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PhD research proposal: Stages of microbial evolution with regards to accessibility to the human immune system

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Abstract

Microbial evolution may represent a highly complex subject in the biomedical scientific subjects of microbiology, immunology and vaccinology. Given that the microbe and the host represent living forms, the laws of life and intelligence apply in both cases. As a result, the laws of evolution automatically apply equally for the human host and the germ, and given the fact that the immune system represents a major element of the terrain, it can be suggested that it is both the terrain and the germ that can be calibrated to an increased extent. Given the progress of the direct and indirect microbial impact upon various major elements of human immunity, it may be hypothesized that microbes have been evolved in stages, in relation to the two major departments of the immune system; the innate and the adaptive. Namely, throughout the previous centuries, the majority of microbes have evolved by producing diverse accessory proteins to suppress the interferon system in various stages, which plays a central role in stimulating and modulating major immune responses. Toward the end of the 20th century, a new virus emerged in the environment and it was found to directly infect central elements of the immune system; CD4+ and CD8+ T-lymphocytes. Interestingly, Hepatitis C Virus (HCV) and Nipah Virus (NiV) were indicated, via in-vitro studies, to have some mechanisms of directly infecting T-lymphocytes. Another clinical study indicated that the interferon system of T-Lymphocytes is suppressed by the NiV infection. In the beginning, microbes evolved to gain access to the profound, foundational layers of human immunity; the first-line immune system. As time went by, microbes then evolved to gain direct access to the central layer of immunity; the adaptive immune system, which is deemed as the department of the immune system with a memory. With regards to intelligence, it is the adaptive immune system that has a central importance. Nevertheless, the innate immune system has a central importance with regards to the support in evolutionarily outcompeting microbes altogether, as it may be stimulated over time to produce faster signals and prevent them from silencing their key genes, which include Interferon Genes (INGs) and Interferon-Stimulated Genes (ISGs). This may show that foundations are sometimes actually situated at the periphery, and the data collected in this study may hint that such an analogy applies to evolutionary immunology. When the innate immune system is repeatedly outcompeted, there may be a risk that microbes evolve to directly target major elements of the adaptive immune system. The aim of this study involves highlighting the

importance of a wider inclusion of the interferon system in the major vaccinology-related efforts to contain present epidemics and prevent the onset of future deadly epidemics and pandemics.

Introduction

Pathogenic agents represent living multicellular, unicellular or particle-like formations that contain a genetic code, meaning that they contain their own long-term intelligence system that comprises an evolutionary pattern in relation to their opposition side, which comprises the host immune systems. An immune system constitutes an antimicrobial defense system that is situated within a living organism. Any living cell or particle that is pathogenic in nature or that contributes to processes of pathogenesis and pathogenic distribution is automatically deemed as toxic and likewise becomes a target of immunity. Sometimes, however, the immune system targets its own elements in error, and this phenomenon is known as autoimmunity. Induced autoimmunity most likely represents a major element of strategy used in the process of microbial evolution, and the recent COVID-19 pandemic may represent an important sign of this. Sometimes, human errors in the clinical environment may facilitate microbial evolutionary patterns. The genetic background, the frequency of genetic mutation, the reproductive rate, the incubation period, the means of transmission and the extent of capability to evade first-line immune recognition represent aspects of prime importance in the observation of the extent of evolution in certain pathogenic agents. A prolonged incubation period may often represent a relevant sign of advanced pathogenic evolution, given the displayed lack of adequate immune action against the microbial load/count prior to the onset of clinical symptoms. Microbial self-camouflage represents a relevant problem in modern-day immunology as it leads to a more frequent incidence of severe holo- and auto-immunological disease.
Polarized views in society and incomplete scientific discernment facilitated the spread of pseudoscientific ideas on multiple dimensions, and it is perhaps perception that plays a major role in shifting focus to various pieces of information that are more or less accurate in nature. The truth may be that, whilst a significant number of toxins have a tremendous potency to cause severe disease and lethality, there are certain layers of the immune system that can be calibrated and given tremendous evolutionary advantage over such microbes on a long-term basis if the accurate intellectual and research tools are used, and the evolution of human intelligence applies abundantly to the matter of immune evolution. All layers of the existing matter are profoundly and yet subtly interconnected, and Science has recently proven that the Universe is not locally real, around a century and a few decades after the same Science had proved that all elements of physical existence are relative, which ultimately indicates the highly complex nature of the relativity of the germ and the terrain in the life-sustaining environment, although relativity may appear as a simplistic system at first. Interestingly, the fact that it is scientifically correct to mention that the Universe is real and that the University is not real simultaneously ultimately indicates that the diversity of human perspective is far broader than we might have previously thought. Hence, it is a perspective as such that can still make a tremendous difference in research and innovation, despite the reputed scientific aims for exact methodology and results. With regards to the applications of principles from natural immunity into vaccinology, there are now considerable hints that placing a more voluminous importance

upon the direct sensitization of the interferon system could be equivalent to building an immunological highway in the discipline and industry of vaccinology.

