Review

Countering and Tackling Advanced First-Line IMMUNE Evasion: The Most Feasible and Accurate Approach to Control and Eradicate Rabies?

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Review

Countering and Tackling Advanced First-Line Immune Evasion: The Most Feasible and Accurate Approach to Control and Eradicate Rabies?

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Abstract: Despite being a rare disease worldwide, rabies has the highest morbidity and mortality rates, with nearly all symptomatic cases leading to coma and death. Rabies represents an infectious disease caused by the Rabies virus (RABV), which is part of the Lyssavirus group and the Rhabdoviridae family, and it mainly spreads through the bite and scratch of an infected mammal, but particularly of wild animals, such as bats, foxes, wolves and racoons, and of domestic animals, such as dogs and cats, in rabies-prone areas of the world. Airborne transmission has been deemed as extremely rare, and no clinical case as such has been recorded worldwide yet, except in the enclosed environment, such as research laboratories and caves where infected bats are present. Domestic mammals, such as dogs and ferrets, represent other important reservoirs of disease transmission, and the human cases of Asia and Africa amount approximately 95% of all human cases worldwide. Infected animals most commonly start transmitting the virus once the first symptoms have occurred, and if they experience disease aggravation and death within 10 days, a case of rabies is registered, more easily if the incidence occurred in the urban area and then, any person or animal that had been potentially exposed are strongly recommended to receive the inoculation. It is rare for asymptomatic mammals to transmit the illness. Most First-World and several Second-World countries have recently been declared dog rabies-free by the World Health Organization. The disease can only be treated prophylactically, with three doses of a vaccine containing an inactivated form of RABV, or with five doses of the vaccine and two doses of anti-RABV immunoglobulins within 28 days if the patient is believed to have been exposed to the virus beforehand. It has been projected that, once the viral load reaches elements of the central nervous system, prophylactic approaches are no longer effective, even if symptoms have not begun yet, and this highlights the urgent trait of the medical condition, strongly recommending exposed people to receive the prophylactic doses immediately after the potential exposure to the virus. The pathogen first infects the bodily fluids, before reaching the peripheral nervous system, from where it will gradually move toward the spinal cord or the encephalon, at a speed of movement ranging from 1 to 40 cm per day. It was also found, in extremely rare circumstances, to infect the nasopharyngeal cavity and the lungs. The primary cause of a successful, gradual advance of the viral load toward the point of clinical no-return for the patient - the CNS - is a complex mechanism of induced innate immune evasion, with the interferon system being heavily targeted and silenced by RABV proteins. The 'Milwaukee' protocol is locally believed to decrease the mortality rate of the clinical illness to approximately 80%, although significantly more research is required in this sense. First-line immune evasion represents the central mechanism developed by viruses during their evolutionary process to gain control over human immunity, so it could be the development and adjustment of a counter-offensive to this evolutionary operating system that could address the core elements of the problem. Human recombinant Type I and Type III Interferons were found to be significant vaccine adjuvants and to considerably delay the clinical onset of the disease. Despite their central role in natural immunity-based prophylaxis, vaccine support and, in often cases, vaccination per se, a local administration of IFNs as such may not be enough to tackle the core problem of the endemic disease, and a specific and systemic treatment of potential host cells with IFN I and III, as well as IFN-stimulating proteins, may constitute a major research requirement in the coming years of disease investigation, as the inoculation efforts with the inactivated virus and immunoglobulin administration continue. The administration of a relatively low dosage of somatic Natural Killer cells, gamma-interferon and perhaps, of somatic helper CD4+ and somatic cytotoxic CD8+ T-lymphocytes treated with alpha-, beta- and lambda-interferon could be merged with the administration of a similar dosage of alpha-, beta- and lambda-interferon during the efforts to develop an effective and less costly prophylactic vaccine against rabies. A

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combination of a nasal substance containing a low dosage of IFN I and III with a reduced concentration of neutralised RABV copies, and/or with a low dose of anti-RABV IgA antibodies, could also be tested for humans for the purposes of pre- and post-exposure prophylaxis. It is important to acknowledge that are existing clinical signs that early alpha-interferon-based therapies alone are not effective in curbing the mortality rate of the disease, which could be explained by the fact that, once symptoms begin, the virus has already reached and started distributing its load in critical areas of the host organism - the Central Nervous System (CNS) - and that the vast majority of the cases are detected after clinical symptoms start occurring. Nonetheless, researchers did find a broader window of opportunity with regards to early antiviral therapy, with methods such as RNA interference, as well as the development of the Favipiravir antiviral agent and of new targeted molecular therapies, which perhaps could be used alongside recombinant Type I, Type II and/or Type III Interferons, as well as the other mentioned innate and adaptive immune elements mentioned. Overall, there could be an application of a concept called "a United Immune System" into the practical, clinical world, that will significantly help in mounting an effective, long-term evolutionary response against advanced microbial immune evasion, changing the connection between innate and adaptive immunity from a "national road" to a "motorway".

