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The c.305del3 in *IL-2* gene in Homonoidea theoretically affects IL-2/IL-2Rα interaction as well as lymphocyte homeostasis

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ABSTRACT

Interleukin-2 (IL-2) is a well-known monomeric T-cell growth factor that is produced primarily by activated CD4+ T cells following exposure to antigen. IL-2 structural analysis among primates showed a few polymorphisms as well as a 3-nucleotide deletion (c.305del3) in Hominoidea superfamily including *Homo sapiens*. On the other hand, the interaction of IL-2 with its alpha subunit of the receptor (IL-2Rα) is the first step for assembly of the whole IL-2R and considered as a species-specific phase. Four models of human IL-2, IL-2Rα, and their ancestral forms were made and were used for molecular dynamics (MD) simulation. Subsequently, the final structures were docked to each other and finally, the complexes were used for MD simulation. Our results showed that the above mentioned deletion led to weaker interaction of human IL-2 to its receptor relative to ancestral IL-2. Association study of lymphocyte counts, as an indicator of IL-2 function, in 78 primate species with or without this deletion showed significant association of this deletion with their overall lymphocyte counts ($P < .01$). Therefore, it can be suggested that p.81delThr in IL-2 in Hominides superfamily interfered with interaction of IL-2 and IL-2Rα and led to overall decrease in lymphocyte counts in this superfamily of primates in comparison with other primates.

1. Introduction

IL-2 is a member of a cytokine family including IL-4, IL-7, IL-9, IL-15 and IL-21. Interleukin-2 (IL-2) plays a key role in the proliferation of T lymphocytes and natural killer cells. IL-2 receptor (IL-2R) expressed by lymphocytes is a complex consist of alpha, beta and gamma chains [1]. The alpha subunit of IL-2R (IL-2Rα) has low affinity for IL-2 but after binding to the β and ϒ subunit it can increase the IL-2R affinity up to 100-fold. Heterodimerization of the β and ϒ subunits of IL-2R is essential for the signaling in T-cells [2].

It has been reported that polymorphisms in the IL-2 gene are associated with various cancers [3]. Among cytokines of T-helper cells, IL-2 plays a unique and important regulatory and effector role. Therefore, defects in IL-2 leads to the lack of $CD4^+CD25^+$ regulatory T cells, which resulted in the occurrence of T-cell mediated autoimmune diseases [4]. For example, IL-2 is required for the generation and maintenance of regulatory T-cells (Treg) to provide lifelong protection from autoimmune disease [5]. The decreased production of IL-2 in patients with autoimmune disease triggers various immune defects, such as decreased production of Treg cells, decreased activation-induced cell death (AICD) and decreased cytotoxicity of cytotoxic lymphocytes [6]. In many individuals with autoimmune disease, IL-2 is the case for autoimmune diseases such as type 1 diabetes [7], rheumatoid arthritis [8] and systemic lupus erythematosus [9,10].

The receptor for IL-2 is a complex protein, which is composed of integrated multimers in the cell membrane. Two forms of this receptor are biologically active and exist with different affinities for IL-2 [11]. The high-affinity one is a heterotrimer contained the IL-2Rα, IL-2Rβ and γc subunits, but the other with intermediate affinity is a heterodimer made only of the IL-2Rβ and γc chains. Although the IL-2Rα chain does not play a role in intracellular signaling because of its very short cytoplasmic tail but involved in receptor complex formation [12]. The IL-2R β subunit is shared with the IL-15 receptor (IL-15R) complex, and the γc chain is shared with the IL-4, IL-7, IL-9, and IL-15 receptors. Therefore, specificity of ligand binding is based on the presence of an α subunit that is unique to each receptor complex. Each of the individual IL-2R subunits has specific IL-2 binding properties that contribute to the overall properties of the multimeric receptor forms [13]. For example, the IL-2Rα subunit binds to and dissociates from IL-2 rapidly [14,15], whereas the IL-2Rβ subunit dissociates from IL-2 significantly more slowly [11]. Consequently, the net binding phenotype of the αβ heterodimer is a composite of the properties of each subunit that favors

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ligand binding. Hence, the heterodimer is characterized by a rapid association rate (a characteristic of the alpha subunit) and a slow dissociation rate (a property of the β chain) and therefore binds IL-2 with a higher affinity than either component alone [13].

The IL-2 of one species can somehow interact with IL-2R of another species among different evolutionary species. However, some of these interactions are not reciprocal among different organisms. For example, albeit human IL-2 in both human or mouse can effectively bind to its receptor on lymphocytes but murine IL-2 interacts with markedly higher affinity to its receptor in mouse rather than in human. However, it has been demonstrated that IL-2Rα is primarily responsible for this species specificity of IL-2 binding [13].

Therefore, in this study because of the importance of IL-2Rα in the initiation of IL-2 signaling, we decided to study the interaction of human IL-2 (IL-2h) with its IL-2Rα subunit (IL-2Rαh) as well as ancestral IL-2 (IL2a) with its IL-2Rα subunit (IL-2Rαa) via molecular dynamics simulation and associate these interactions with the number of lymphocytes.

2. Methods

2.1. Indel polymorphisms in the IL-2 gene among primates

To find more effective changes in IL-2, and IL-2Rα structure among higher mammals all available primates in NCBI database were selected. Then their *IL-2* mRNA sequences were checked by Clustal Omega as an usual multiple alignment tools to identify any exonic indel polymorphisms.

