

Review

Not peer-reviewed version

---

# How the Next Pandemic Could Be Handled— Studying the Risks of (A)H5N1 Influenza Human Spillover

---

[Theodor-Nicolae Carp](#) \*

Posted Date: 16 October 2024

doi: 10.20944/preprints202303.0477.v5

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



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Review

# How the Next Pandemic Could Be Handled— Studying the Risks of (A)H5N1 Influenza Human Spillover

Carp Theodor-Nicolae

Affiliation: Independent Researcher, Freelance

**Abstract:** The Influenza A Virus (IAV) represents an enveloped, positive-sense and 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 favour 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 colonise 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 favoured 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 colonising vital areas of the human body, and this aspect is tricky, as microbial colonisation as such 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 approximately 60% was projected by the World Health Organization, as the virus was shown to favour 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 neighbouring uninfected cells, thereby preventing them from responding to the virus in the first place. Additionally, it was determined that NSP1 induces the apoptosis of the cells it is produced in and the neighbouring ones it enters via induced paracrine signalling, and such an aspect may only emphasise upon the critical role that early innate immune stimulation plays in preventing the development of severe respiratory illness afterward, given the context of increased viral ability of suppressing the production of first-line and second-line signals. 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 a 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. Somatic natural and adaptive lymphocytes treated with IFN I and III could also constitute a substantial approach of immunisation and heavily favour an indefinite shift in the evolutionary battle between the host organism and microbes of public health concern, as IFN I and III could improve the immunity of T-lymphocytes against HIV infection and even improve the performance of the

mentioned types of immune cells during other types of infections. Likewise, a combination of all intellectual and clinical efforts implicating a shift of scientific and medical focus toward natural immunity in vaccine-related research could be a wise method in making the best efforts to prevent or alleviate the effects of a possible future pandemic, given the highly significant and diverse abilities of microbial “intelligence” to effectively hijack the natural immune system, many times causing severe implications upon the quality of the overall immune response against such microbes.

**Keywords:** H5N1; Influenza A; microbial evolution; first-line immunity; interferon; mucosal antibodies; immune evasion; sialic acid receptor; haemagglutinin; natural lymphocytes; adaptive lymphocytes; monocytes; macrophages; cytokines

---

## 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 generalised 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). NS1 also was found to induce the apoptosis of the cells in which it is produced or enters through paracrine signalling, through the catalysis of caspase, implicating an effect of “trace erasing”. In other words, the developed viral “intelligence”, via the translation of NS1, aims to ensure that infected cells are lysed and that the viral load increases in an exponential manner without a present inflammatory response (Zhang C. et al., 2010).

Behind such a viral machinery of hijacking major innate immune pathways lies a highly complex evolutionary battleground between diverse microbes and mammal immunity that has been occurring particularly over the past couple of centuries. Namely, viruses have undergone mutations, not only leading to the expression of proteins that target genes encoding major Type I and even Type III Interferon glycoproteins, but also leading to the expression of proteins increasingly more capable of tricking mammal host cells into confusing the viral genomes with cellular RNAs, causing host cells to fail in recognising Pathogen-Associated Molecular Patterns (PAMPs) and Damage-Associated Molecular Patterns (DAMPs), which represent certain genetic features specific of pathogenic agents and toxins produced by the viral genomes respectively (Carp T. et al., 2024). It is evident that major delays in the activation process of major innate immune pathways will impact the timing and quality of the adaptive immune activation, often leading to the eventual production of responses that are exaggeratedly inflammatory in nature, whilst displaying a decreased extent of precision with regards to microbial clearance, often due to the fact that delays in the process of immune activation facilitate the reproduction and spread of the microbial load/count throughout entire tissues, making it more difficult for the immune system to clear an increased concentration of microbe. Such a scenario may also explain why there is an increased number of clinical cases of various modern-day infectious diseases in which the duration of recovery from symptomatic disease tends to be higher than average, alongside the considerable probability that there is a relationship of causal proportionality between the ability of a microbe to transiently silence first- and second-line immunity, and the duration of pre-symptomatic stages of infectious diseases (i.e., stage commonly known as asymptomatic disease in COVID-19). With the occurrence of the past two viral pandemics - HIV-1-induced AIDS and SARS-CoV-2-induced COVID-19 - it has become more evident that the extent of microbial evolution is likely advanced enough that updates in the therapeutic and vaccine-based clinical research strategies are becoming needed. An approach or set of approaches involving overall a persistent sensitisation-like catalysis of innate immune signalling following viral exposure at the cellular level could represent “the bridge” to a more advanced and precise area of clinical research implicating vaccine innovation and development; one that is more inclusive of major elements of natural immunity (Carp T., 2024).

## Discussion

Recently, it was discovered that the natural immune system also contains its own “memory”, and the concept of natural immunity gaining “memory” is now regarded as “trained immunity” (Dagenais A. et al., 2023). The interferon system constitutes the central element of the natural immune system and plays a core importance with regards to mounting a robust cellular defence system against microbes of diverse types. It comprises the molecular elements responsible with 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 an 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 antagonises them.

