Article

Expression of concern with regards to the current stages of avian Influenza A (H5N1) zoonotic spillover into humans

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Abstract: The Influenza A Virus (IAV) represents a positive-sense, single-stranded RNA-based virus that infects mammals mainly via the respiratory system, although other bodily systems are also infected and undergo various extents of inflammatory pathogenesis. There are two well-known strains of IAV that cause life-threatening disease in mammals; H1N1 and H5N1, and the first strain caused the 1918 IAV H1N1 pandemic that claimed between 30 and 50 million human lives. Due to the significant ability of IAV to evade important immune recognition, the virus was observed to favor the onset of secondary microbial infections (i.e. bacterial or fungal), as the overall performance of the immune system became transiently weakened during the viral infection. During the IAV H1N1 pandemic, many patients died as a result of bacterial pneumonia, as pathogenic bacteria, such as Streptococcus pneumoniae and Haemophilus influenzae, gained a wider opportunity to colonize and infect vital areas of the lower respiratory tract, and such a phenomenon led to the excessive, prophylactic usage of antibiotics due to the increased levels of panic, which in turn favored the natural selection of bacteria with genes that became resistant to such antibiotics. Antibiotics might be required for usage solely when bacteria are known to be colonizing vital areas of the human body, and this aspect is tricky, as colonization is asymptomatic and screening is consequently rare. Recently, new variants of the avian IAV H5N1 strain were transmitted from live, infected birds to mammals, including humans in some isolated cases, and given that there have already been several zoonotic spillover events overall since the beginning of 2023, we are rapidly approaching the time when a zoonotic spillover into humans will mark the first epidemic outbreak of the avian flu in humans. A lethality rate of 60% was projected by the World Health Organization, as the virus was shown to favor the development of life-threatening hyper-inflammatory responses at the levels of alveolar tissues constituted by Type II pneumocytes. There are hints that novel variants of H5N1 are capable of infecting the intestinal layer, as recently, two dolphins died as a result of ingesting infected birds within the area of the British Isles. IAV is known to suppress the production and transmission of Type I Interferons by expressing various non-structural proteins (NSPs), such as NSP1, which was found to be also packaged into exosomes and transmitted to neighboring uninfected cells, thereby preventing them from responding to the virus in the first place. A more pronounced rate of innate immune evasion would probably be observed in H5N1 IAV infection than in the infection caused by recent variants of H1N1 IAV. The H5N1 strain of IAV was also found to secrete a higher concentration of NSP1 than SARS-CoV-2, indicating the existence of an association to the greater mortality rate of H5N1 IAV infection. A direct, prophylactic stimulation of the interferon system using a reduced oral or nasal dosage of recombinant anti-inflammatory and anti-viral interferon glycoproteins may represent the most viable approach to prevent an emergence of a life-threatening H5N1 IAV pandemic. A similar non-invasive approach could be developed for an Marburg Virus (MARV) and a Nipah Virus (NiV) infection of humans, as risks of the emergence of a Marburg epidemic and also of a Nipah epidemic may be substantial at this stage as well. Clinical testing of clinical approaches as such could be of critical importance at the moment. Animals could also benefit from related clinical approaches.

Keywords: H5N1, Influenza A, first-line immunity, interferon, mucosal antibodies, immune evasion, sialic acid receptor, natural lymphocytes, adaptive lymphocytes, monocytes, macrophages, cytokines

