Review

Turning Pathogens into Vaccines via Loss-of-Function Research and Interferon Gene Insertion: Trampling Death by Death?

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Posted Date: 30 January 2025

doi: 10.20944/preprints202501.1699.v4

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Review

Turning Pathogens into Vaccines via Loss-Of-Function Research and Interferon Gene Insertion: Trampling Death by Death?

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Abstract: Throughout several centuries, infectious pathogenic agents have been used as models for the ongoing efforts of vaccine development, which saved hundreds of millions of lives from life-threatening infectious diseases worldwide. Nonetheless, there has been a missing gap that various polymorphic microbes have been taking advantage of in their evolutionary pathway: the interferon system, which often prevented the timely activation of second and third-line host immunity, leading to chaotic and mismatching immune responses. The phenomenon of increased incubation period of various infectious diseases may be a result of the increased abilities of such microbial agents to directly and indirectly undergo molecular self-camouflaging, which prevents the activation of Type I and Type III Interferon-encoding genes (INGs) in indirect and direct manners respectively, and cleaves the mRNA molecules encoding such interferon glycoproteins, often causing major delays in the process of autocrine and paracrine signalling of Type I and Type III Interferon glycoproteins, which in turn allows an unrestricted, exponential increase of the microbial load/count, giving rise to a statistical probability that the quality of the delayed immune response will be low and contributory to the processes of pathogenesis and pathophysiology. Apprehending the foundational layer of the current problems in evolutionary microbiology, epidemiology and public health studies is most likely crucial for the course of immunological, pharmaceutical and vaccine-related clinical research. In the current case, it is the complex set of molecular capabilities to suppress Type I and Type III Interferon-based signalling displayed by several polymorphic microbes of public health concern, and it may be that the rates of immunopathogenesis induced by such microbes are directly proportional with such pathogenic abilities of induced interferon suppression. Proportional medical responses could include the development of approaches involving low dosages of human recombinant Type I and Type III Interferon glycoprotein and perhaps also of protollin in the nasopharyngeal cavity, potentially bringing an example of putting a novel concept of a "United Immune System" into practice. Furthermore, similar dosages of such interferons could be administered into human immune cells including plasmacytoid dendritic cells, as well as natural and adaptive lymphocytes, to optimise their immune function and integrity against various environmental hazards. Ultimately, clinical researchers may isolate the pathogenic agents, attenuate them through the process of loss-of-function laboratory research, before performing gene editing to insert Type I and Type III Interferon-encoding, perhaps as well as Pattern Recognition Receptor (PRR) Agonist-encoding genes that specifically match the PRR targeted by the implicated microbes, into their genomic profile and potentially releasing the genetically-modified pathogens back into the environment transmissible factories of Type I and Type III Interferons, perhaps as well as of specific PRR Agonist proteins. It may be important to mention that the process of clinical weakening of the isolated microbes would be aimed at reducing the activity of microbial genes implicated in pathogenesis and pathophysiology, and perhaps not as much microbial genes involved in transmission. Such a change may bring various pathogenic agents into a path of evolutionary self-destruction, as they would start producing and sending signals to the proximal, innate immune system as soon as they enter the first host cells, making their same processes of induced innate immune suppression ineffective, and several dilemmas in microbial evolution could ultimately be tackled as a result, possibly even at least

 doi:10.20944/preprints202501.1699.v4

attenuating the phenomenon of acquired antibiotic resistance by various pathogenic bacteria. A clinical approach as such is likely based on the model of increasing the accessibility to insulin-based treatment against Diabetes Mellitus via insulin-encoding gene insertion into the genomes of harmless bacteria prior to their administration into human host organisms, which saved millions of lives worldwide. Processes of shrinkage of any level of limitations to potential efficacy would include the manual utilisation of inhalators, oral drops and/or injectable serums containing such modified microbes to ensure that such an immunising effect would be conferred simultaneously with exposure to the artificially-changed genetic version of the microbe. A set of clinical responses involving all such pathways may ultimately bring a promise of a health-related "Golden Age" throughout the world.

