УДК 636.082.1

Functional genomics of adenosine deaminase in immune response

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Key words: adenosine deaminase, isoenzymes, genetics, genomes, mutations, immune system, polymorphism, interleukines

Adenosine deaminase (ADA) [1] is a key enzyme in metabolism of purines. It catalyzes the irreversible hydrolysis of adenosine into inosine and ammonia. It has a certain role in maintaining immune competence. The consequence of some point mutations in active catalytic center of ADA resembles a phenotypical representation of Severe Combined Immunodeficiency Syndrome (SCID). According to Wilson, D.[2] the 6-hydroxyl-1,6-dihydropurine ribonudeoside (HDPR) and zinc ion are bound in the deep pocket and located at the COOH-terminal end of the β barrel. ADA has a 8 barrel structural motif and it is a metallo-enzyme (β/α), containing Zn atom in the catalytic pocket, which is coordinated by five atoms: three Nε2 atoms of His15, His17, and His 214, the 0 δ2 of Asp 295, and the 0-6 of HDPR. Three Nr2 atoms exhibit tetrahedral geometry with 0δ2 and 0-6 sharing the remaining site.

It was shown that in humans ADA activity occurs mainly in two distinct isoenzymes and they are referred to as adenosine deaminase 1 (ADA*1) and adenosine deaminase 2 (ADA*2). ADA*1 exists in two major forms: a monomer of molecular weight 33,000 (small form) and a dimer-combining protein complex with a total molecular weight of 280,000 (large form), earlier called ADA-S and ADA-L. This complex has no signifcant influence on its catalytic activity. ADA*2 exists only as a monomer with molecular weight of 100,000 [3,4].

It is worthy to mention the diagnostic role of ADA activity in diseases. One of the most pronounced increase of enzymes activity was discovered by the group from H. Buniatian Institute of Biochemistry, NAS RA leaded by Mardanian S.S[5].

Expression of ADA in accordance with the first path of the literature is controlled by locus with 2 codominant alleles ADA*1 and ADA*2 located on the long arm of chromosome 20. Each allele is responsible for production of one from two of existing ADA isoenzymes, ADA*1 and ADA*2, which have different cellular and tissue distribution, as well as kinetic parameters [6].

The other group of the investigators (and they are multiple) think that ADA*1 and ADA*2 are located within the same gene locus [7-9].

And, finally, there is one highlighted publication, included into the NIH Gene database (CECR 1 gene) evidencing about different chromosome localization of ADA*1 and ADA*2 [10]. However, it is necessary to mention, that this single publication wasn't supported by other scientists in this field after 2005.

The most pronounced functional representation of ADA Single Nucleotide Polymorph (SNP) mutations is the SCID, as it was mentioned above. This phenomenon makes ADA a desirable molecule for the research and treatment of SCID. Missense mutations of ADA active center amino acids make this molecule biochemically not active, which serves as a basis not only for the development of SCID but also in a variety of other diseases including acquired immunodeficiency syndrome, anemia, various lymphomas, and leukemias [4,11-13]. ADA gene therapy is the first trial which was carried out in the patients with SCID caused by the defect of ADA gene. Also it is necessary to mention that ADA is used alone or in combination with the other drugs, as antimetabolic, antineoplastic, and antibiotic agents [14,15].

The main immunological function of ADA is regulation of T,B- cells differentiation as well as B-cells proliferation [3]. These processes occur in complex ways, when the other enzymes, such as kinase and phosphatases, are activated. It is necessary to mention that interleukins, which have peptidic nature, are involved into this process.

However, the main functional interaction of ADA gene with the ILs or other immune response representative genes are not fully determined and utility of collective bioinformatical data approaches might highlight and fulfill these gaps.

The most of the analyzed data are related with the ADA gene located on the 20th chromosome.