Interferons represent glycoproteins that are produced by various cells in response to early stages of cellular and tissue-related 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 favouring 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 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 hypothesised 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. It is important to mention that somatic NK cells and somatic T-Lymphocytes also play significant roles in immunisation, and it is such three elements of human immunity that could be merged during the efforts of developing a life-saving vaccine.

It is important to mention that T-lymphocytes alone could not bring sufficient long-term immunising effects due to the polymorphic nature of the H5N1 strain of IAV, just as they do not sufficiently bring long-term immunoprotective support against the polymorphic SARS-CoV-2 +ssRNA pathogen. As a result, it could be wise to combine T-lymphocytes with NK cells and anti-inflammatory and antiviral interferon glycoproteins in the attempt to protect human life from the devastating effects of the avian flu. Moreover, both somatic NK cells and somatic T-lymphocytes could be treated with IFN I and III beforehand to better prepare them as potential vaccine components. The treatment of helper CD4<sup>+</sup> and cytotoxic CD8<sup>+</sup> T-lymphocytes with IFN I and III could also represent the ultimate immunological “punch of self-defence” against HIV, as the retrovirus gained final evolutionary abilities, which directly antagonise and neutralise the central elements of human immunity; adaptive lymphocytes. It is thereby probable that such a treatment of the lymphocytes will improve their performance during major microbial infections. A treatment of somatic lymphocytes as such with the interferon glycoproteins in cause could effectively turn them into “super-lymphocytes” and bring unprecedented aid in the evolutionary counter-offensive of human immunity against microbes of clinical concern; particularly in the context of infection by HIV. Furthermore, somatic primary dendritic cells, which are also deemed as “the brain of the immune system”, could be administered by means of aiding the production and distribution of IFN I and III in the organism in the efforts of disease prevention and early therapy. There are various traditional therapeutic approaches used to indirectly sensitise the production and signalling of IFN 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 neighbouring tissues, as the stimulation of the rapid production of IgA immunoglobulins represents an important repertoire in the development of an effective defence 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.

The treatment and administration of fetal NK cells, T-lymphocytes and/or primary dendritic cells could be unethical, as human life has been clinically demonstrated to start at the moment of fertilization by the joining of the “winner” sperm cell with the egg cell. It is universally known and scientifically agreed upon that the most fundamental unit of life represents the living cell. During fertilization, two living cells join together to form a zygote. Life represents a concept that is more complex than the human mind can naturally apprehend, and it is at the moment the zygote is formed when the energies that constitute human life are fulfilled; energies that come partially from the maternal side, via the egg cell, and partially from the paternal side, via the “winner” sperm cell. By the moment of fertilization, the transition from meiosis to mitosis takes place, and the intracellular level of Ca<sup>2+</sup> rises sharply following a trigger by Inositol Triphosphate (InsP3) (Whitaker M., 2006). The beginning of such an exponential growth in the concentration of Ca<sup>2+</sup> ions by InsP3 inside the new cell could mark biophysical energy fulfillment. As a result, by the exact moment of fertilization, human life begins. To apprehend much wider details regarding the elements of life, biophysical concepts require to be researched as well, and scientific perspective should not be solely relied upon molecular and cell biology, given the foundational characteristics of physics, which is also known as an instrument studying the first degree of matter. Overall, fetuses who were affected by induced pregnancy termination were not even capable of consenting to have their life ended and as a result, treatment using cells of aborted fetuses is definitely not situated within the circle of bioethics.

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 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.

Despite the promising success that mRNA have been scientifically projected to bring, 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, as well as by the unusually multi-lateral pathophysiological effects 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 microstructures that it reaches, favouring the onset of hyper-inflammatory responses. Whilst it is necessary to acknowledge that world-class researchers placed efforts beyond the threshold the validity to make the vaccine spike protein less pathogenic and more immunogenic, by performing a double Proline insertion in its S2 subunit, it may also now be realistic enough to suggest that the spectrum of intersection between the threshold of the minimum count of spike protein bringing an effect of immunisation and the threshold of the maximum count of spike protein not causing immunopathology is significantly narrower than projected during the initial phases of the COVID-19 pandemic (Carp T., 2024). 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 catalysed 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 at least hypothesised 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 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. Determining the ratio between benefits and risks constitutes a major step in holding fast to scientific and medical progress, and the final step is the development of one approach that is both uniquely effective and presents minimal risks of adverse events of any type.

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. Tumour-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, favouring 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.

Interestingly, multiple subtypes of IgA immunoglobulins were shown to be effective in aiding the development of precise and flexible innate and adaptive immune responses alike and against many types of infectious agents of significant concern, and they could likewise count as adjuvants of natural and adaptive immunity-based vaccines. Specifically, they were particularly shown to provide significant efficacy if used in the nasal and the oral cavities. Likewise, anti-H5N1 IAV IgA immunoglobulins could be collected and administered nasally for the purpose of prophylaxis and early treatment. In case of necessity, such an approach could be merged with the administration of recombinant IFNs and of “super-lymphocytes”. Nevertheless, the administration of natural and adaptive lymphocytes treated with IFN I and III could be more important in more advanced stages of the disease, when the viral load is distributed in multiple organ systems, and in the cases of HIV-induced AIDS, as a direct and proportional evolutionary response to the manner the HIV virus specifically targets helper CD4+ and cytotoxic CD8+ T-lymphocytes. If the disease is in more advanced stages, it would be feasible to apply a treatment scheme implicating a restart of the immune system, by suppressing the hyper-activated pathways via the usage of zinc ionophores and corticosteroids, prior to the stimulation of the suppressed immune system to produce the innate antiviral and anti-inflammatory products of ISGs. This could aid the immune system in a more precise and effective localization and neutralisation of the remaining viral copies. Likewise, researchers could utilise this fresh opportunity by collaborating and creating new teams of clinical “brainstorm” to join the recently created intellectual forces and provide potentially with the greatest resistance against the increasing risk of future life-threatening epidemics and pandemics.