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1. Introduction

The H5N1 strain of IAV represents the version of the viral microbe that is among the most concerning on Earth, with strong hints that a final zoonotic spillover event leading to an epidemic outbreak in humans would cause around 60% of the infected persons to lose their lives, according to projections made by leading scientific researchers and authorities. The hemagglutinins (HAs) of the regular variants of IAV infecting the human host (i.e. of the H1N1 strain) generally bind to alpha2,6-linked sialic acid (SA) receptors on the surface of human host cells before they are granted entry into the cells. Nevertheless, avian IAV strain were shown to bind to alpha2,3-linked SA receptors, and this event was shown to be associated with a higher extent of suppression of Interferon-encoding gene and Interferon-Stimulated Gene expression in human primary dendritic cells, such as plasmacytoid dendritic cells (pDCs), and consequently, of pro-inflammatory cytokine secretion, given that more severe and prolonged delays of the activation of the interferon system allows the viral load to replicate with a poorer resistance, thereby resulting in a much higher synthesis rate of Type I Interferons in latter stages of the infection due to the exponential nature of the viral load increase, and likewise, in a much higher extent of pro-inflammatory cytokine synthesis and secretion. It was also observed that pro-inflammatory genes were more broadly expressed in human primary macrophages and respiratory epithelial cells (i.e. Type II pneumocytes) in the case of the viral strain binding the alpha2,3-linked SA receptors, and the overall differences as such were observed not to be based upon the extent of viral replication at all, indicating therefore that the avian strain of IAV causes more generalized and severe inflammatory responses, and consequently, a higher incidence of severe respiratory disease (Ramos I. et al., 2011). Nevertheless, it was also highlighted that both H1N1 and H5N1 strains of IAV infects other areas of the human body as well, such as pancreatic tissues, macrophages evolved from the bone marrow, as well as central nervous system (CNS) components. Furthermore, it was discovered that the two IAV genes encoding NS1 and NS2 altogether play a major role in determining the intensity and extent of pathogenesis following an infective event, but NS1 is known to be a bigger determinant of severe Influenza A disease. NS1 and NS2 play a major role in cleaving the mRNA encoded by the Interferon Genes (ING), leading to a suppressed extent of Type I and Type III Interferon translation, packaging and exocytosis. NS1 was shown to undergo single nucleotide polymorphisms and to substantially aid in the overall process of viral evolution and induced immune evasion. With each round of NS1-encoding gene mutation, the H5N1 strain of IAV was found to regulate the ability of the innate immune system to regulate, in turn, the extent of viral replication. Furthermore, two proteins were found to decrease the ability of IAV to undergo RNA-splicing, thereby inhibiting their ability to replicate and express their proteins that help the virus camouflage itself; the NS1-binding proteins (NS1-BPs) and heterogeneous nuclear ribonucleoproteins (hRNPs). In other words, NS1-BP not only was given such nomenclature for its known function of targeting NS1, but also plays a major role in preventing the translation of NS1 by the IAV genome (Tsai P. L. et al., 2013). Following an experiment of quantitative proteomic analysis, NS1 was found to display an antagonistic activity toward the PACT protein, which is activated by the RIG-I in human host cells by means of significantly aiding the expression of Type I IFNs (Tawaratsumida K. et al., 2014). Moreover, the IAV NS1 was recently found to be targeted by Proteasome Subunit Beta type 4 (PSMB4) following a molecular screening procedure, and the interaction between the two proteins was confirmed using two additional experiment methods; co-immunoprecipitation assay and confocal microscopy through mammal cell lines. NS1 is probably also degraded by PSMB4, although more evidence needs to be found to state this with certainty (Chee-Hing Y. et al., 2022).

2. Discussion

The interferon system constitutes the central element of the natural immune system and plays a core importance with regards to mounting a robust cellular defense system against microbes of diverse types. It comprises the molecular elements responsible with