Methods

To analyze the functional genomes of ADA and its relevance with the functional immune system, to reveal its point mutational associative diseases, we have used series of the web-sites and databases. The main source of identifications was performed by utility of genome bioinformatic resource [16]. Functional properties of such point mutations were withdrawn by the utility of the web-site [17], which was created by Indiana University and was called as Center for Computational Biology and Bioinformatics. It is necessary to mention, that entire analysis of ADA functional genomics was performed with human genome based databases. To reveal the functional relevance of ADA with the other immune responses representing genes we have worked with OMIM [18]. Expression profile of ADA in immune representing cells was appreciated by newly developed web-site [19]. Moreover, this web-site helps to find out the relevance of ADA with the genes of kinases and phosphatases as well as transcriptional factors. RAPID [20] allowed to determine possible functional connection between Primarily Immunodeficiency Genes (PID) [3], which was ADA in our work, non PID Genes, but expressed in immune cells, non PID Genes but expressed in non immune cells,

as well to understand the lethality and/or immune/hematopoetic phenotype. The general [21] web-site served to classify the general cell biological functions, where ADA has an undoubted role. We have worked with the LIB & DAVID Bioinformatics [22] to find out the functionally relevant genes of Immune Response, mainly cytokines, with ADA.

Results and Discussion

ADA SNPs. ADA, a protein whose deficit leads to severe combined immunodeficiency, binds to the cell surface by means of CD 26, A1 adenosine receptors, or A2B adenosine receptors.

To evaluate SNPs of ADA we have used the primary repository for SNPs data of NIH [16, 23]. This web-site allowed appreciating the entire spectrum of ADA missense mutations. After all, we have used the data base created by Indiana University Center for Computational Biology and Bioinformatics (see methods part) to evaluate deleterious functional abilities of ADA SNPs. Authors characterized SNPs by probability value based on sequence homology and conservation. This value serves as a prediction for clarification whether the mutation will be deleterious or not [24]. Those values colored red on this web-site predict deleterious, those in green are tolerated phenotype. The results of our on-line analysis pointed to the fact that the most of exonic SNPs (73.4%) served as a basis for phenotypical SCID manifestation. It was interesting that after precise comparison of the mentioned on this web-site outcome of the analysis of existing for ADA SNPs and much more earlier published paper, mentioned above, about ADA active center main amino acids' SNPs, we couldn't find any identical matches (SNPs). Thus, to have a clear view of the entire information related with missense mutation it is necessary to analyze all of the existing databases, which might be even not a guarantee for such a new scientific direction, which is Bioinformatics.

ADA Relevant Genes. To analyze genetic relevance of ADA with the main molecules of Immune Response-cytokines, we have tried to utilize specialized for immune response as well as not specialized web-sites. Submission of 35 genes symbols of cytokines and ADA, LIB & DAVID Bioinformatics (see the link in part-methods) didn't bring any correlation between them. DAVID database helped to identify pathways and functional categories, where cytokines and ADA were involved (percentage of the involvement of the genes calculated from the total amount of them). However, we couldn't find any of the pathways where there were involved any from the cytokines and ADA; it wasn't noticed any pathway indicating the correlation between ADA and the other 35 cytokine representing gene symbols (**Fig.1**).

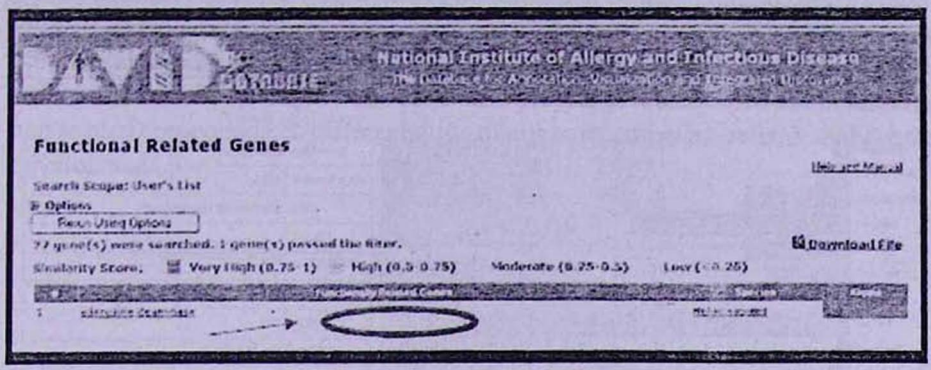

Fig.1. Utility of DAVID Bioinformatics to find out ADA relevant genes.