Keywords: rabies; RABV; PRV; single-stranded RNA; RNA-dependent RNA polymerase; viral self-camouflaging; glycoprotein; innate immunity; interferon system; natural lymphocytes; adaptive immunity; adaptive lymphocytes; dendritic cells; IgA; IgM; IgG; primary dendritic cells; macrophages; united immune system

Introduction

Rabies virus (RABV) represents an enveloped negative-sense, single-stranded ribonucleic acid-based virus that infects mammals, although the degree of transmission varies from species to species. The virus has a length that generally varies from 100 to 300 nm and a diameter of approximately 75 nm in the majority of the cases. It first needs to express RNA-dependent RNA Polymerase before its genome becomes activated for distribution, meaning that, the less sensitive the viral genome is - due to various external factors - the less capable it is to express its activation enzyme. RABV-induced encephalitis was first documented around 2,000 years B.C. and represents one of the most complicated and life threatening illnesses mankind has ever come across. Despite the fact that humans primarily become infected as a result of a direct transmission of the virus from infected dogs, the primary reservoirs of the virus are not in domestic animals, such as dogs and ferrets, but in wild animals, such as bats, foxes and raccoons. The virus is mainly transmitted directly from the saliva of infected animals through bites and deep scratches, transmission of infected saliva through the intact mucosal layer of the host is uncommon and airborne transmission has been deemed as virtually impossible, particularly if the environment is natural and in the open air. As soon as the virus is suspended through micro-drops of infected saliva into the dry air, it quickly becomes neutralised and its structure and genetic sequence are then disintegrated. Once the virus infects a host organism, it then travels toward the encephalon at a median speed of 5-10 cm per day, although it highly varies from case to case. It seems that the speed of the viral migration in the nervous system is proportional with the quality and extent of the innate immune sensitivity, meaning that, generally speaking, the virus migrates faster with age, and likely this also applies with the health background status of the patient. Nevertheless, around 60-70% of all human cases are in children, mainly due to their common interaction with domestic dogs in rabies-prone world areas. Moreover, the speed of the viral load migration could be proportional with the amount of time it was exposed to environmental factors during the process of transmission. The virus typically survives in fluids and at temperatures ranging from 27 to 37 degrees Celsius. The average reproductive rate of the virus (R0) is estimated to be at 1.2, meaning that, for each infected mammal, there will most commonly be only a sole host mammal that will become infected, although in some occasions, two or three other mammals can become infected. Non-bite and non-abrasive transmission routes, but particularly airborne transmission, that also do not implicate a direct transmission of the saliva into the mucous membranes of the host, are

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so rare that hundreds or thousands of infected mammals would have to simultaneously transmit the disease to one healthy mammal. On the other hand, bitten animals and humans have around 40-60% chances of becoming ill with the disease, and the probability may become even higher when it is wild animals, such as bats, foxes and racoons, that have their saliva infected.

Due to the fact that the virus is enveloped and its ssRNA is negative-sense, it is particularly sensitive to the outside environment. UV radiation was found to lyse the virus within seconds if found on dry material, whilst dry air alone at a temperature outside of the 27-37 degree Celsius range was found to weaken and lyse the virus robustly as well. RABV was found to survive longest on glass and metal surfaces, and this may be due to the longest sustainability of water molecules on surfaces as such. Although airborne transmission is almost taken out of the question of transmission and no cases as such have ever been documented in the open environment, everybody who encountered a potentially rabid animal receives the public health recommendation to receive post-exposure prophylaxis (PEP), which currently includes a 5-dose course of rabies vaccination, through the upper arm's deltoid muscle, and a one or two-dose course of prepared anti-RABV IgM and IgG immunoglobulins at the site of the initial potential transmission of the virus. The recommendation is not based upon the rarity of transmission, but upon the virtual certainty of death in case symptoms of the disease develop. Despite the fact that a safe and effective clinical approach exists, there is still a wide extent of evidence to be determined with regards to the duration of prophylactic immunity against the disease. One clinical case of rabies occurred in a patient who had been immunised around 13 years beforehand, raising a few worries with regards to the length of immunity against the disease. Furthermore, there is evidence to suggest that the clinical disease can become unpreventable even with a full course of prophylaxis that is administered several weeks and months before the first symptoms occur, as studies indicate that, once the viral load reaches elements of the central nervous system or even principal pathways of the peripheral nervous system, no currently available clinical approaches can stop its advance to the encephalon and mount a firm immunological barrier alongside the blood-brain barrier. The blood-brain barrier prevents chemicals from crossing into the brain, including broad-spectrum antivirals that are normally highly effective in clearing the viral load, and the viral agent also causes the immune system to react against itself in the end, resulting in the destruction of vital neuronal tissues. Once it reaches its ultimate target organ, the viral load starts increasing substantially and viral copies are distributed to other organs, including the salivary glands. The first signs and symptoms of RABV-induced encephalitis are similar to the symptoms of the flu, and then, as the disease progresses further, neurological symptoms appear and develop, with anxiety, hydrophobia, aerophobia, psychosis and mania as key examples of the aggressive type of the symptomatic disease, which occurs in approximately 80% of all cases. The non-aggressive type of symptomatic rabies implicates a progressively widespread phenomenon of paralysis, which ultimately leads to death as a result of the inability of the patient to swallow and breathe. As a result, the vast majority of the medical responses against clinical cases have only been palliative in nature. Usually, it takes 7-10 days from the first symptoms to the onset of systemic organ failure. It is uncommon, though not impossible, for asymptomatic mammals to spread the virus, provided that the stage of the infection is proximal to the moment the first symptoms occur (up to 10-14 days beforehand).

