1-IL1Ra complex is contributed by interacting residues occurring on the active site. Analysis of the surface interaction of the IL1R1-IL1Ra complex suggested that presence of more hydrophobic residues was seen than polar residues. This is suggestive of the fact that the presence of hydrophobic residues contributes to a more tight complex formation by expelling the water out of the active site. The presence of hydrophobic amino acids participating in interaction could also be the reason for the minimal free energy of the complex. Comparing the surface interaction residues of the IL1R1-IL1Ra complex with IL1R1-IL1Ra_SNP complex, it was observed that an increase in polar residues contributed to surface interaction. The presence of polar residues increases the van der Waals force, which could be the contributing factor to increase in free energy and thereby to decrease the stability. The SNP occurrence interferes with the overall conformation of the complex and could influence the regulation on the cytosolic part of the receptor and thereby influencing its function.

The overall interacting surface area is critical in maintaining the activity of the IL1R1-IL1Ra complex. In the IL1R1-IL1Ra_SNP complex, a strategy for re-establishing the total surface area was performed, by targeting the allosteric site of IL1R1, using a known inhibitor imiquimod. The total surface energy improved considerably for the IL1R1-IL1Ra_SNP+Imiquimod complex. Analysis of interacting surface revealed that upon binding the inhibitor, the number of buried atoms participating in the interacting region increased compared to the IL1R1-IL1Ra_SNP complex without the inhibitor. Also, the change in the surface area shifted towards the IL1R1-IL1Ra complex. This approach suggests that targeting allosteric sites in SNP variants could establish conformational stability in receptor-ligand complex and thereby regulating the downstream signalling process. Although allosteric sites have been previously targeted for inhibiting receptor-mediated signal [27], no approach to use small molecule has been reported in inducing conformational stability in SNP variants.

CONCLUSION

SNP-induced conformational instability can cause dysregulation of the IL1 system. A strategy for establishing conformational stability by targeting allosteric sites in IL1R1-IL1Ra_SNP complex helped in regaining the conformational stability of the complex. Exploring other binding pockets of TLR with similar computationally driven strategy can also provide conformational stability and thereby increasing the binding affinity of the SNP variants.

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CONFLICT OF INTERESTS

Declared none

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