Relevance of ADA with genes of kinases and phosphatases transcriptional factors as well as cytokines, their interactive connections were analyzed by the Immunogene web-site (see, please, the link in methods part). Unfortunately, as with the non-immune response-specified database, DAVID, by utility of Immunogene we were not successful enough to find any relation of ADA with cytokines. Authors explain it in their publication that ILs (interleukins) are expressed in negligible amount which makes it almost undetectable in immune cells by the general tools utilized by them [25].

However, utility of Immunogene was beneficial to determine the expression profile of ADA in immune representing cells (**Fig. 2**). It is clear from analysis that the most pronounced amount of ADA is synthesized or accumulated by/in human cord blood-derived mast cells differentiated over 7-10 weeks using IL-10, IL-6 and stem cell factor; macrophages differentiated from isolated monocytes in the presence of GM-CSF; LPS-stimulated for 4 h., peripheral blood natural killer cells; unstimulated.; unstimulated basophils; human peripheral blood effector memory T cells; human peripheral blood central memory T cells; human Th1 cells.

RAPID [20] allowed to determine possible functional connection between Primarily Immunodeficiency Genes [3], which were in our case ADA, non PID Genes but expressed in immune cells, non PID Genes – not expressed in immune cells, as well as to understand the lethality and/or immune/hematopoetic phenotype in mice.

This is one of those rare newly developing, even not completed web-sites, which is devoted to the genomics of Immune System. However, even in this database we couldn't find completed source of information related with interaction of ADA gene with the other, immune response related genes. For instance, there was no any information about possible relation of ADA and cytokines, any of them. Thus, the main source of information for today, as we know, is the analysis of the literature trough PUBMED and OMIM.