Keywords: innate immunity; adaptive immunity; evolutionary microbiology; evolutionary immunology; virus; bacterium; yeast; pattern recognition receptors; interferon system; lymphocyte system; polymorphic microbes; single-nucleotide polymorphism; molecular self-camouflaging; loss-of-function research; gene editing; antibiotic resistance; cytosol; medical ethics; prophylaxis; therapeutics; gene expression; protein synthesis; autocrine signalling; paracrine signalling

Introduction

Recently, several public health incidents have occurred and significantly impacted the health state of both animal and human host organisms. The highly diverse phenomena of molecular self-camouflaging displayed the causative microbial agents may represent a foundational factor for the induced severity of infectious clinical disease in both animal and human hosts, which seems to surpass the current version of vaccine-based clinical responses, despite their high rates of efficacy displayed throughout the past centuries. It may be that there is an existing gap of potential update in the domain of vaccinology - one that would directly antagonise such a foundational factor of induced severe infectious illness. Given the fact that natural immunity has recently been shown to exhibit traits of specificity and even its own, distinct "specific memory" as well makes it possible for the efforts of vaccinology to be updated through a wider inclusion of both first-line and second-line, natural immune elements. Such an aspect may only confirm the high rates of efficacy and safety displayed by recently-developed prophylactic and early therapeutic approaches involving low dosages of human recombinant Type I and Type III Interferon glycoproteins into the nasopharyngeal cavity. Interestingly, results indicate that such concentrations of Type I and Type III Interferon glycoprotein brought effects of immunostimulation, immunomodulation and even whole effects of immunisation against multiple diseases, including COVID-19, flu, AIDS and various oncological diseases. Moreover, it has been suggested that protollin brings similar immunostimulatory and immunomodulatory effects in the case of Alzheimer's Disease, by the recruitment of adaptive lymphocytes to areas of the Central Nervous System, where they will in turn activate microglial cells and oligodendrocytes before misfolded alpha-synuclein and beta-amyloid toxins start causing clinical signs and symptoms (Frenkel D. et al., 2008). Scientists theorised that protollin brings an immunisation effect against the pathogenesis and early pathophysiology of Alzheimer's Disease, potentially giving further rise to the probability that Type I and Type III Interferons bring similar effects (Frenkel D. et al., 2005). A similar outcome may occur for the case of Retinitis Pigmentosa, which is caused by misfolded Rhodopsin toxins in the process of a progressive destruction of retinal cells with rods, which is clinically manifested as a progressive loss of vision to the point of the patient reaching a state of complete blindness, often by mid-age adulthood and, in such cases, a medical approach involving the administration of chaperones specific to the retinal tissue may be required as well. Furthermore, it is possible for plasmacytoid Dendritic Cells (pDCs), Natural Killer (NK) Cells, helper CD4+ T-Lymphocytes and cytotoxic CD8+ T-lymphocytes to be treated with a low dosage of Type I and Type III Interferons, perhaps alongside protollin, to improve both their efficacy and integrity against environmental hazards, and such an approach would effectively represent a form

 doi:10.20944/preprints202501.1699.v4

of “immunisation of immunising agents” and could potentially turn adaptive lymphocytes into “super-lymphocytes” in efforts to protect human immunity from the long-term and life-threatening danger of HIV-1-induced AIDS, and essentially conferring a proportional “punch of immunological self-defence” against the virus (Carp T., 2024).

Given the fact that the interferon system may be calibrated likewise to help human immunity develop a proportional evolutionary response that would preserve human health over microbial self-camouflaging capabilities, it is possible for vaccine researchers and developers to include Type I and Type III Interferon-encoding genes into live-attenuated pathogens or pathogenic fragments. Furthermore, it is possible for researchers to perform similar gene editing procedures in isolated microbes to turn microbial agents into signalling facilities for the host innate immunity, which would essentially mean that pathogens as such would be effectively transformed into vaccines, as they would become unable to cause disease given the automatic microbial autocrine and paracrine signalling of both Type I and Type III Interferon glycoproteins once it undergoes the first series of receptor-mediated endocytosis. Given the fact that the first and the third classes of the interferon system profoundly stimulate and modulate major immune responses, essentially representing the foundation for the adequate activation of the entire immune system following the first stages of infection, it may be that such a scenario may apply even for pathogenic agents causative of diseases of more significant public health concern. Likewise, with the unprecedented threats made by recent developments contained within the evolutionary path of pathogenic agents, there seems to be a small, but considerable window of opportunity that may bring unprecedented hope, with the possibility of artificially inducing genetic manipulation of newly-selected variants of microbial agents that constitute a concern for both human and animal public health, ultimately making it unnecessary for the pharmaceutical industry to develop nasopharyngeal spray or drop-based vaccine approaches, let alone traditional needle-based ones. In other words, the ultimate stage of vaccine evolution may involve a silent “infection” of human and animal hosts with attenuated microbes that contain active Type I and Type III Interferon-encoding genes, which produce a number of interferon glycoprotein that significantly crosses a threshold level characterised by the ability of the same pathogen to antagonise them. Poetically, just as advanced stages of microbial evolution hijacked and suppressed the quality of human immune responses via favouring the development of autoimmunity, so artificial interventions will lead to advanced stages of human and animal immune evolution by the induction of microbial activities that antagonise each other. If problems of inefficacy occur due to a lack of a threshold level of human-to-human and animal-to-animal transmission of such attenuated, interferon-encoding microbial agents, then inhalators or injectable serums containing low concentrations of such microbial copies may be administered to patients during the first weeks of the fall season to manually induce an immunising effect where gaps preventing the reach of herd immunity may exist.