Discussion

Section 1: Centuries of innate immune escape led to a final, adaptive immune targeting

Microbial agents have a Universal ability to transiently suppress the first-line immunity of their host unicellular and multicellular organisms, and immune suppression as such comprises three principal methods: the methylation of the 5’ end of the microbial genome, the enhancement of the production of reactive oxygen species, which goes hand-in-hand with the manipulation of the mitochondrial activity, as well as the usage of produced cytoplasmic tunneling nanotubes (TNTs) to evade signaling recognition by various pattern recognition receptors (PRRs), which include Toll-Like Receptors (TLRs) 3, 4 and 8, Retinoic acid Inducible Gene-I-Like receptors (RLRs) and C-type Lectin Receptors (CLRs), resulting overall in a lower extent of Type I and Type III Interferon production rate and in a lower activation rate of Interferon Stimulated Genes (ISGs) during critical stages of microbial reproduction and distribution (Li D. and Wu M., 2021). Numerous viral pathogenic agents contain genes that encode various non-structural proteins, which are also known as accessory proteins, and open reading frame products that antagonize the interferon system in all its stages of function and distribution of function. To exemplify, the genomes of Influenza A and SARS-CoV-2 variants of public health concern translate non-structural proteins 1, 2, 4, 6, 10 and 16, as well as open reading frame products 6, 8 and 9b, which play major roles in desensitizing the interferon system in stages that include the prevention of Toll-Like Receptor 3 and 7 recognition of various Pathogen-Associated Molecular Patterns, the prevention of the binding of cGAS with STING, the translation of interferon-alpha and interferon-lambda-encoding mRNA, the prevention of the polymerization of STAT proteins in neighboring cells that contain the IFNAR1/2 and IFNLR1/IL10R2 receptors, the restriction of ISG activation extent, as well as the lysis of various anti-inflammatory cytokine proteins that are produced by ISGs. As a result, a transient inhibition of the interferon system during critical stages of microbial load or count increases very often leads to a restricted extent of adaptive immune activation, as well as to a significant disruption of the stimulatory-modulatory spectrum during adaptive immune activation. Likewise, there is an increased probability that a wider focus upon the sensitization of the core element of first-line immunity, the interferon intracellular and intercellular system, rather than upon continuing to directly offer the adaptive immune system the genetic information of numerous pathogenic agents and risking an exaggerated focus upon the direct stimulation of adaptive immunity by a pathogen with a specific genetic information, will favor a major shift of the evolutionary battle between the depths of human immunity and the ability of microbes to suppress them, and rather directly as well. In short, the clinical research community may be required to change the strategy of preventing and containing epidemic and pandemic crises, by paying a greater attention to the potential opportunity of effectively encouraging the immune system to build fast-paced first-line defense mechanisms before and during threatening public health crises, as pathogenic agents have continued to build

increasingly tricky methods of evading cellular recognition during the stages of microbial distribution in which the load increases sharply and exponentially. A matter of proximal certainty can be observed, that novel infectious diseases will continue to emerge and spread in the human population, as a result of new zoonotic spillover events, and the continuing to offer the adaptive immune system direct information with regards to the genetic information of new pathogens may ultimately overload the adaptive immune memory with new information and actually start inducing delays in its overall developmental rate. Interestingly, the acquired immune system seemingly contains its own intelligence and the information contained by the genomes of live-attenuated, inactivated and dead microbes is transferred to its memory for the scope of preventing the development of hyper-inflammatory immune responses and consequently, of severe disease. Hence, information probably lies within the foundational layers of physical matter, alongside energy. There is a continuous process of information and energy exchange occurring within each living organism, and it is ultimately probable that information constitutes a form of energy, as it seems to accurately fit under the First Law of Thermodynamics. The process of immune learning ought to be as natural as possible, and as the current stages of immune evolution indicate, continuing to directly offer the adaptive immune memory information about specific pathogenic genomes may cause it to lose the focus upon the big image of the current stages of microbial evolution, thereby resulting in the onset of an evolutionary decay of human immunity in relation to modern-day living pathogens.