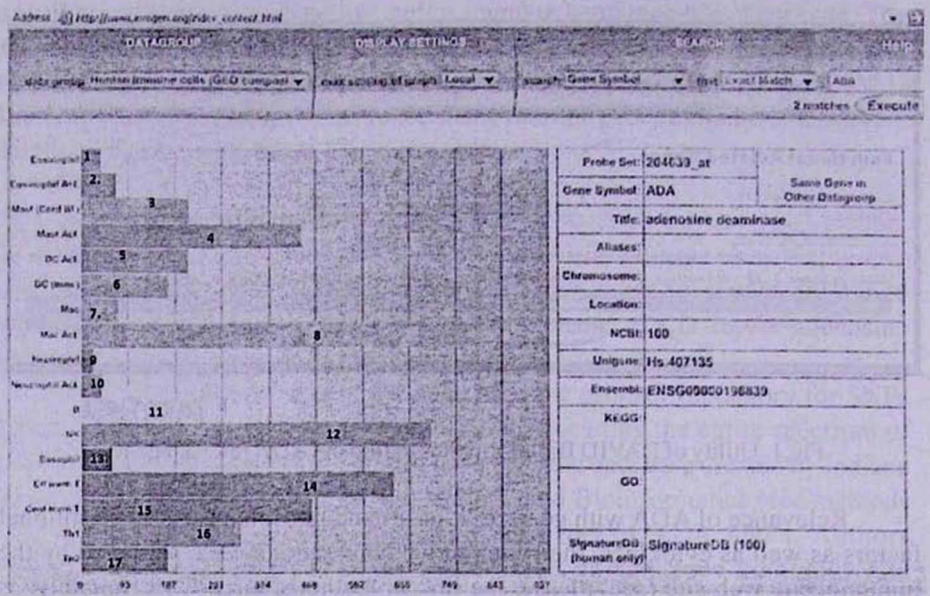

Fig.2. ADA expression profile in Immune Response representative cells. ADA expression profile in every cell type is demonstrated as a separate column. 1. **Eosinophil.** Human peripheral blood purified eosinophils. 2. **Eosinophil Act.** Human peripheral blood purified eosinophils (PMA-stimulated for 2 hours).3. **Mast.** Human cord blood-derived mast cells differentiated over 7-10 weeks using IL-10, IL-6 and stem cell factor; unstimulated. 4. **Mast Act.** Human cord blood-derived mast cells differentiated over 7-10 weeks using IL-10; IL-6 and stem cell factor; IgE-stimulated for 2 hours. 5. **DC Act.** Human peripheral blood dendritic cells differentiated from CD14+ peripheral blood monocytes using IL4 and GM-CSF; then stimulated for 48 hours with LPS. 6. **DC (imm.).** Human peripheral blood dendritic cells differentiated from CD 14+ periperal blood monocytes using IL-4 and GM-CSF; Immature. 7. **Mac.** Macrophages differentiated from isolated CD14+ monocytes in the presence of GM-CSF; unstimulated. 8. **Mac. Act.** Macrophages differentiated from isolated CD14+ monocytes in the presence of GM-CSF; LPS-stimulated for 4 h. 9. **Neutrophil.** Human Cd 16+; CD62L high; CCR3-peripheral blood neutrophils; unstimulated. 10. **Neutrophil Act.** CCR3-peripheral blood neutrophils; LPS-stimulated for 1 h. 11. **B unstimul.** Human peripheral blood B cells; unstimulated. 12. **NK.** Human CD16+CD56+; peripheral blood NK cells; unstimulated. 13. **Basophil.** Human peripheral blood CCR3+ basophils; unstimulated. 14. **Eff mem T.** Human peripheral blood CD4+CD45RO+CCR7-effector memory T cells. 15. **Cent mem T.** Human peripheral blood CD4+CD45RO+ CCR7+ central memory T cells. 16. **Th1 (T-helper 1).** Th1 unstimul. Human Th1 cells. 17. **Th2 (T-helper 2).** Th2 unstimul. Human Th2 cells.

Utility of OMIM web-site [21] had the main impact on our literature search. Literature overview via this web-site allows finding out genetically determined, SNPs based information related with functional disbalance in man. We have narrowed our search by the topic, which will be describing the relevance of ADA

and cytokines. Also, we would like to mention that besides the literature search OMIM has ready database of defined main function characteristics of many compounds. In our case, OMIM helped us to evaluate vitally important processes, where ADA is imposed as a main molecular player: 1. metabolism of nucleotides and related compounds; 2. differentiation of immunocompetent cells; 3. embryonic development.

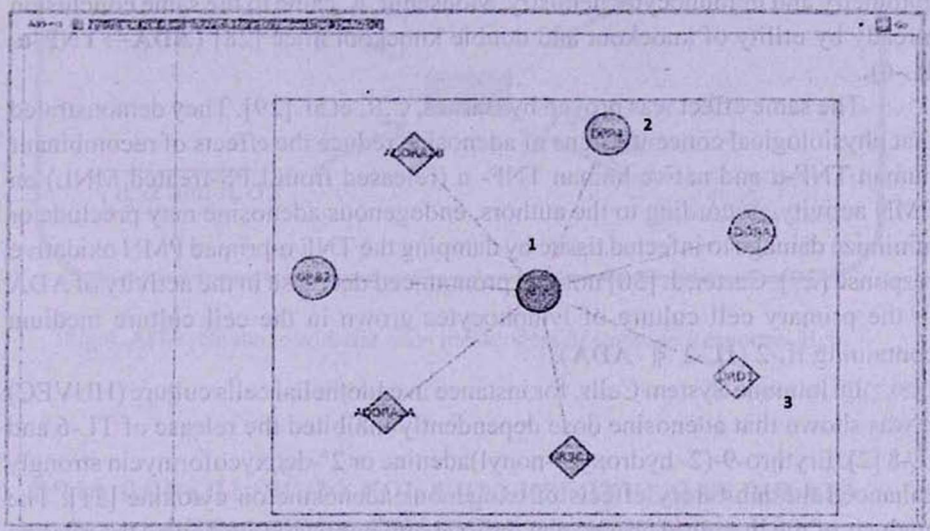

Fig. 3. **Analysis of ADA releavance with the other non/Primarily Immunodeficiency Genes [3] expresed/not expressed in Immune Cells.** (1). PID genes, (2) non PID genes but expressed in Immune Cells, (3) PID genes not expressed in Immune Cells, rectangle points to the lethality and/or immune/hematopoetic phenotype in mice.