Discussion

Tackling the complex microbial machinery of induced immune evasion most likely represents the primary objective of public health and vaccine innovation-based pharmaceutical, scientific and clinical research. There is a highly diverse group of candidate clinical approaches that can help the human immune system outcompete the novel extents of induced immune evasion by several polymorphic microbes, and such approaches may be used even in combination to foster the production of utmost qualitative and long-lasting results for the human and animal immune systems alike. It may be that the ultimate solution to the dilemma of viral and bacterial immune evasion is the isolation, attenuation and genetic editing of epidemic microbes during their initial stages of distribution throughout human and animal populations respectively, which commonly occurs during the first weeks of the fall season. Despite the fact that microbial agents utilise highly diverse methods of inducing cellular and tissue-level pathogenesis and pathophysiology, there seems to be a Universal method of immune evasion utilised by the majority of such microbes in their preparation

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for inducing clinical disease. The machinery of induced immune escape generally consists of three distinct pathways, which all ultimately point to the common result of significantly suppressing the production and signalling of Type I and Type III Interferon glycoproteins. The first pathway constitutes a direct form of microbial self-camouflaging and involves the double methylation of the 5′ end of the microbial genome by two viral non-structural protein complexes (NSP10/14 and NSP10/16 respectively, with NSP10 representing the activator protein and NSP14 and NSP16 representing the effector proteins), which leads to the prevention of Pattern Recognition Receptor (PRR)-based recognition of Pattern-Associated Molecular Patterns (PAMPs) on the microbial genome, as well as of Damage-Associated Molecular Patterns (DAMPs), which represent toxin proteins synthesised by the microbial genome once it has undergone receptor-mediated endocytosis without significant restriction. Given the existence of indirect, transient immunosuppressive methods as such, active genes encoding PRR agonists specific to the type of PRR inactivated by the pathogenic microbe could also be inserted into the microbial genome, perhaps to ensure proportion in the interferon-stimulatory and interferon-stimulated signalling rates in all cases. The second pathway represents an indirect form of microbial self-camouflaging, which however involves the direct antagonism of Type I and Type III Interferon-encoding genes (INGs), as well as of Interferon-Stimulated Genes (ISGs) through various methodologies of mRNA cleaving and induced protein disposal - particularly by translated non-structural proteins (NSPs) 1 and 2. The third pathway involves the facilitation of the viral protein-based paracrine signalling through channeling nanotubes, which are produced by host cells with the original purpose of transmitting immune signals as soon as the first infection stages occur. Likewise, microbial agents of individual and public health concern have generally developed highly profound networks of immune evasion and even suppression, stimulating scientific and pharmaceutical researchers to develop unprecedented, world-class methodologies of clinical responses that “outsmart” such networks contained by the evolutionary machinery of viruses, bacteria and even yeasts.

