STRONG MOLECULAR IMMUNOLOGY EVIDENCES ALLOW FOR HYPOTHESIS OF AN EXISTENCE OF A REVERSE PATHWAY (RP) WHICH PASSES GENETIC INFORMATION FROM POLYPEPTIDE ANTIGENS TO VDJ SECTION OF Ig GENE IN B-LYMPHOCYTES

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Abstract

Current Clonal Selection Theory fails to adequately explain the presence of a "hyper mutation" particularly in 3 CDR sections of a V part of Ig genes, which are responsible for recognition and binding of antigens with a high affinity, the degree of wastefulness of random rearrangements of Ig genes in B- lymphocytes and a standard formation of four special recognition palindromic sequences after the1st V-D-J rearrangement.

The precision of the process allows for a hypothesis of an existence of a Reverse Pathway(RP) in B-lymphocytes which passes the exact genetic information from polypeptide antigens back to DNA, permitting for versatility and quick reaction in B-lymphocytes in response to foreign antigens.

The discovery of such a pathway will, undoubtedly, challenge the current Clonal Selection Theory of Immunology as well as the (current) Central Dogma of Molecular Biology which has established the DNA⇔RNA=>Protein pathway of genetic information. It is our hope that our pioneering Reverse Pathway Research Project will prove that the genetic information can follow by both directions: DNA⇔RNA⇔Protein.

The discovery of such a reverse pathway will allow for a new, more effective and affordable treatment methods for curing many of the immune related diseases as well as cancer.

Molecular Immunology Evidences that Speak on Behalf of an Existence of a Reverse Pathway:

The following evidences point to an existence of a Reverse Pathway mechanism which passes an exact genetic information to VDJ (VJ) region of rearranged V parts of Ig genes:

1. "Hypermutation" of Ig gene is not clearly explainable (1,2,3)
2. "Hypermutation" of Ig genes is localized mostly in 3 CDRs of V parts of H and L chains of Ig genes (1, 2)
3. Creations of 2 and 4 standard Palindromic Recognition Segments (PRS) in VDJ and VJ regions of rearranged Ig genes also show existence of undiscovered yet RP mechanism. (2).
4. Wastefulness of "random" rearrangements of VDJ and VJ regions of Ig genes of all B-Lymphocytes also speak against accepted point of view.
5. At high concentrations TI-1 antigens are stimulating proliferation and antibody secretion by as many as one third of all B-cells also couldn't explain by random rearrangements theory . (2)

Explanation of Main Steps of Reverse Pathway Hypothesis:

Pattern (first) re-arrangement:

According to our hypothesis, the first rearrangement of V-D-J sequences of V parts of Ig Genes in pro- and pre-Lymphocytes that occurs in a bone marrow is not a "random" rearrangement, but a Pattern rearrangement!

Because of some of pattern antigens encounter repeatedly in some of the Microbes groups and are the same and characteristic for these group of Microbes, evolution of Immune System had created Toll Like Receptors (TLRs) for quick reacting to these pattern antigens.

According to our hypothesis existing of numerous v-exons in the V parts of H and L chains in B-Lymphocytes are also pattern exons corresponding to different pattern antigens! Therefore after getting corresponding TLRs pathway signaling through cytokines, chemokine produced by Macrophage and Stromal Cells of Bone Marrow, V parts of Ig genes of pro, pre-B-Lymphocytes exactly choose corresponding v-exon from the existing repertoire of V section of Ig genes and undergo to first pattern rearrangement! It is not random rearrangement!

Such rearrangement produces specific IgM for the type of pattern antigens, however it lacks the high degree of affinity towards to many unknown polypeptide antigens on microbes .

The first pattern rearrangement of V(D)J sequences of Ig genes creates 2-4 Palindromic Signal Sequences which later allow mature lymphocytes to replace nucleotides between palindromic sequences to nucleotides which code for higher affinity amino acids sequences to an antigen using genetic information coming though Reverse Pathway (will explain later). (fig.1) (2)

Figure 1. VDJ sequence after 1st rearrangement

- After making stalk between 2 palindromic heptamers and 2 nonamers (1-term RSS+ 2 term RSS) and cutting them together with intervening sequences of 12+23 bps (7bp+9bp +12bp+23 bp)=51 bpx2=102bp by Recombinase (RAG-1;-2 encoded enzymes) between V_H---D---J_H, endonucleases cut hairpins and repair enzymes generates 4 palindromic bps and **TdT** adds 2 N- nucleotides (up to 15 nucleotides) between

V_H-p-N- p-D-p-N- p-J_H

- Since this diversity occurs at VDJ coding joints, it is localized in **CDR-3** of the Heavy chain genes!

Reverse Pathway Replacement (Affinity Maturation):

RP replacement of nucleotides in V(D)J regions of Ig genes to yield a higher affinity immunoglobulin, takes place in germinal centers of secondary lymphoid tissues, such as lymph nodes or spleen, based on the exact genetic information received from MHC-2:antigen (or MHC-1:antigen?) complexes on B-lymphocytes by undiscovered yet Reverse Pathway mechanism.

The proposed mechanism of a Reverse Pathway, although needs further research confirmation, is as follows. Initially mature B-cells internalize the antigen by it's new but less affine membrane bound IgM. In endosome-lysosome of B-cell antigen is digested by hydrolytic enzymes to small polypeptides and connected with MHC-2 in B-cells. Then, in special endosome, small antigen polypeptide on MHC-2 create non-covalent affinity connections with activated amino acids-t-RNA complexes. Following that, a unknown yet RNA –Protein Enzyme Complex (Reverse Ribosome?!) cuts and connect anti-codons of t-RNA of matching activated amino-acids to antigen amino-acids and that way creates an exact RNA template of polypeptide of high affinity to antigen (fig.2), which connects with two nuclear receptors dimers and moves to nucleus of B-lymphocytes. In the nucleus, it connects with the two Palindromic Recognition Sequences (PRS) of VDJ (VJ) parts of Ig Genes. Then, an endonuclease cuts Genomic DNA between 2 palindromic sequences in VDJ (VJ) section of Ig genes and DNA repair enzymes replace nucleotides using the template RNA which is carrying high affinity genetic information about the antigen to VDJ (VJ) segments of Ig genes. After that, there is going class switching and transcription of high affinity IgG m-RNA and translation of high affinity IgG to given antigen (see: fig.3).

Figure 2. A proposed mechanism for creation of an antigen-based template RNA.

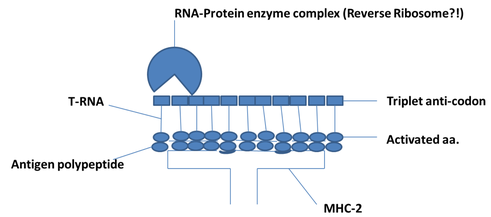
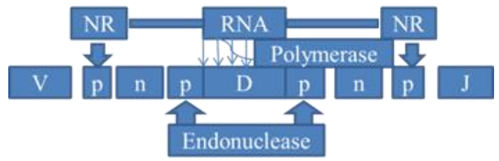


Figure 3. Replacing of the sequences coding for a high affinity Ig into the VDJ section of Ig gene



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Future Directions:

After discovering of RP will be begun experiments inflicting of damages to different steps of RP and corrections of these damages. Results of this research will bring new more effective treatment methods of many immune related diseases, including cancer.