It was shown and proved that in the cells of the immune response ADA colocalizing with adenosine receptors on dendritic cells interact with CD26 expressed on lymphocytes and this costimulation brings to the marked increase of proinflammatory cytokines, which are IFN- γ, TNF-α, and IL-6 and to the production of T helper 1 (Th1) [26] (**ADA .→IFN- γ, TNF-α, IL-6).**

On the other hand, it was shown that utility of IL-3 or IL-7 significantly improved the maintenance of in vitro B cell progenitors from ADA-SCID BM cells and allowed the efficient transduction of B and NK cell progenitors [1] (**IL-3 or IL-7 →ADA**).

By means of flow cytometry, immunofluorescence, and immunoblotting techniques, it was found that IL-2 and IL-12 up-regulate ecto-ADA and CD26 expression [27] (**IL-2 and IL-12 → ADA**). In the other study, where there were used mice deficient in the purine catabolic enzyme ADA and developed pulmonary inflammation and mucous metaplasia in association with adenosine elevations, as well as by utility of ADA/A(2A)R double knockout mice, it was shown that there

was an enhanced inflammation comprised largely of macrophages and neutrophils, mucin production in the bronchial airways, and angiogenesis. In addition, levels of the chemokines monocyte chemoattractant protein-1 and CXCL1 were elevated, whereas levels of cytokines such as TNF-α and IL-6 were not. These results are in absolute agreement with above mentioned work, described in the paper from R. Pacheco [26], related with IL-6 overexpression. If R. Pacheco, et al. [26] were demonstrating such a phenomenon on the functional level by utility of flow cytometry and immunocytochemistry, Mohsenin, A. came to the same conclusion already by utility of knockout and double knockout mice [28] (**ADA→TNF-α, IL-6**).

The same effect was proved by Barnes, C.R. et al. [29]. They demonstrated that physiological concentrations of adenosine reduce the effects of recombinant human TNF-α and native human TNF- α (released from LPS-treated MNL) on PMN activity. According to the authors, endogenous adenosine may preclude or minimize damage to infected tissue by damping the TNF α-primed PMN oxidative response [29]. Carter, J. [30] noticed pronounced decrease in the activity of ADA in the primary cell culture of lymphocytes grown in the cell culture medium containing IL-2 (**IL-2 ⫤ ADA**).

In Immune System Cells, for instance in endothelial cells culture (HUVEC) it was shown that adenosine dose dependently inhibited the release of TL-6 and IL-8 [2]. Erythro-9-(2- hydroxy-3-nonyl)adenine or 2'-deoxycoformycin strongly enhanced the inhibitory effects of exogenous adenosine on cytokine [31]. The analysis of the literature proved that only several publications showed the relation of ADA (non-RNA specific form) and interleukins interaction from the stand point genetic alterations.

Zeta chain of the T-cell antigen receptor plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways. Caplan and co-authors [32] presented evidence that the zeta chain, while expressed on the T-cell surface, is associated with the cytoskeletal matrix. A 42-amino acid region in the intracytoplasmic domain of the zeta chain proved crucial for maximal interaction between zeta chain and the cytoskeleton. Rieux-Laucat [33] noted that the finding of somatic mutations of a germline mutation in the CD3Z gene recalled somatic mutations of the adenosine deaminase gene, the interleukin-2 receptor gamma-c gene, the recombination-activating gene-1, the Wiskott-Aldrich syndrome protein, and the NEMO gene. These somatic changes could cause the mutant gene to revert to a wildtype gene or to a sequence compatible with expression of the corresponding protein (**ADA↔IL-2**).

To determine whether IL-13 has an impact on the levels of adenosine and ADA activity as well as to estimate whether adenosine has an impact on the production of IL 13 M. R. Blackburn [34] used ADA null mice. Experimental design of this work allowed to understand clearly whether cytokines might influence the level of adenosine, as well as that adenosine and adenosine signaling contribute to and influence the severity of IL-13–induced tissue responses in lungs.