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the synthesis and expression of Type I and Type III Interferons, as well as the molecular elements responsible with the activation and expression of Interferon-Stimulated Genes that altogether comprise a significant amount of antiviral cytokines and a balanced anti-inflammatory to pro-inflammatory ratio of secreted cytokines. IAV strains and variants of concern represent a major example of viruses manipulating the cellular machinery of recognition and sensitive defense, and the outcome of such a cellular immune escape is the latter exacerbated Type I and Type III Interferon response, as the viral load increases in an exponential manner and there would be far more cells producing immunological glycoproteins as such, resulting overall in the production of exaggerated amounts of pro-inflammatory cytokines before a substantial amount of anti-inflammatory cytokines may be produced. As a result, the activity of the produced anti-microbial cytokines will have a lower quality and the systemic distribution of the virus would lead to a continuation of the phenomenon of innate immune escape in immune cells, such as plasmacytoid dendritic cells and primary macrophages. In other words, the ability of viruses to evade detection of pattern recognition receptors and to directly cleave the mRNA encoding central elements of first-line immunity leads to the development of a immunological and temporal "vicious cycle", and the immunity of the infected host tissues would require to make extra effort to fit in the latent stage of viral load replication. Namely, a prolonged incubation period is likely caused by first-line immune evasion and by the time the viral load is starting to approach its peak, the onset of the first clinical symptoms would often have just occurred. Likewise, it would be only rational to project that, the higher the ability of IAV strains to produce and distribute NS1 and NS2, the higher the incidence of severe Influenza A disease would ultimately be. The existence of very firm associations between the interferon system and the adaptive immune system displays the existence of a dependency of the core elements of adaptive immunity to the innate immune system, despite the non-specific nature of innate immune mechanisms. In other words, it is sometimes the peripheral side of a structure that constitutes its core foundations, displaying likewise the possibility that the immune system has a slightly "poetic" nature. The fact that the innate immune system was also and only recently found to display its own "memory" further indicates the necessity for its wider inclusion in the modern-day efforts of vaccinology (Gourbal B . et al., 2018). Overall, it could be the innate immunity escape and the prolonged incubation period of the disease that would often allow opportunistic secondary infections to occur and spread, meaning that first-line immune evasion could often result in the development of a transient phenomenon of immunocompromisation, particularly in patients with underlying health conditions predisposing their immune system to such conditions. The fact that this is likely also the case with other infections, such as SARS-CoV-2 infection and Hepatitis B and C infections, may be allowing the research communities to project a higher resolution of the current stages of pathogenic evolution with regards to the elements of the host immunity they have direct regulatory access to. For example, the ability of pathogenic agents to directly and actively infect and compromise the core elements of the immune system, B- and T-lymphocytes, would represent the final stage of pathogenic evolution, displaying the ultimate "objective" of microbes, which is a direct and complete subversion of all systems that restricts their evolution and distribution throughout the environment, and systems as such normally develop mechanistic action that antagonizes them.

Interferons represent glycoproteins that are produced by various cells in response to early stages of cellular and tissular infection. Interferons were discovered by Alick Isaacs and Jean Lindenmann in 1957, and they were given a nomenclature as such due to their unique nature of interfering with microbial entry and reproduction in the cytoplasm of host cells. A lack of a phenomenon of microbial interference as such results in the development of late and aberrant adaptive immune signals, thereby favoring scenarios of severe and life-threatening infectious illness. There are three overall classes of interferons: Type I, Type II and Type III. The first and the latter classes of IFNs mainly stimulate the production and exocytosis of anti-inflammatory cytokines, whilst the second class of IFNs mainly stimulate the production and exocytosis of pro-inflammatory cytokines. Many

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interferon proteins have a glycosylation site, either in their Nitrogen-terminus, on the left-hand side of their primary structure or in their Oxygen-terminus, on the right-hand side of their primary structure, and it is the glycosylated IFNs that have a higher access to host cells, via the IFNAR1/2, IFNGR or IFNLR1/IL10R2 receptor complex, as their glycosyl group interacts with the carbohydrate molecules on the surface of cells, speeding up the process of protein recognition and receptor activation (M. W. Taylor, 2022). Interferons have often been shown to play a major role as vaccine adjuvants, and given the new scientific and clinical evidence, it can be hypothesized that they often play a major role as vaccines as well. Perhaps, a clinical approach as such could be merged with the insertion of somatic Natural Killer (NK) Cells to aid the immunostimulatory and immunomodulatory effects of IFNs I and III, particularly in people with specific comorbidities, and improve the overall efficacy of the potential vaccinological efforts as such to prevent the emergence of life-threatening epidemics. There are various traditional therapeutic approaches used to indirectly sensitize the production and signaling of IFNs I and III, including a regular intake of minerals, such as cholecalciferol and ascorbic acid. Novel prophylactic aims include the development of nasal sprays aiming to prevent the early spread of viral copies to neighboring tissues, as the stimulation of the rapid production of IgA immunoglobulins represents an important repertoire in the development of an effective defense system against viruses with advanced capabilities of transient self-camouflage. At this moment, much of the specific information with regards to the association of each IgA subtype with its particular activity and locality remains unclear; nevertheless, numerous clinical studies suggest they play a major role in mounting a firm prophylactic immune response against local viral loads that are replicating and spreading to an exponential number of host cells.

