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RNA interference: the role in antiviral immunity and immune memory

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Abstract

The role of innate immunity in neutralization of viral infections (including COVID-19) and forming long-lasting and specific immune memory is considered. It is assumed that antiviral protection is generated by the mechanism of RNA interference (RNAi) and is based on the presence of specific viral patterns in the DNA library of the host cells.

"Memory" of innate immunity and antiviral response

It is well known that innate immunity also has the ability to memorize and learn (1-10). Plants are capable of forming the so-called systemic acquired resistance (SAR). It has been demonstrated that a primary infectious agent causes systemic resistance to repeated use of the same or similar agents (1). In invertebrate metazoans, protective innate immune responses are also modulated by previous exposure to various infectious agents (2–5). Interestingly, such enhanced reactivity of the invertebrate immune system can last a long time and has been found active in the offspring up to the third generation (7). Furthermore, among vertebrates, it has been clearly demonstrated that the innate component contributes to the formation of immune memory. An increase insensitivity of macrophages, monocytes and NK-cells to the repeated exposures of the pathogens is well documented phenomenon. Epigenetic changes in these effector cells are noted (8,9).

When considering antiviral defense mechanisms in the living kingdoms bacteria with its very effective yet smart CRISPR-Cas system has vowed scientists in our understanding of the complexity of cellular capacity to fight bacteriophages, bacteria's viruses. A close analogue of such a protective system in eukaryotes is RNA interference (11). Plants and invertebrates make extensive use of this mechanism against viruses (12-15). Vertebrates, on the other hands, in addition to the antiviral RNA-i system (16-19) employ an interferon-activated antiviral system (20, 29-30). This protein-based defense system effectively combats viral infections, but it also blocks the operation of the anti-virus RNA-I system (21, 31-34). An analysis of published data allows us to shed a light to this contradiction and explain the phenomenon of long-term antiviral memory.

Hypothesis of generation specific antiviral memory by innate immune system

It has been shown that exogenous viral RNAs are cleaved in the cytoplasm by the Dicer enzyme into short fragments of about 20 bases in length, called small interfering RNAs (siRNAs) (22). Notably, these siRNAs by themselves act as active components of RNA-i. However, in order to

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form long-lasting memory those fragments must be reverse-transcribed and then inserted into host-cell DNA. The very existence of such mechanisms has been described in the research of retrotransposons and pseudogenes (23-24), where intracellular reverse transcriptase ORF2 has been implemented in transforming cytoplasmic RNA and retroelement transcripts into complementary DNA parts. Moreover, those elements account to almost half of human DNA in the cells (25, 26). It is tempting to assume then that at least some parts of human DNA are coded DNA fragments of virus genome. This is some sort of "black list" of nucleotide sequences, prohibited for cell translation. After transcription of these sequences mRNA is then cleaved by the Drosha nuclease into micro RNA (miRNAs) sized by 21-23 length fragments. Micro RNAs has been frequently observed during infections caused by both DNA and RNA viruses (16). Those interfering RNAs no matter how they were formed, either by the Dicer (siRNA) activity or by Drosha (miRNA), would bind with RNA-induced silencing complex (RISC). Active RISC ensures binding between interference RNA and a complementary viral sequence and consequently potentiates cutting a viral RNA by aid of Argonaute protein, ultimately inhibiting translation and/or RNA deadenyaltion (27, 28). When re-infected RISC system is readily alert to degrade viral RNA or to inhibit viral protein translation. This is a proposed mechanism of antiviral immune memory in a nutshell.

In regards to the above mentioned antagonism between interferon and RNA-i protective system, again a closer look into localization of virus infection might give a clue. When viruses enter the cells interferon release is triggered. Interferons signal cascades of protective responses including apoptosis (29-30). Neighboring cells react to the interferon signals by switching them into "alarm mode", which is usually blocks DICE/Drosha-RISC mechanism (31-34). However, as it turns out, this is not a case with actively proliferating and unipotent cells. (35). First line of cells that encounter virus entry is usually well differentiated surface epithelial or endothelial cells. They deal with the small virus load via interferon induced protection (29-35) and they do not participate in forming specific infection memory. True immune memory is formed only in unipotent precursor cells when either virus enters in them or interference RNAs. Only de novo formed differentiated surface cells will possess specific antiviral memory giving them a powerful tool to effectively eliminate additional loads of virus. Maturation of the novel endothelial and epithelial cells usually takes several days which constitutes a required time for forming specific antiviral memory. It also mean that this memory is local in its nature, and in order to have a systemic memory multipotent cells should be involved. Furthermore, for innate immunity being able to protect further generations or off-spring, germ line cells should be impacted as well.

Therefore, in fact there is no any antagonism in the two antiviral protection systems of innate immunity. It all depends on the level of cell differentiation and location of these cells.

Discussion

The above described mechanism of forming immune memory, by all means does not down value development of acquired immunity via specific CD4, CD8 cells along with humoral antibody-based antiviral immunity. This function of acquired immunity is well known, although the main role of this additional mechanisms in vertebrates might lie with the preservation of the integrity of their own cells, control over their change during aging and destruction processes (36-38). From this point, antibodies are produced against all antigens that a body encounters, including any viral proteins. However, sometimes such "antiviral" antibodies are rather more harmful then

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protective. This is evident with the phenomenon of antibody-dependent enhancement (ADE) of the infection (39-42). In ADE virus not only infects susceptible cells through appropriate receptor but is able to highjack virus-specific antibodies to easily traffic virus bodies inside the monocytes/macrophages, granulocytes, platelets, mast and many more host cells through interaction with Fc and/or complement receptors (40). There are numerous examples of ADE, triggered by alpha- and beta-coronaviruses (43,44). Primates, vaccinated with modified Ankara vaccine virus encoding the full-length SARS-CoV glycoprotein, despite of low viral loads suffered from severe lung injury due to ADE (44). ADE phenomenon has been observed on the animal models of SARS and MERS (40). It has been shown that SARS-CoV-1 is capable to enter macrophages via antibody dependent route and even replicate in those cells (45, 46).

In connection with the foregoing, it is necessary to make adjustments to the assessment of herd immunity to COVID-19, which is at the moment traditionally based only on the measurement of the neutralizing antibodies titers. Another tool for this assessment should be DNA tests confirming the formation of new sequences in human cells corresponding to SARS-CoV-2 RNA. Future vaccines against SARS-CoV-2, the prospects of which are still remain elusive (49, 50), will also have to pass the same evaluation of effectiveness. It is necessary to draw the attention of medical community, especially practitioners to the role of innate immunity, which is especially important in the current COVID-19 pandemic.

Conclusion

To form long-term and specific antiviral memory the human body actively uses the mechanism of RNA interference, inserting for this purpose the nucleotide sequences corresponding to encountered viruses into the DNA of host cells.

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