## 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 sensitise 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 polarisation 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 favour 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 prioritise approaches that fulfil 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. Risking an incomplete focus upon the necessity to address the evolutionary aim of pathogens of concern to evade first-line immune recognition and signals may contribute to an increasing allowance of pathogens to further evolve their mechanisms of pathogenesis and, ultimately, an increasing probability of the onset of a life-threatening pandemic not far in the future. 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, 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.

## References

1. Dagenais A, Villalba-Guerrero C and Olivier M (2023) Trained immunity: A "new" weapon in the fight against infectious diseases. *Front. Immunol.* 14:1147476. doi: 10.3389/fimmu.2023.1147476
2. Ramos, I., Bernal-Rubio, D., Durham, N., Belicha-Villanueva, A., Lowen, A. C., Steel, J., & Fernandez-Sesma, A. (2011). Effects of receptor binding specificity of avian influenza virus on the human innate immune response. *Journal of virology*, 85(9), 4421–4431. <https://doi.org/10.1128/JVI.02356-10>
3. Luczo, J. M., Stambas, J., Durr, P. A., Michalski, W. P., & Bingham, J. (2015). Molecular pathogenesis of H5 highly pathogenic avian influenza: the role of the haemagglutinin cleavage site motif. *Reviews in medical virology*, 25(6), 406–430. <https://doi.org/10.1002/rmv.1846>
4. Sriwilaijaroen, N., & Suzuki, Y. (2022). Roles of Glycans and Non-glycans on the Epithelium and in the Immune System in H1-H18 Influenza A Virus Infections. *Methods in molecular biology (Clifton, N.J.)*, 2556, 205–242. [https://doi.org/10.1007/978-1-0716-2635-1\\_16](https://doi.org/10.1007/978-1-0716-2635-1_16)
5. Scheibner, D., Salaheldin, A. H., Bagato, O., Zaack, L. M., Mostafa, A., Blohm, U., Müller, C., Eweas, A. F., Franzke, K., Karger, A., Schäfer, A., Gischke, M., Hoffmann, D., Lerolle, S., Li, X., Abd El-Hamid, H. S., Veits, J., Breithaupt, A., Boons, G. J., Matrosovich, M., ... Abdelwhab, E. M. (2023). Phenotypic effects of mutations observed in the neuraminidase of human origin H5N1 influenza A viruses. *PLoS pathogens*, 19(2), e1011135. <https://doi.org/10.1371/journal.ppat.1011135>
6. Taylor M. W. (2014). Interferons. *Viruses and Man: A History of Interactions*, 101–119. [https://doi.org/10.1007/978-3-319-07758-1\\_7](https://doi.org/10.1007/978-3-319-07758-1_7)
7. Chan, R. W., Yuen, K. M., Yu, W. C., Ho, C. C., Nicholls, J. M., Peiris, J. S., & Chan, M. C. (2010). Influenza H5N1 and H1N1 virus replication and innate immune responses in bronchial epithelial cells are influenced by the state of differentiation. *PloS one*, 5(1), e8713. <https://doi.org/10.1371/journal.pone.0008713>