Section 2: The natural selection of HIV and challenges for Modern Science

As the general members and audience of the scientific community are aware, the human immunodeficiency virus directly targets central elements of the human adaptive immunity, including helper CD4+, cytotoxic CD8+ T-lymphocytes and B-lymphocytes, whence important types and sub-types of immunoglobulin antibodies are expressed and matured. The selection of retroviruses may have revealed the final evolutionary objective of microbes, which is to directly and completely subjugate all biological systems and micro-systems that are deemed as part of the opposing side, even if such a side only functions in defending its own organism. The fact that other contemporary viruses have a less widespread mechanism of action implicating a direct hijacking of adaptive immunity may indicate that, prior to the selection of the retrovirus, there may have been other evolutionary events of microbial competition, as other viruses managed to directly reach central elements of human immunity as well. For example, NiV was found to infect T-lymphocytes and inhibit their Type I Interferon responses, leading to a phenomenon that may be deemed as innate immune escape within an adaptive immune escape. The complexity of the concept of immune evasion is, likewise, indicated to be higher than previously projected, even by the brightest of the minds in the scientific realm. Hence, it may be more crucial than ever before to develop mechanisms of innate immune sharpening, as there is now growing evidence of a tremendous positive implication in the efforts of vaccinology. A few theories also suggest that human intelligence may have been used within areas of the clinical environment in ways that catalyzed microbial evolution. Conclusive evidence remains unclear, as we are still situated in the beginning stages of immunological and microbiological research. It is important to mention that even the most localized or isolated mistaken effort in

clinical research worldwide could end up catalyzing microbial evolution, and possibly not catalyzing human immunological evolution as much. During times of infiltration of personal opinion and ideology in the scientific discipline, the most important action one could take is to strongly attain neutrality of opinion and likewise, objectivity. Moreover, whilst knowledge of all sides of the major problem is crucial, it is widely possible that scientific efforts should be focused not on the possible deep and hidden shadows of the health problems that have been crippling numerous members of society, but on the possible solutions, whose light are far more powerful than the darkness of the possible hidden roots of problems.

Section 3: How a sharp microbial evolution played a role in disrupting the normal rate of neuronal and systemic development

A sharp microbial evolution over the past few centuries was made possible particularly by the increasing ability of first-line immune evasion. As a result, the onset of significant forms of infectious disease in expecting mothers and also in young children that are undergoing critical stages of neuronal, immunological and systemic development is likely associated with an exponential increase of the incidence level related to human developmental delays, via induced neurodevelopmental diseases. The problem seems to be in its initial stages, as the number of related cases seems to have only begun to sharply increase. Namely, the existence of a fine and profound association between developmental adaptive immunity and developmental neurology makes it plausible that the onset of moderate and severe infectious disease will disrupt the developmental rates of important encephalic sub-regions, leading to uneven levels of development in major brain sub-regions, which may explain the nature of autism spectrum disorder, which does not characterize an equally underdeveloped set of neuronal regions, but rather by a set of neuronal regions that are unevenly overdeveloped and underdeveloped respectively (i.e. overdeveloped brain sub-region that is related with cognition and underdeveloped sub-region that is related to speech). Given that the nervous system co-ordinates all functions of the organism, the induction of more severe cases of neurodevelopmental disruptions result in the induction of systemic developmental disruptions as well. With regards to infected expecting mothers, it is important to highlight that the regular transfer of IgG antibodies from the mother to the fetus via the umbilical cord makes it rather likely that the fetal neuronal developmental rates become affected via a passive, maternal infection. It is likewise the advanced and widespread level of microbial immune escape that is the primary factor for an increased incidence of neurodevelopmental delays in babies in modern and contemporary history (Carp T. and Metoudi M., 2022). A possible change in the overall strategy of vaccination could likewise not only prolong human lifespan by modulating metabolic processes and by decreasing the general demand for energy consumption as a result of less infectious disease of significant intensity, but also prevent many cases of induced neurodevelopmental and systemic disruptions. In short, it may be the advanced and weakly controlled pathogenic evolution that caused the majority of the major health problems the contemporary society has been facing.