Engineered IAV variants containing NS1 genes that underwent loss-of-function mutations were shown to cause far milder forms of the infectious disease, and the results of a clinical study implicating the examination of a live-attenuated H5N1 strain of IAV as a potential avian influenza vaccine further indicate that NS1 plays a determining role with regards to the causality of Influenza A disease severity. A clinical approach as such may tremendously help the human immune system develop its memory against future variants of H5N1 IAV, although the procedure may require to be repeated once in a few years, and adjuvant therapies implicating the direct targeting of all viral proteins responsible with hijacking the interferon system would need to be developed as an adjuvant as well. The overall approach would still not constitute a set of interventions with a thorough effect of improving the evolutionary efforts of natural immunity against microbes with advanced capabilities of immune evasion, as the interferon system would still not be directly stimulated to build more robust mechanisms of recognition and association with the development of further immune signals. It may also be important to acknowledge that recent evidence with regards to the central dogma of biology has caused an unprecedented shift of research perception, as it was discovered that numerous human cell lines produce several polymerase enzymes, such as DNA Theta Polymerase, which reverse transcribe the cellular mRNA into double-stranded DNA and the purpose of a molecular activity as such is DNA repair and the prevention of the onset of life-threatening cancer, including metastatic melanoma. The enzyme represents an important therapeutic target in diseases as such (Chen X. et al., 2021). However, such enzymatic activities have a “double-edged sword”-like nature, as the reverse transcription of mRNA nucleotides, including the nucleotides of the UV-attenuated H5N1 IAV proteins, which contain the genetic information that is foreign of the individual genome, may lead to indirect and partial additions to non-coding and gene-related DNA alike. Risks are probably more reduced because the UV-attenuated virus is locally inserted into the deltoid muscle and it does not share the nature of an mRNA molecule, as it would require translation of RNA-dependent RNA Polymerase prior to expressing the rest of its proteins, like in the cases of inoculation with other negative-sense single-stranded RNA viruses, as well as a few inactivated positive-sense single-stranded RNA, and double-stranded DNA viruses.

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The COVID-19 mRNA vaccines have often been deemed as uncertain and controversial due to the unprecedented number of short-term and long-term vaccine-induced adverse reactions, which are substantially diverse in nature. The most important fault of prophylactic vaccines may mostly be caused by the uncertain origins of SARS-CoV-2 and implicitly, of the spike protein, although the functional nature of a foreign and nucleoside-modified mRNA molecule may also contribute visibly to the development of such faults, as the mRNA molecule is set to last for longer, thereby entering and exiting a more significant number of host cells. The spike glycoprotein was found to damage virtually all bodily structures and micro-structures that it reaches, favoring the onset of hyper-inflammatory responses. A combination of uncertainty with regards to the duration of functioning of the nucleoside-modified mRNA molecule and a significant intensity of pathogenesis induced by the spike glycoprotein probably constitute the explanation to why the COVID-19 mRNA vaccines led to a more usual development of pathogenesis than previous vaccines. Inflammatory Monocytes and Macrophages and T-Lymphocytes were found to be aberrantly recruited following an ACE2-mediated viral endocytosis, and the bioavailability of the receptor in numerous organ systems indicate the high accessibility of the spike glycoprotein into many human cell lines. The technology of mRNA vaccination is indeed based upon more recent foundations and many scientific questions likely are still not completely answered, despite the tremendous features of prophylaxis and therapy. It seems that the single-stranded RNA molecules that are fully functional, or active, are implicated the most in the equation of the discovered reverse transcription phenomenon that considerably infiltrates the central dogma of biology, and that inactive single-stranded RNA molecules are likewise rarely reverse transcribed, as they do not present a genetic message to be translated into a proteic message. Nonetheless, all ssRNA viruses contain at least one active region and numerous others that can be activated following the expression and catalysis of RNA-dependent RNA Polymerase.