8. Yu, W. C., Chan, R. W., Wang, J., Travanty, E. A., Nicholls, J. M., Peiris, J. S., Mason, R. J., & Chan, M. C. (2011). Viral replication and innate host responses in primary human alveolar epithelial cells and alveolar macrophages infected with influenza H5N1 and H1N1 viruses. *Journal of virology*, 85(14), 6844–6855. <https://doi.org/10.1128/JVI.02200-10>
9. Huo, C., Xiao, K., Zhang, S., Tang, Y., Wang, M., Qi, P., Xiao, J., Tian, H., & Hu, Y. (2018). H5N1 Influenza A Virus Replicates Productively in Pancreatic Cells and Induces Apoptosis and Pro-Inflammatory Cytokine Response. *Frontiers in cellular and infection microbiology*, 8, 386. <https://doi.org/10.3389/fcimb.2018.00386>
10. Siegers, J. Y., van de Bildt, M. W. G., Lin, Z., Leijten, L. M., Lavrijssen, R. A. M., Bestebroer, T., Spronken, M. I. J., De Zeeuw, C. I., Gao, Z., Schrauwen, E. J. A., Kuiken, T., & van Riel, D. (2019). Viral Factors Important for Efficient Replication of Influenza A Viruses in Cells of the Central Nervous System. *Journal of virology*, 93(11), e02273-18. <https://doi.org/10.1128/JVI.02273-18>
11. Chan, R. W., Leung, C. Y., Nicholls, J. M., Peiris, J. S., & Chan, M. C. (2012). Proinflammatory cytokine response and viral replication in mouse bone marrow derived macrophages infected with influenza H1N1 and H5N1 viruses. *PloS one*, 7(11), e51057. <https://doi.org/10.1371/journal.pone.0051057>
12. Short, K. R., Kedzierska, K., & van de Sandt, C. E. (2018). Back to the Future: Lessons Learned From the 1918 Influenza Pandemic. *Frontiers in cellular and infection microbiology*, 8, 343. <https://doi.org/10.3389/fcimb.2018.00343>
13. Zhao, H., Zhou, J., Jiang, S., & Zheng, B. J. (2013). Receptor binding and transmission studies of H5N1 influenza virus in mammals. *Emerging microbes & infections*, 2(12), e85. <https://doi.org/10.1038/emi.2013.89>
14. Li, K., McCaw, J. M., & Cao, P. (2023). Enhanced viral infectivity and reduced interferon production are associated with high pathogenicity for influenza viruses. *PLoS computational biology*, 19(2), e1010886. <https://doi.org/10.1371/journal.pcbi.1010886>
15. Peiris, J. S., Cheung, C. Y., Leung, C. Y., & Nicholls, J. M. (2009). Innate immune responses to influenza A H5N1: friend or foe?. *Trends in immunology*, 30(12), 574–584. <https://doi.org/10.1016/j.it.2009.09.004>
16. Malik, G., & Zhou, Y. (2020). Innate Immune Sensing of Influenza A Virus. *Viruses*, 12(7), 755. <https://doi.org/10.3390/v12070755>
17. Gourbal, B., Pinaud, S., Beckers, G. J. M., Van Der Meer, J. W. M., Conrath, U., & Netea, M. G. (2018). Innate immune memory: An evolutionary perspective. *Immunological reviews*, 283(1), 21–40. <https://doi.org/10.1111/imr.12647>
18. Palmieri, B., Vadala', M., & Palmieri, L. (2021). Immune memory: an evolutionary perspective. *Human vaccines & immunotherapeutics*, 17(6), 1604–1606. <https://doi.org/10.1080/21645515.2020.1846396>
19. Scarcella, M., d'Angelo, D., Ciampa, M., Tafuri, S., Avallone, L., Pavone, L. M., & De Pasquale, V. (2022). The Key Role of Lysosomal Protease Cathepsins in Viral Infections. *International journal of molecular sciences*, 23(16), 9089. <https://doi.org/10.3390/ijms23169089>
20. Us D. (2008). Kuş gribinde sitokin fırtınası [Cytokine storm in avian influenza]. *Mikrobiyoloji bulteni*, 42(2), 365–380.
21. Nogales, A., Martinez-Sobrido, L., Topham, D. J., & DeDiego, M. L. (2018). Modulation of Innate Immune Responses by the Influenza A NS1 and PA-X Proteins. *Viruses*, 10(12), 708. <https://doi.org/10.3390/v10120708>
22. Li, Z., Jiang, Y., Jiao, P., Wang, A., Zhao, F., Tian, G., Wang, X., Yu, K., Bu, Z., & Chen, H. (2006). The NS1 gene contributes to the virulence of H5N1 avian influenza viruses. *Journal of virology*, 80(22), 11115–11123. <https://doi.org/10.1128/JVI.00993-06>
23. Wang, J., Zeng, Y., Xu, S., Yang, J., Wang, W., Zhong, B., Ge, J., Yin, L., Bu, Z., Shu, H. B., Chen, H., Lei, C. Q., & Zhu, Q. (2018). A Naturally Occurring Deletion in the Effector Domain of H5N1 Swine Influenza Virus Nonstructural Protein 1 Regulates Viral Fitness and Host Innate Immunity. *Journal of virology*, 92(11), e00149-18. <https://doi.org/10.1128/JVI.00149-18>
24. Bornholdt, Z. A., & Prasad, B. V. (2008). X-ray structure of NS1 from a highly pathogenic H5N1 influenza virus. *Nature*, 456(7224), 985–988. <https://doi.org/10.1038/nature07444>
25. Carrillo, B., Choi, J. M., Bornholdt, Z. A., Sankaran, B., Rice, A. P., & Prasad, B. V. (2014). The influenza A virus protein NS1 displays structural polymorphism. *Journal of virology*, 88(8), 4113–4122. <https://doi.org/10.1128/JVI.03692-13>
26. Kerry, P. S., Ayllon, J., Taylor, M. A., Hass, C., Lewis, A., García-Sastre, A., Randall, R. E., Hale, B. G., & Russell, R. J. (2011). A transient homotypic interaction model for the influenza A virus NS1 protein effector domain. *PloS one*, 6(3), e17946. <https://doi.org/10.1371/journal.pone.0017946>
27. Evseev, D., & Magor, K. E. (2021). Molecular Evolution of the Influenza A Virus Non-structural Protein 1 in Interspecies Transmission and Adaptation. *Frontiers in microbiology*, 12, 693204. <https://doi.org/10.3389/fmicb.2021.693204>
28. Long, J. X., Peng, D. X., Liu, Y. L., Wu, Y. T., & Liu, X. F. (2008). Virulence of H5N1 avian influenza virus enhanced by a 15-nucleotide deletion in the viral nonstructural gene. *Virus genes*, 36(3), 471–478. <https://doi.org/10.1007/s11262-007-0187-8>