Generally, it is known that the cytokine system of the innate immune system constitutes the root of the entire process of adaptive immune activation and signalling that is proportional to the extent and severity of the microbial reproductive rates within the host organism. Nonetheless, it may be important to differentiate the first and the third classes of the interferon system from the second class, due to the fact that the production and signalling of Type II Interferons is directly dependent upon the production and signalling of Type I and Type III Interferons. Namely, it is known that Interferon-Stimulated Gene products, which are signalled as a direct result of adequate Type I and Type III Interferon signalling, are responsible for the recruitment of Natural Killer Cells, which constitute factories for Type II Interferons. Likewise, it may be more contextual for the research communities to deem Type I and Type III Interferon glycoprotein as pre-cytokine innate immune elements and potentially raise clinical awareness about the particularly high importance such particular interferon glycoprotein types brings in the activation process of the immune system, as they constitute a foundational factor for the adequate activation of the cytokine system itself. Moreover, the fact that the innate immune system displays considerable extents of “specific memory”, as well as considerable traits of specificity in their signalling processes, ultimately indicates the existence of adaptive immunity-like “purpose” even within its first line of defence, which comprises the PRR system, as well as the pre-cytokine networks of INGs and ISGs. Given the fact that innate immunity has shown to display considerable extents of “specific memory”, as well as specificity in their activation and signalling processes, Likewise, innate immunity may also be used significantly in the process of immune system-based vaccine innovation and development, despite the development of the initial theory that important elements of the innate immunity may only be used as vaccine adjuvants (Carp T., 2024). Such developments ultimately indicate that principal elements of first-line, innate immunity also play visible roles in whole processes of immunisation as well, and not solely the second line of natural immunity. Hence, infectious pathogens may be isolated, undergo loss-of-function research in various laboratory settings, specifically by having their pathogenesis-inducing and pathophysiology-maintaining genes substantially attenuated, whilst probably not having their

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genes involved in transmission substantially attenuated as well, prior to having Type I and Type III Interferon glycoprotein-encoding genes inserted into their genome, and being released back into the surrounding environment as transformed, immunising agents that have become factories for Type I and Type III Interferons, and that may be transmitted in an airborne manner. There may still be some existing limitations in such a case, as there ought to be some form of transmission in order for herd immunity to be reached, and that may only occur if there is some extent of clinical symptoms occurring following such microbial exposures, and the interferon-encoding genes may prevent the development of symptoms, potentially making a significant number of the copies of the genetically-modified microbes unable to be transmitted. Perhaps, a low concentration of genetically modified microbial copies can be inhaled nasally by and/or administered via oral drops to a given number of human and animal recipients in order for herd immunity to be manually reached if necessary. In other cases, low concentrations of genetically modified microbial agents as such may be placed into an injectable serum, prior to being administered intramuscularly, in a similar fashion to traditional, intramuscular vaccination. Another advantage of such an overall set of potential approaches represents the fact that human interferon-alpha, -beta and -lambda-encoding genes contain an approximate total of 1,250 base pairs (bp), which generally represents a proportion of 0.1-10% of major microbial genomes, meaning that the probability of the existence of limitations with regards to potential negative effects to microbial genomic capacity is pronouncedly low, even if genes encoding agonists of human and animal Pattern Recognition Receptors (PRRs) are included in the process of microbial gene insertion. In any, most likely remote cases of limitations, one or two interferon subtype-encoding genes may be inserted instead of three, for example. The ultimate objective of such candidate vaccination approaches is to help both humans and animals outcompete the gained evolutionary capabilities of several microbial agents through direct and indirect methods of molecular self-camouflaging whilst keeping the extent of safety above the threshold level established by the Universal principles of medical ethics. Such a candidate clinical approach is likely based on the model used in past efforts to exponentially increase the bioavailability and biodistribution of insulin for patients suffering from Type I and Type II Diabetes Mellitus, which occurred via the utilisation of harmless bacteria containing recombinant genes encoding insulin, effectively transforming them into “mobile factories” of insulin (Riggs A. D., 2021). Through such a procedure, millions of lives were saved worldwide, as bacterial genes encoding insulin were reproduced and distributed sharply with each round of bacterial binary fission. Likewise, such a process of bacterial gene editing utilised for prophylactic or therapeutic approaches in humans and animals is not completely foreign to the scientific and medical communities. Perhaps, insertion of genes encoding Type I and Type III Interferons, as well as protollin, chaperones that play a role in the maintenance of retinal integrity, as well as wild-type Rhodopsin can be inserted into the genomes of such harmless bacteria, before they would be administered through eye drops or nasal sprays for the purpose of attempting prophylactic and/or early therapeutic approaches against the extracerebral proteinopathy of Retinitis Pigmentosa, for example (Kosmaoglou M. et al., 2008). Such an approach could at least sometimes even prevent the onset of the disease.