Given the significant error presented by the reverse transcription activity that is catalyzed by the Theta Polymerase enzyme and its significant functional similarity with HIV-1 Reverse Transcriptases, it is rather likely that the insertion of mRNA that is foreign of the genome of the individual will lead to mutations and genomic toxicity, particularly in people with one or more genetic and immunological predispositions, and particularly in the cases where the mRNA molecules that are functional in nature; that is, when the entire mRNA molecule encodes one or more identical or distinct proteins (Chandramouly G. et al., 2021) and also, to a lesser degree, when the genome of an attenuated form of a virus is a positive-sense single-stranded RNA (+ssRNA) molecule (i.e. SARS-CoV-2), as the virus would not need to translate RNA-dependent RNA Polymerase to translate its proteins, meaning that it would be more capable, to a certain extent, of translating its pathogenic proteins. As a result, hopes of mRNA vaccination breakthrough against several diseases of concern seem to have been decreased substantially with the change of perception with regards to the solidity of the central dogma of biology, leaving protein-based vaccines as the category that looks to be safer and more promising, despite the high probability of significant breakthrough from genetic and infectious diseases, particularly in people without major genetic and immunological predispositions. Given the history of traditional vaccines containing attenuated versions of single-stranded RNA viral genomes (i.e. measles), it can be hypothesized that the described risks mostly cover the prophylactic and therapeutic approaches based on functionally-active mRNA molecules. Even if the success rate of ssRNA-based prophylactic approaches as such exceeds 99.9% and the rate of illness drops substantially for a considerable number of years, a serious error rate of under 0.1% could not only destroy the credibility of the approach, but cause numerous cases of severe illness and death following genetic adverse events, and cripple health systems, at least on a local basis. It is also important to observe that an event of medical failure implicating an exchange of genetic mutations would have long-term effects, which would often not display phenotypic effects often for at least 5-10 years, thereby leading patients into

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the erroneous belief that they experience a complete breakthrough from their previous illness for several years, and a future incidence of disease would be more difficult to be associated with a possible medical error. It is well-known that medicine operates by the statement "First, do no harm" (Carp T., 2023). It is important to acknowledge that medical risks could sometimes involve a few decades of duration until they begin to manifest in a thorough, widespread manner and likewise, a few, local cases of genetic collateral damage may have been infiltrated through cases of long-term underlying health conditions that are either oncological or immunological in nature.