29. Kato, Y. S., Fukui, K., & Suzuki, K. (2016). Mechanism of a Mutation in Non-Structural Protein 1 Inducing High Pathogenicity of Avian Influenza Virus H5N1. *Protein and peptide letters*, 23(4), 372–378. <https://doi.org/10.2174/0929866523666160204124406>
30. Kajihara, M., Sakoda, Y., Soda, K., Minari, K., Okamatsu, M., Takada, A., & Kida, H. (2013). The PB2, PA, HA, NP, and NS genes of a highly pathogenic avian influenza virus A/whooper swan/Mongolia/3/2005 (H5N1) are responsible for pathogenicity in ducks. *Virology journal*, 10, 45. <https://doi.org/10.1186/1743-422X-10-45>
31. Li, W., Wang, G., Zhang, H., Xin, G., Zhang, D., Zeng, J., Chen, X., Xu, Y., Cui, Y., & Li, K. (2010). Effects of NS1 variants of H5N1 influenza virus on interferon induction, TNF $\alpha$  response and p53 activity. *Cellular & molecular immunology*, 7(3), 235–242. <https://doi.org/10.1038/cmi.2010.6>
32. Park, E. S., Dezhbord, M., Lee, A. R., & Kim, K. H. (2022). The Roles of Ubiquitination in Pathogenesis of Influenza Virus Infection. *International journal of molecular sciences*, 23(9), 4593. <https://doi.org/10.3390/ijms23094593>
33. Lamotte, L. A., & Tafforeau, L. (2021). How Influenza A Virus NS1 Deals with the Ubiquitin System to Evade Innate Immunity. *Viruses*, 13(11), 2309. <https://doi.org/10.3390/v13112309>
34. Jiang, J., Li, J., Fan, W., Zheng, W., Yu, M., Chen, C., Sun, L., Bi, Y., Ding, C., Gao, G. F., & Liu, W. (2016). Robust Lys63-Linked Ubiquitination of RIG-I Promotes Cytokine Eruption in Early Influenza B Virus Infection. *Journal of virology*, 90(14), 6263–6275. <https://doi.org/10.1128/JVI.00549-16>
35. Ferraris, O., Casalegno, J. S., Frobert, E., Bouscambert Duchamp, M., Valette, M., Jacquot, F., Raoul, H., Lina, B., & Ottmann, M. (2018). The NS Segment of H1N1pdm09 Enhances H5N1 Pathogenicity in a Mouse Model of Influenza Virus Infections. *Viruses*, 10(9), 504. <https://doi.org/10.3390/v10090504>
36. Liu, S., Zhang, L., Yao, Z., Xing, L., & Liu, K. (2017). In vitro and in vivo characterization of a novel H1N1/2009 influenza virus reassortant with an NS gene from a highly pathogenic H5N1 virus, isolated from a human. *Archives of virology*, 162(9), 2633–2642. <https://doi.org/10.1007/s00705-017-3408-z>
37. Yang, C. H., Hsu, C. F., Lai, X. Q., Chan, Y. R., Li, H. C., & Lo, S. Y. (2022). Cellular PSMB4 Protein Suppresses Influenza A Virus Replication through Targeting NS1 Protein. *Viruses*, 14(10), 2277. <https://doi.org/10.3390/v14102277>
38. Zhang, C., Yang, Y., Zhou, X., Yang, Z., Liu, X., Cao, Z., Song, H., He, Y., & Huang, P. (2011). The NS1 protein of influenza A virus interacts with heat shock protein Hsp90 in human alveolar basal epithelial cells: implication for virus-induced apoptosis. *Virology journal*, 8, 181. <https://doi.org/10.1186/1743-422X-8-181>