Section 4: Possible challenges of modern-day mRNA vaccines and acknowledging new evidence

Toward the end of the 20th century, scientists discovered a novel method that vaccines could be developed and rolled out; via the production of mRNA molecules, which are set to be genetically modified (by changing uridine nucleotides into pseudouridine nucleotides in order to make them more stable) and protected by an outer layer of lipid nanoparticles (LNPs) to temporarily resist cellular RNase enzymes and be able to enter multiple cells. The new discovery looked promising, with scientific projects aiming to prevent significant forms of cancer and infectious diseases. The entire scientific community knew that, once mRNA molecules have been expressed by genes, no nucleotide would become DNA nucleotide again, so it would not go back into its gene. Likewise, we became conditioned by our increased state of certainty that transcription is a one-way system and ideas questioning this theory would not be taken seriously. Then, retroviruses became naturally selected and viral reverse transcription was discovered. Following the natural selection of HIV, it was also discovered that the human organism encodes approximately 17% of the Long-Interspersed Nuclear Elements 1 (LINE-1) regions, meaning that human host cells have the resources that retroviruses require to have their single-stranded RNA nucleotide chains reverse transcribed into double-stranded DNA. In short, microbes evolved according to the “weaknesses” of the human genetic code.

Recently, scientists discovered that numerous human cells encode enzymes known as RNA theta-polymerases, which not only are capable of reverse transcribing mRNA molecules back into DNA molecules and of favoring insertion of such newly-reverse transcribed DNA into both non-coding and coding regions of the cellular DNA, but they were also found to have a significant incidence of error, leading to events of gene mutation. Other types of polymerases with similar functions were also found to exist. Such a set of discoveries seems to be demolishing the hope that mRNA vaccination can eradicate significant forms of cancer, as they now seem to be giving extra genetic information to both non-functional and functional areas of the human cellular genome indiscriminately, and it is Universally known that a point mutation in a central area of a gene causes a major change to the genetic information of such a gene, leading either to a significant loss-of-function of its protein, or to the production of short and ineffective proteins. Even the development of a vaccine candidate with a non-”self” mRNA molecule encoding an element of human immunity may interfere with the cell’s genetic information, as the human genome and the genetic background of its proteins are highly diverse. Normally, RNA theta-polymerases function in repairing DNA segments that contain errors and they constitute a well-known therapeutic target in cancer (Chen X. et al., 2021). Nevertheless, a fine line between DNA repair and DNA mutation might exist, and the insertion of mRNA genetic information that is foreign to the genetic information contained by the host DNA may cross that line more often than one may first project (Chandramouly G. et al., 2021). Patients with genetic and immunological risk factors may be at a particular risk of developing long-term genetic adverse events via the occurrence of frameshift mutations and/or genomic toxicity.

The definition of a safe and effective therapy and vaccine is the agent that does not interfere with the host cell’s genetic information in any manner and in any dimension. Whilst it is essential to give all the credits to the merits of previous innovative research and observe the potential beneficial effects of mRNA vaccination for many people, it can be observed that, in a substantial number of cases, there would be a system of functional genetic mutation exchange and

likewise, it is only ethical to state that protein-based vaccines may look safer and possibly more promising, at this stage, than mRNA-based vaccines.

Conclusion

In this manuscript, the highlight is on the knowledge of all roots of modern-day problems that led to the advanced natural selection of pathogenic agents causing crippling disease to numerous members of the global society, as well as on the possible solutions that could bring the advance of the human species with centuries on the evolutionary scale. The development of natural immunity-based therapeutics and vaccines (i.e. recombinant human interferon-alpha and interferon-lambda with plant proteins stimulating the healthy expression of interferon-encoding genes) would not only cancel the effects of innate immune evasion and of direct adaptive immune targeting, but could also prolong the general human lifespan with decades on a medium-to-long-term basis, as there are profound regulatory effects upon the cellular metabolic machinery as well. Namely, an overall sharpening of the interferon system could prevent several microbes from dysregulating the activity of mitochondria, which in turn would prevent the aberrant synthesis of reactive oxygen species (ROS) and ultimately, of excessive induced cell death pathways. As a result, the overall demand for energy consumption could significantly decrease, leading to a slower cellular and tissular aging process. Recombinant immune proteins containing N-terminal or O-terminal glycosylation sites are probably more effective as therapeutic agents, given their higher accessibility into cells due to the presence of carbohydrate macromolecules on their plasma membrane, and such proteins could also play a role in modulating glucose-related intra-cellular metabolic processes. Overall, microbial evolution may have played a major role in the progressive decay of human metabolism, which involved increasing demands for energy consumption and, consequently, an increased speed of cellular and tissular aging. The knowledge of the exact steps that microbes used throughout entire centuries to gain direct access to the core elements of human immunity may have given the scientific community the necessary knowledge to stimulate the counter-evolution of human immunity from its core foundations onward. As Isaac Newton stated in his Third Law of Motion, to every reaction, there is always an equal counter-reaction. It may also be important to note the difference between a response, which seeks to remove a problem with its usually-complex core, and a reaction, which only seeks to directly target the weapons developed and used by the problem, which only comprise its overall surface.