2.2. Ancestral IL-2, and IL-2Rα model reconstruction

Since usually indel polymorphisms show more significant effects on protein function we decided to check interaction of ancestral IL-2, and IL-2Rα with and without c.305del3 in the *IL-2* gene. As deletion polymorphism existed in Hominoidea superfamily (Catarrhini parvorder), another member of the same parvorder (*Macaca fascicularis*) lacking that deletion was selected for reconstruction of the ancestral IL-2, and IL-2Rα protein structure by employing the Clustal Omega web server (Supplementary Fig. S1). Comparison of current human with ancestral proteins showed deletion of Thr81 in the IL-2 and five mutations (Pro11, Thr66, Met126, Met205 and Ser212) in the IL-2Rα (without considering signal peptide sequence). Subsequently, the interaction of ancestral IL-2 with its IL-2Rα was compared with the interaction of human IL-2 with its IL-2Rα using docking and molecular dynamics simulation.

2.3. Homology modeling

In order to obtain a suitable structure for human and ancestral IL-2, and IL-2Rα, four homology modeling were performed. The target sequences of human IL-2 and IL-2Rα were obtained from NCBI site and used for the two modeling separately. In addition, their reconstructed ancestral forms were used in another two modeling separately. The templates in all modelings were obtained from PDB bank with PDB code: 2erj and 2b5i that contain IL-2 and α, β and γc chains of IL-2R. Then, Modeller software 9.16 [16] was used for multiple template modeling using two mentioned templates. One thousand models have been made in each modeling. The best model which had the least dope energy was chosen from each modeling [17]. The quality of the various models was assessed using Procheck software by Ramachandran plots. In these plots, the number of residues on allowed or disallowed areas determines the quality of the created protein model [18]. In addition, energy plots were created by Verify 3D site (http://services.mbi.ucla.edu/Verify_3D/).

2.4. Molecular dynamic simulation of human IL-2 and its receptor

The best models for human and ancestral IL-2 and IL-2Rα that were obtained from homology modeling were subjected to MD simulation separately. MD simulation and molecular mechanic minimizations were performed using GROMACS 5.1 package [19] under Gromos force field (G43A1) [20]. The systems were neutralized by adding 15 Na^+ and 15 Cl^- ions and about 5944 water molecule with SPC216 models for human IL-2 and 46 Na^+ and 46 Cl^- ions and about 17630 water molecules for IL-2Rα in order to obtain 140 mM ionic strength that is similar to physiological ionic strength[21]. Because IL-2Rα exists in extracellular part of the membrane, MD simulation of it in water is not incorrect. Four MD simulations in which 20 ns MD simulation run were performed at NPT ensemble at 300 K and time step of 2 fs. These simulations were performed with the same procedures and details that were mentioned in our previous works to obtain the final structures [22].

2.5. Docking

HADDOCK software (http://www.bonvinlab.org/software/haddock2.2), as a popular docking program which uses the data-driven approach and support for a wide range of experimental data, was used for docking study [23]. This software can also integrate information derived from biochemical, biophysical or bioinformatics methods to enhance sampling and scoring. We ran Haddock with constraints, the active and passive residues or ab initio data were our constraints.

In the IL-2Rα the residues (without considering signal peptide sequence) of Glu1, Leu2, Asp4, Asp6, Met25, Asn27, Glu29, Cys30, Arg35, Arg36, Lys38, Ser39, Ser41, Leu42, Tyr43, Leu45, Asn57, Ser64, Ile118, and His120 are in contact with the IL-2 residues (without considering signal peptide sequence) of Lys35, Thr37, Arg38, Thr41, Phe42, Lys43, Phe44, Tyr45, Glu61, Glu62, Lys64, Pro65, Glu68, Leu72, and Tyr107 (according to 2erj template) [24]. The final structures obtained from MD simulation for human IL-2 and its receptor IL-2Rα was docked to each other using HADDOCK software. The active residues for docking were the same interface mentioned residues.

2.6. Molecular dynamics of complexes

After obtaining a complex of human IL-2 with its receptor as well as ancestral IL-2 with its receptor via haddock server, these complexes were used for 20 ns MD simulation separately with the same mentioned conditions and interaction of IL-2 with its receptor was investigate with MD simulation in human and ancestor.

2.7. Comparison of lymphocyte counts in primates with or without c.305del3

As IL-2 is involved in lymphocyte homeostasis in animals [2] we obtained lymphocyte counts of available primates from Reference ranges for physiological values in captive wildlife produced by International Species Information System physiological data reference values [25]. Briefly, 78 primates consisted of 44 different genera (14 families) entered in this study. Then they were grouped according to their c.305del3 condition. Therefore, nine species (2 families) including Homo sapiens had c.305del3 and other 69 species (12 families) entered the second group. Name of each species, their corresponding reference interval, mean and number of tested individuals are shown in Supplementary Table 1. Subsequently, we analyzed the association of lymphocyte counts, as a probable consequence of Thr81 deletion on the IL-2 function in lymphocyte homeostasis primates with or without c.305del3. Comparison of means carried out by Student's t-Test, by the criteria of one-tailed and unequal variances.