39. Zhang, C., Yang, Y., Zhou, X., Liu, X., Song, H., He, Y., & Huang, P. (2010). Highly pathogenic avian influenza A virus H5N1 NS1 protein induces caspase-dependent apoptosis in human alveolar basal epithelial cells. *Virology journal*, 7, 51. <https://doi.org/10.1186/1743-422X-7-51>
40. Bian, Q., Lu, J., Zhang, L., Chi, Y., Li, Y., & Guo, H. (2017). Highly pathogenic avian influenza A virus H5N1 non-structural protein 1 is associated with apoptotic activation of the intrinsic mitochondrial pathway. *Experimental and therapeutic medicine*, 14(5), 4041–4046. <https://doi.org/10.3892/etm.2017.5056>
41. Tsai, P. L., Chiou, N. T., Kuss, S., García-Sastre, A., Lynch, K. W., & Fontoura, B. M. (2013). Cellular RNA binding proteins NS1-BP and hnRNP K regulate influenza A virus RNA splicing. *PLoS pathogens*, 9(6), e1003460. <https://doi.org/10.1371/journal.ppat.1003460>
42. Tawaratsumida, K., Phan, V., Hrincius, E. R., High, A. A., Webby, R., Redecke, V., & Häcker, H. (2014). Quantitative proteomic analysis of the influenza A virus nonstructural proteins NS1 and NS2 during natural cell infection identifies PACT as an NS1 target protein and antiviral host factor. *Journal of virology*, 88(16), 9038–9048. <https://doi.org/10.1128/JVI.00830-14>
43. Engel D. A. (2013). The influenza virus NS1 protein as a therapeutic target. *Antiviral research*, 99(3), 409–416. <https://doi.org/10.1016/j.antiviral.2013.06.005>
44. Lin, C. Y., Shih, M. C., Chang, H. C., Lin, K. J., Chen, L. F., Huang, S. W., Yang, M. L., Ma, S. K., Shiao, A. L., Wang, J. R., Chen, K. R., & Ling, P. (2021). Influenza A virus NS1 resembles a TRAF3-interacting motif to target the RNA sensing-TRAF3-type I IFN axis and impair antiviral innate immunity. *Journal of biomedical science*, 28(1), 66. <https://doi.org/10.1186/s12929-021-00764-0>
45. Wang, T., Wei, F., Jiang, Z., Song, J., Li, C., & Liu, J. (2022). Influenza virus NS1 interacts with 14-3-3 $\epsilon$  to antagonize the production of RIG-I-mediated type I interferons. *Virology*, 574, 47–56. <https://doi.org/10.1016/j.virol.2022.07.002>
46. Tam, E. H., Liu, Y. C., Woung, C. H., Liu, H. M., Wu, G. H., Wu, C. C., & Kuo, R. L. (2021). Role of the Chaperone Protein 14-3-3 $\epsilon$  in the Regulation of Influenza A Virus-Activated Beta Interferon. *Journal of virology*, 95(20), e0023121. <https://doi.org/10.1128/JVI.00231-21>
47. Gabriel, G., Czudai-Matwich, V., & Klenk, H. D. (2013). Adaptive mutations in the H5N1 polymerase complex. *Virus research*, 178(1), 53–62. <https://doi.org/10.1016/j.virusres.2013.05.010>
48. Steel, J., Lowen, A. C., Pena, L., Angel, M., Solórzano, A., Albrecht, R., Perez, D. R., García-Sastre, A., & Palese, P. (2009). Live attenuated influenza viruses containing NS1 truncations as vaccine candidates