Reference list

1. Li, D., Wu, M. Pattern recognition receptors in health and diseases. *Sig Transduct Target Ther* 6, 291 (2021). https://doi.org/10.1038/s41392-021-00687-0
2. Carp, T.; Metoudi, M. Profound Associations between Maternal Infectious Disease and Fetal Neurodevelopmental Delays. Preprints 2022, 2022120190. https://doi.org/10.20944/preprints202212.0190.v1.

3. Winkler, R., Gillis, E., Lasman, L., Safra, M., Geula, S., Soyris, C., Nachshon, A., Tai-Schmiedel, J., Friedman, N., Le-Trilling, V. T. K., Trilling, M., Mandelboim, M., Hanna, J. H., Schwartz, S., & Stern-Ginossar, N. (2019). m6A modification controls the innate immune response to infection by targeting type I interferons. *Nature immunology*, *20*(2), 173–182. https://doi.org/10.1038/s41590-018-0275-z
4. Skardasi, G., Chen, A. Y., & Michalak, T. I. (2018). Authentic Patient-Derived Hepatitis C Virus Infects and Productively Replicates in Primary CD4+ and CD8+ T Lymphocytes *In Vitro*. *Journal of virology*, *92*(3), e01790-17. https://doi.org/10.1128/JVI.01790-17
5. Gerberick, A., DeLucia, D. C., Piazza, P., Alaoui-El-Azher, M., Rinaldo, C. R., Sluis-Cremer, N., & Rappocciolo, G. (2021). B lymphocytes, but not dendritic cells, efficiently HIV-1 *trans* infect naive CD4 + T cells: Implications for the viral reservoir. *MBio*, *12*(2). https://doi.org/10.1128/mbio.02998-20
6. Fu, Y., Zhang, Z., Yang, Z., Jiang, Y., Han, X., Xu, J., Chu, Z., Ding, H., He, S., & Shang, H. (2021). CD27−CD38+ B cells accumulated in early HIV infection exhibit transitional profile and promote HIV disease progression. *Cell Reports*, *36*(2), 109344. https://doi.org/10.1016/j.celrep.2021.109344
7. Hu, W. S., & Hughes, S. H. (2012). HIV-1 reverse transcription. *Cold Spring Harbor perspectives in medicine*, *2*(10), a006882. https://doi.org/10.1101/cshperspect.a006882
8. Cervantes-Ayalc, A., Ruiz Esparza-Garrido, R., & Velázquez-Flores, M. Á. (2020). Long Interspersed Nuclear Elements 1 (LINE1): The chimeric transcript L1-MET and its involvement in cancer. *Cancer genetics*, *241*, 1–11. https://doi.org/10.1016/j.cancergen.2019.11.004
9. Chen, X. S., & Pomerantz, R. T. (2021). DNA Polymerase θ: A Cancer Drug Target with Reverse Transcriptase Activity. *Genes 12*(8), 1146. https://doi.org/10.3390/genes12081146
10. Chandramouly, G., Zhao, J., McDevitt, S., Rusanov, T., Hoang, T., Borisonnik, N., Treddinick, T., Lopezcolorado, F. W., Kent, T., Siddique, L. A., Mallon, J., Huhn, J., Shoda, Z., Kashkina, E., Brambati, A., Stark, J. M., Chen, X. S., & Pomerantz, R. T. (2021). Polθ reverse transcribes RNA and promotes RNA-templated DNA repair. *Science advances*, *7*(24), eabf1771. https://doi.org/10.1126/sciadv.abf1771
11. Daniel Garisto (2022), The Universe Is Not Locally Real and the Physics Nobel Prize Winners Proved It, available at: https://www.scientificamerican.com/article/the-universe-is-not-locally-real-and-the-physics-nobel-prize-winners-proved-it/