There are multiple existing environmental approaches of weakening specific microbes, by physical, chemical, biological and/or genetic manners, to make them more tolerable by the host organisms, with the purpose of encouraging the production of a herd immunity level without the causation of individual, life-threatening forms of infectious disease in the process. Nonetheless, few would barely pass the bioethical screening procedures because the ultimate purpose of medicine is to first not cause any form of harm. Nonetheless, it has become possible to utilise such approaches in the specific context of added Type I and Type III Interferon-encoding genes into the genomic profile of the microbial agent, as there would be no harm induced any longer due to the fact that the pathogen would automatically produce the glycoprotein molecules that produce the adequate anti-microbial signals whilst maintaining the adequate balance between produced anti-inflammatory and pro-inflammatory signals. Such approaches would require due clinical testing if a direct, separate administration of Type I and Type III Interferon glycoproteins does not bring the required long-term

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effects of immunisation whilst keeping financial expenditure to a level as low as the case of the vaccination campaigns against various epidemic illnesses that have been occurring for the past century. Interestingly enough, it is such a missing "piece of puzzle" existent in research ideas concerning loss-of-function microbial research that seems to fill in a proportional gap in human and animal vaccinology, as the host interferon system represents the primary target of microbial adaptation via multiple single-nucleotide polymorphism events in various functional areas of their genome. Another example of a clinical application may be in the tackling of antibiotic-resistant bacterial infections due to the fact that the foundation of the issue lies within evolutionary biology, like the dilemma of evolved, interferon-evading microbial mechanisms. It may ultimately be less financially demanding for such an application to be widely performed in antibiotic resistant bacteria of individual and public health concern, by having their pathogenic genes attenuated and two or three subtypes of genes encoding Type I and Type III Interferons inserted into the bacterial genome. In short, due to the foundational role played by first-line immunity, it may be that a widespread utilisation of Type I and Type III Interferon-based clinical applications may tackle complex modern-day health-related problems that include acquired antibiotic resistant by bacteria, perhaps due to an existing level of excess antibiotic usage and distribution in several areas of the world and particularly in hospital settings, where secondary bacterial infections are deemed as common and safety often turns to be placed above the necessity of medical solutions to be projected and applied according to the matched aetiological context of the involved clinical disease.

Conclusion

The evolutionary battle between human and animal immune systems and polymorphic pathogenic agents has reached a critical stage, with the current existence of highly profound and firm microbial networks that are evasive of first-line and second-line, natural immunity. It seems that the development of recent epidemic and pandemic diseases has heavily depended upon such evolutionary capabilities of direct and indirect molecular self-camouflaging of the causative pathogens in front of the host interferon system. The fact that there are existing therapeutic approaches designed to target microbial gene products directly or indirectly responsible for the suppression of the host interferon system displays an existing level of scientific awareness to the existing phenomenon of natural immune evasion by numerous microbes. According to the latest stages of scientific, pharmaceutical and clinical research of the human immunity and microbial evolution, there is a paradoxical existence of both unprecedented threats to the integrity of human and animal public health, as well as of novel horizons of hope, as there is a possibility for clinical researchers in almost any geographical area of the world to turn threatening pathogenic agents into immunising factories for foundational, innate immune signals that will automatically activate the adaptive immune system in a manner that is proportionate to the microbial count or load. An update of the current course of therapeutic and vaccine-based research and innovation may likewise involve a proportional inclusion of first-line and perhaps also second-line, natural immunity, to merge a considerable extent of such immune departments with the central, adaptive immune elements, with the overall purpose of naturally stimulating the human immune system to outcompete the highly developed interferon-suppressive evolutionary responses developed by polymorphic microbial agents through numerous rounds of single-nucleotide polymorphism (SNP) in diverse important genes. Such an approach may illustrate the concept of "United Immune System" put in clinical practice. Loss-of-function microbial research may represent a controversial form of research if it is not accompanied by viable methods to turn microbial agents into immunising agents whilst causing no harm in the process, and gene editing via the insertion of active Type I and Type III Interferon-encoding genes may represent the accompanying factor that may turn such a research procedure into a thoroughly ethical one for both the medical and the biological domains. And the current context of advanced microbial evolution may be causing the production of sentiments of urgency within major research communities regarding the development of innovative solutions to proportionately

counteract such microbial genetic "intelligence". Utilising a set of combined approaches, particularly in groups of patients where prophylactic immune support is needed more, may bring the utmost effect of immunisation and long-term immunity. The ultimate objective of such hypothesised and proposed updates in the known methods of immunisation, prophylaxis and early therapeutic approaches is to at least gradually decrease the probabilities of occurring limitations toward the point of zero. Currently, it is the responsibility of the scientific communities to assess and distribute any existing piece of scientific evidence that is relevant to such hypotheses and novel developments into clinical research, with the ultimate purpose of encouraging clinical researchers to assess novel candidate approaches that cross the initial threshold levels of safety and efficacy that are necessary for initial clinical trials to occur.

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