- against H5N1 highly pathogenic avian influenza. *Journal of virology*, 83(4), 1742–1753. <https://doi.org/10.1128/JVI.01920-08>
49. Wang, B. X., & Fish, E. N. (2017). Interactions Between NS1 of Influenza A Viruses and Interferon- $\alpha/\beta$ : Determinants for Vaccine Development. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*, 37(8), 331–341. <https://doi.org/10.1089/jir.2017.0032>
  50. Brambati, A., Barry, R. M., & Sfeir, A. (2020). DNA polymerase theta (Pol $\theta$ ) - an error-prone polymerase necessary for genome stability. *Current opinion in genetics & development*, 60, 119–126. <https://doi.org/10.1016/j.gde.2020.02.017>
  51. Chen, X. S., & Pomerantz, R. T. (2021). DNA Polymerase  $\theta$ : A Cancer Drug Target with Reverse Transcriptase Activity. *Genes*, 12(8), 1146. <https://doi.org/10.3390/genes12081146>
  52. 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). Pol $\theta$  reverse transcribes RNA and promotes RNA-templated DNA repair. *Science advances*, 7(24), eabf1771. <https://doi.org/10.1126/sciadv.abf1771>
  53. Reuther, P., Giese, S., Götz, V., Kilb, N., Mänz, B., Brunotte, L., & Schwemmler, M. (2014). Adaptive mutations in the nuclear export protein of human-derived H5N1 strains facilitate a polymerase activity-enhancing conformation. *Journal of virology*, 88(1), 263–271. <https://doi.org/10.1128/JVI.01495-13>
  54. Perrone, L. A., Plowden, J. K., García-Sastre, A., Katz, J. M., & Tumpey, T. M. (2008). H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS pathogens*, 4(8), e1000115. <https://doi.org/10.1371/journal.ppat.1000115>
  55. Karo-Karo, D., Bodewes, R., Restuadi, R., Bossers, A., Agustini, A., Stegeman, J. A., Koch, G., & Muljono, D. H. (2022). Phylodynamics of Highly Pathogenic Avian Influenza A(H5N1) Virus Circulating in Indonesian Poultry. *Viruses*, 14(10), 2216. <https://doi.org/10.3390/v14102216>
  56. Smith, G. J., Naipospos, T. S., Nguyen, T. D., de Jong, M. D., Vijaykrishna, D., Usman, T. B., Hassan, S. S., Nguyen, T. V., Dao, T. V., Bui, N. A., Leung, Y. H., Cheung, C. L., Rayner, J. M., Zhang, J. X., Zhang, L. J., Poon, L. L., Li, K. S., Nguyen, V. C., Hien, T. T., Farrar, J., ... Guan, Y. (2006). Evolution and adaptation of H5N1 influenza virus in avian and human hosts in Indonesia and Vietnam. *Virology*, 350(2), 258–268. <https://doi.org/10.1016/j.virol.2006.03.048>
  57. Aleith, J., Brendel, M., Weipert, E., Müller, M., Schultz, D., Ko-Infekt Study Group, & Müller-Hilke, B. (2022). Influenza A Virus Exacerbates Group A Streptococcus Infection and Thwarts Anti-Bacterial Inflammatory Responses in Murine Macrophages. *Pathogens (Basel, Switzerland)*, 11(11), 1320. <https://doi.org/10.3390/pathogens11111320>
  58. Lahariya, C., Sharma, A. K., & Pradhan, S. K. (2006). Avian flu and possible human pandemic. *Indian pediatrics*, 43(4), 317–325.
  59. Kuchipudi, S. V., Nelli, R. K., Gontu, A., Satyakumar, R., Surendran Nair, M., & Subbiah, M. (2021). Sialic Acid Receptors: The Key to Solving the Enigma of Zoonotic Virus Spillover. *Viruses*, 13(2), 262. <https://doi.org/10.3390/v13020262>
  60. Lange, C. M., Gouttenoire, J., Duong, F. H., Morikawa, K., Heim, M. H., & Moradpour, D. (2014). Vitamin D receptor and Jak-STAT signaling crosstalk results in calcitriol-mediated increase of hepatocellular response to IFN- $\alpha$ . *Journal of immunology (Baltimore, Md. : 1950)*, 192(12), 6037–6044. <https://doi.org/10.4049/jimmunol.1302296>
  61. Gal-Tanamy, M., Bachmetov, L., Ravid, A., Koren, R., Erman, A., Tur-Kaspa, R., & Zemel, R. (2011). Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. *Hepatology (Baltimore, Md.)*, 54(5), 1570–1579. <https://doi.org/10.1002/hep.24575>
  62. Kondo, Y., Kato, T., Kimura, O., Iwata, T., Ninomiya, M., Kakazu, E., Miura, M., Akahane, T., Miyazaki, Y., Kobayashi, T., Ishii, M., Kisara, N., Sasaki, K., Nakayama, H., Igarashi, T., Obara, N., Ueno, Y., Morosawa, T., & Shimosegawa, T. (2013). 1(OH) vitamin D3 supplementation improves the sensitivity of the immune-response during Peg-IFN/RBV therapy in chronic hepatitis C patients-case controlled trial. *PloS one*, 8(5), e63672. <https://doi.org/10.1371/journal.pone.0063672>
  63. Iqtadar, S., Khan, A., Mumtaz, S. U., Livingstone, S., Chaudhry, M. N. A., Raza, N., Zahra, M., & Abaidullah, S. (2023). Vitamin D Deficiency (VDD) and Susceptibility towards Severe Dengue Fever-A Prospective Cross-Sectional Study of Hospitalized Dengue Fever Patients from Lahore, Pakistan. *Tropical medicine and infectious disease*, 8(1), 43. <https://doi.org/10.3390/tropicalmed8010043>
  64. Huang, F., Zhang, C., Liu, Q., Zhao, Y., Zhang, Y., Qin, Y., Li, X., Li, C., Zhou, C., Jin, N., & Jiang, C. (2020). Identification of amitriptyline HCl, flavin adenine dinucleotide, azacitidine and calcitriol as repurposing drugs for influenza A H5N1 virus-induced lung injury. *PLoS pathogens*, 16(3), e1008341. <https://doi.org/10.1371/journal.ppat.1008341>
  65. Martineau, A. R., Jolliffe, D. A., Greenberg, L., Aloia, J. F., Bergman, P., Dubnov-Raz, G., Esposito, S., Ganmaa, D., Ginde, A. A., Goodall, E. C., Grant, C. C., Janssens, W., Jensen, M. E., Kerley, C. P., Laaksi, I., Manaseki-Holland, S., Mauger, D., Murdoch, D. R., Neale, R., Rees, J. R., ... Hooper, R. L. (2019). Vitamin