The direct stimulation of Type I and Type III IFN synthesis represents an essential step in the adequate recruitment of natural and adaptive lymphocytes, which then results in the normal synthesis of gamma interferons and likewise, in the development of balanced pro-inflammatory immune activity. An exacerbated synthetic rate of pro-inflammatory cytokines is caused by the activation of ISGs in numerous cells following a transient suppression of the entire interferon system, and results in the aberrant recruitment of Natural Killer cells (NK cells) and T-lymphocytes. An initial suppression of IFN I and III-based signaling is often associated with disrupted M1/M2 macrophage recruitment ratios, usually implicating a more abundant recruitment of M1 macrophages, which are associated with pro-inflammatory activity and, consequently, an uncontrolled, prolonged and often a systemic level of inflammation. Tumor-Necrosis Factor-alpha (TNF-alpha), Interleukin-1 (IL-1), IL-6, Macrophage Inflammatory Protein 1 (MIP-1), Monocyte Chemoattractant Protein 1 (MCP-1) and Interferon-gamma-inducible Protein 10 (IP-10) represent other relevant examples of pro-inflammatory cytokines that are overexpressed (Us D., 2008). The worst case scenario following a systemic activation of ISGs represents the tremendous accumulation of pro-inflammatory cytokines, including of gamma interferons, favoring the onset of an immunological condition known as hemophagocytic lymphohistiocytosis, or in short as cytokine storm, which is often life-threatening. On a different note with regards to gamma interferon, the activation of Type I IFNs was found to inhibit Type II IFN-related immune responses during mycobacterial infection (Teles R. M. et al., 2013). Likewise, the development of prophylactic and early therapeutic approaches remain the wisest and most feasible approaches to at least substantially decrease the life-altering and life-threatening effects of potential future respiratory epidemics caused by a definitive zoonotic spillover event of viruses, like the H5N1 strain of IAV. Late therapies are often highly complex in nature due to the reflection of the highly complex nature of the latently-activated micro- and macro-immunological pathways that comprise a systemic immunological hyper-activation and likewise, bring significant logistical, financial and resource-related demands. Ascorbic acid and cholecalciferol were found to boost the interferon system before and during the early stages of the IAV infection. The first focuses more upon the regulation of the release of the reactive oxygen species (ROS) by the mitochondria, whilst the latter directly stimulates the synthesis of an accurate quantity of Type I Interferons per one infected host cell. The mechanism of action by cholecalciferol was nonetheless discovered to be inhibited by released Type I IFNs, indicating the existence of a counter-regulatory relationship between the mineral and the immunological glycoproteins as such. It is important to mention that Vitamin D3 was also found to regulate excessive IFN I signals during moderate phases of IAV-induced infectious disease and COVID-19. Moreover, antibiotics and antifungal agents may be effective against severe respiratory disease when the patients are suffering from secondary infections by bacteria or fungi respectively. Likewise, as it is important to hold fast to clinical discernment to avoid risks of antibiotic resistance development by concerning pathogenic bacteria, it may be important to develop rapid clinical testing approaches aimed to detect the presence of bacteria or of fungi in the sputum of patients with the flu.

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3. Conclusion

The H5N1 strain of IAV represents a highly pathogenic and concerning variant of the respiratory virus and the increase of incidences implicating zoonotic spillover events from live birds to mammals and humans should alarm the health researchers and authorities; not in the manner of stimulating the production and perpetuation of an unhealthy sentiment of panic, but in the manner of thinking outside the box, calmly and positively, and of defying the toxicity of excessive financial gains of mainstream and multi-billionaire health corporations, which do not seem to have an active interest in investing in cheap, nature-friendly drug-like compounds that directly train and sensitize the interferon system, thereby playing a tremendous role in sharpening vaccinology-related efforts. The increasing threat of zoonotic spillover events regarding multiple pathogens of concern should stimulate all scientists to think outside of the box, attain the law of common sense and push for the rapid and thorough testing of clinical approaches as described above, regardless of whether the investment of such approaches would fill in the pockets of multi-billionaire health corporate leaders or not. A problem can never be resolved with the same mindset as used when it first occurred, according to Albert Einstein. Not abiding by principles that might be directly and firmly attached to the very values that the democratic civilization has been built upon may condemn us to repeating harmful history that, in the author's humble point of view, seriously needs no repeating. The current exponential sharpening of the polarization of wealth-related conditions worldwide, which seems to be breeding unprecedented ideological toxicity, seems to constitute a primary factor of the inability to perform thorough medical research and favor a systemic rollout of a widely bioavailable, nature-friendly, financially accessible and rather clinically non-invasive prophylaxis and early therapy for public health crises that may threaten thousands of human lives. In an ideal scenario, the medical drug regulatory bodies should prioritize approaches that fulfill all such criteria over approaches that seem not to. The prime aim of public health-related medicine is to prevent the occurrence of outbreaks that would massively impair human health and cause many people to lose their lives, when we ought to acknowledge that the primary cause of crippling disease as such can be calibrated tremendously. The priority steps in addressing potential dangers with regards to future public health scenarios would include saving a progressively higher number of lives, until at least the majority of human lives are preserved in the end. A low dose of glycosylated Interferon I and III proteins could be enough to prevent life-threatening outbreaks of infectious disease, and the financial demands for an overall preventive and early therapeutic clinical approach as such seem to be pronouncedly modest.

Author's Note: This study is independent and it aims to cover recent scientific evidence in its profound-most layers and it does not contain subjectivity. The author does not present any conflict of interest.

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