By utility PUBMED and OMIM and analysis of our results we have created a new interactive scheme, which demonstrates ADA relevance with the main messengers of immune response- ILs (**Fig.4.**).

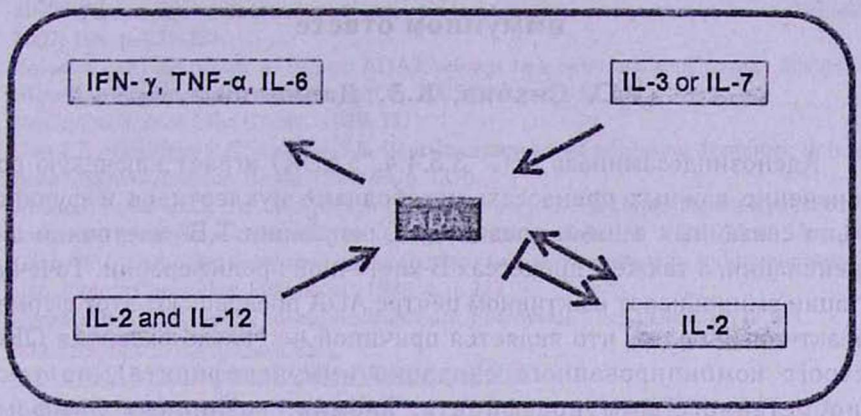

Fig.4. ADA relevance with the main messengers of Immune Response- ILs

Поступила 25.11.09

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Հ.Ս.Սնխչյան, Ք.է.Դանիելյան

Ադենոզինդեզամինազը (EC "3.5.4.4."; ADA) կարևորագույն դեր ունի մի շարք կենսական պրոցեսներում՝ նուկլեոտիդների և նրանց հետ ֆունկցիոնալ կապակցված նյութերի մետաբոլիզմում, T,B-բջիջների դիֆերենցման, ինչպես նաև B-բջիջների պրոլիֆերացիայում: Կետային մուտացիաները ADA-ի ակտիվ կենտրոնում նպաստում են վերջինիս ինակտիվացիային, որը հանդիսանում է հիմք սուր համակցված իմունային անբավարարության համախտանիշի, ծեռքբերովի իմունային արատի, անեմիայի, լիմֆոմաների, ինչպես նաև լեյկեմիաների զարգացման համար: Հարկ է նշել, որ ADA-գեն թերապիան առաջին անգամ կիրառվել է կլինիկայում ADA-գենային դեֆիցիտ ունեցող հիվանդների մոտ: ADA-ի հիմնական իմունային հատկությունները սերտորեն կապված են կինազաների, ֆոսֆատազաների ինչպես նաև ինտերլեյկինների (ILs) գործունեության հետ օրգանիզմում: Սակայն հիմնական ֆունկցիոնալ ADA-գենի փոխազդման յուրահատկությունները ILs գեների հետ հայտնաբերված չեն: Տարբեր կենսատեղեկատվական աղբյուրների տվյալների հետազոտումը կարող է օգնել բացահայտել այդ կապը և լրացնել գիտելիքները այդ բնագավառում:

Функциональная геномика аденозиндезаминазы при иммунном ответе

Р.С. Снхчян, К.Э. Даниелян

Аденозиндезаминаза (EC "3.5.4.4."; ADA) играет ключевую роль в жизненно важных процессах: метаболизме нуклеотидов и функционально связанных с ними соединений, регуляции Т,В- клеточной дифференциации, а также в процессах В-клеточной пролиферации. Точечные мутации аминокислот в активном центре ADA превращают этот фермент в неактивный белок, что является причиной не только развития ОКСИ (острого комбинированного синдрома иммунодефицита), но также приобретенного иммунодефицита, анемии, различных лимфом и лейкемии. Именно ADA- ген терапия была впервые проведена в клинике для лечения ОКСИ у больных с дефицитом ADA-гена. Основная иммунологическая функция ADA тесно связана с функциональной активностью киназ, фосфатаз, а также интерлейкинов (ILs). Однако основные функциональные особенности взаимодействия ADA-гена с генами ILs полностью не выяснены, и использование различных биоинформатических баз данных может выявить эту связь и заполнить брешь знаний в этой области.

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