- D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. *Health technology assessment (Winchester, England)*, 23(2), 1–44. <https://doi.org/10.3310/hta23020>
66. Grant, W. B., Lahore, H., McDonnell, S. L., Baggerly, C. A., French, C. B., Aliano, J. L., & Bhattoa, H. P. (2020). Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients*, 12(4), 988. <https://doi.org/10.3390/nu12040988>
  67. Enioutina, E. Y., Bareyan, D., & Daynes, R. A. (2009). TLR-induced local metabolism of vitamin D3 plays an important role in the diversification of adaptive immune responses. *Journal of immunology (Baltimore, Md. : 1950)*, 182(7), 4296–4305. <https://doi.org/10.4049/jimmunol.0804344>
  68. Zhao, Y., Yu, B., Mao, X., He, J., Huang, Z., Zheng, P., Yu, J., Han, G., Liang, X., & Chen, D. (2014). Dietary vitamin D supplementation attenuates immune responses of pigs challenged with rotavirus potentially through the retinoic acid-inducible gene I signalling pathway. *The British journal of nutrition*, 112(3), 381–389. <https://doi.org/10.1017/S000711451400097X>
  69. Gayan-Ramirez, G., & Janssens, W. (2021). Vitamin D Actions: The Lung Is a Major Target for Vitamin D, FGF23, and Klotho. *JBM plus*, 5(12), e10569. <https://doi.org/10.1002/jbm4.10569>
  70. Teles, R. M., Graeber, T. G., Krutzik, S. R., Montoya, D., Schenk, M., Lee, D. J., Komisopoulou, E., Kelly-Scumpia, K., Chun, R., Iyer, S. S., Sarno, E. N., Rea, T. H., Hewison, M., Adams, J. S., Popper, S. J., Relman, D. A., Stenger, S., Bloom, B. R., Cheng, G., & Modlin, R. L. (2013). Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science (New York, N.Y.)*, 339(6126), 1448–1453. <https://doi.org/10.1126/science.1233665>
  71. Matthaei, M., Budt, M., & Wolff, T. (2013). Highly pathogenic H5N1 influenza A virus strains provoke heterogeneous IFN- $\alpha/\beta$  responses that distinctively affect viral propagation in human cells. *PloS one*, 8(2), e56659. <https://doi.org/10.1371/journal.pone.0056659>
  72. Shin, H., Kim, S., Jo, A., Won, J., Gil, C. H., Yoon, S. Y., Cha, H., & Kim, H. J. (2022). Intranasal inoculation of IFN- $\lambda$  resolves SARS-CoV-2 lung infection via the rapid reduction of viral burden and improvement of tissue damage. *Frontiers in immunology*, 13, 1009424. <https://doi.org/10.3389/fimmu.2022.1009424>
  73. Jeon, Y. J., Lim, J. H., An, S., Jo, A., Han, D. H., Won, T. B., Kim, D. Y., Rhee, C. S., & Kim, H. J. (2018). Type III interferons are critical host factors that determine susceptibility to Influenza A viral infection in allergic nasal mucosa. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, 48(3), 253–265. <https://doi.org/10.1111/cea.13082>
  74. Kim, S., Kim, M. J., Kim, C. H., Kang, J. W., Shin, H. K., Kim, D. Y., Won, T. B., Han, D. H., Rhee, C. S., Yoon, J. H., & Kim, H. J. (2017). The Superiority of IFN- $\lambda$  as a Therapeutic Candidate to Control Acute Influenza Viral Lung Infection. *American journal of respiratory cell and molecular biology*, 56(2), 202–212. <https://doi.org/10.1165/rcmb.2016-0174OC>
  75. An, S., Jeon, Y. J., Jo, A., Lim, H. J., Han, Y. E., Cho, S. W., Kim, H. Y., & Kim, H. J. (2018). Initial Influenza Virus Replication Can Be Limited in Allergic Asthma Through Rapid Induction of Type III Interferons in Respiratory Epithelium. *Frontiers in immunology*, 9, 986. <https://doi.org/10.3389/fimmu.2018.00986>
  76. Isomura, S., Ichikawa, T., Miyazu, M., Naruse, H., Shibata, M., Imanishi, J., Matsuo, A., Kishida, T., & Karaki, T. (1982). The preventive effect of human interferon-alpha on influenza infection; modification of clinical manifestations of influenza in children in a closed community. *Biken journal*, 25(3), 131–137.
  77. Saito, H., Takenaka, H., Yoshida, S., Tsubokawa, T., Ogata, A., Imanishi, F., & Imanishi, J. (1985). Prevention from naturally acquired viral respiratory infection by interferon nasal spray. *Rhinology*, 23(4), 291–295.
  78. Hayden, F. G., Winther, B., Donowitz, G. R., Mills, S. E., & Innes, D. J. (1987). Human nasal mucosal responses to topically applied recombinant leukocyte A interferon. *The Journal of infectious diseases*, 156(1), 64–72. <https://doi.org/10.1093/infdis/156.1.64>
  79. Beilharz, M. W., Cummins, M. J., Bennett, A. L., & Cummins, J. M. (2010). Oromucosal Administration of Interferon to Humans. *Pharmaceuticals (Basel, Switzerland)*, 3(2), 323–344. <https://doi.org/10.3390/ph3020323>
  80. Tovey, M. G., & Maury, C. (1999). Oromucosal interferon therapy: marked antiviral and antitumor activity. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*, 19(2), 145–155. <https://doi.org/10.1089/107999099314298>
  81. Dec, M., & Puchalski, A. (2008). Use of oromucosally administered interferon-alpha in the prevention and treatment of animal diseases. *Polish journal of veterinary sciences*, 11(2), 175–186.
  82. Carp, T. N., Metoudi, M., & Ojha, V. (2024). Infection-Simulator, Immunostimulatory and Immunomodulatory Effects of Interferons I and III in Biological Systems: A New Era in Vaccinology and Therapeutics Possible?. Preprints. <https://doi.org/10.20944/preprints202212.0155.v7>
  83. Carp, T. N. (2024). Potential Innovations in Modern-Day Human and Animal Vaccine Development. Preprints. <https://doi.org/10.20944/preprints202407.2158.v4>
  84. Fraiman, J., Erviti, J., Jones, M., Greenland, S., Whelan, P., Kaplan, R. M., & Doshi, P. (2022). Serious adverse events of special interest following mRNA COVID-19 vaccination in randomized trials in adults. *Vaccine*, 40(40), 5798–5805. <https://doi.org/10.1016/j.vaccine.2022.08.036>
  85. Whitaker M. (2006). Calcium at fertilization and in early development. *Physiological reviews*, 86(1), 25–88. <https://doi.org/10.1152/physrev.00023.2005>

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.