The irreversible progression of the disease to coma and systemic organ failure was found to be due to the uncontrolled inflammation of the encephalon, as the virus quickly spread in the organ. Clinicians would then be strongly encouraged to deduce that the virus would be the primary factor of causing brain death. Nevertheless, it may be important to take into consideration the tremendous ability of the virus to evade important pathways of immune activation and major antimicrobial activity. It is particularly the first-line immune system that is likely targeted by the virus during its irreversible advance from the spinal cord to the encephalon, as the virus was found to heavily hijack the interferon system. However, unlike other major clinical illnesses, where administered recombinant human interferon-alpha, -beta and -lambda play a role as immunising components, rabies was only found to have its incubation period prolonged following the administration of such a clinical approach. Although this would mean that interferons as such play only a significant role as

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vaccine adjuvants, this marks the partial tackling of the viral load migration toward the brain. As a result, it may be that seeking further innate immune system stimulating factors in the current research efforts could gradually get the clinical world closer to the point where the migration of the viral load is completely stopped and where the host immune system would be given the necessary tools to detect and lyse the viral copies. In other words, it may be the advanced phenomenon of viral self-camouflaging that is the primary cause of pathogenesis and virtual certainty of disease-induced death. Furthermore, it may be the manner of immune activation in the final stages of viral spread that is inappropriate for the actual context of the disease, as the immune system could be "misled" to fight against a virus that operates completely based on the model of immune activation. In other words, the only solution against the problem is a temporal lack of immune response, as all survivors of the disease were found to become almost fully functional again. RABV could not be targeting the core elements of the central nervous system, but the core elements of immunity that contribute to its discernment. As a result, whilst the 'Milwaukee' protocol could be a significant step toward the discovery of the keys of a complete immune conquest against the virus, the temporary inactivation of the central nervous system could actually not be fully matching to the context of immunopathogenesis. A temporary inactivation of the host immune system would probably be at least more matching to the context, and it would require to be as short-term as possible, and to take place in a septical environment. In the worst case scenario, the patient would be placed into a state of coma that would implicate the transient inactivation of both the immune and the central nervous systems, to ultimately stimulate the production of functional and relevant defensive responses against the virus to the best of the currently known clinical abilities, provided that such levels of inactivation would be as brief as possible and in accordance with the context of the disease.

Discussion

Microbial evasion of the host immune systems could represent a factor of lethal pathogenesis that is of a primary importance. All microbial agents are known to spread in the host organisms through a mechanism of innate immune evasion, and the diversity of such immune evasion is wide. Some pathogens mainly aim to inhibit the interferon system via hijacking the activity of pattern-recognition receptors (PRRs), such as Toll-Like Receptors (TLRs) 3, 7 and 8, and RIG-I-Like Receptors (RLRs), such as Melanoma-Differentiation Associated protein 5 (MDA5), others target the transcription factors for the expression of Interferon-encoding genes (INGs), others cleave the mRNA molecules that encode Type I and Type III IFNs, others prevent the transport of exocytosed IFNs to Interferon-binding receptors, such as IFNAR1/2 and IFNLR1/IL10R2 respectively for the first and the last classes of IFNs, whilst others prevent the activation and/or translation of various Interferon-Stimulated Genes (ISGs). Furthermore, viruses can also prevent the detection of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by double-methylating the 5' end of their genomes, effectively preventing the activation of cellular PRRs, causing host cells to confuse viral genomes with cellular genetic material and bringing an effect of "molecular trace erase". Whilst PAMPs constitute part of the viral genome, DAMPs represent viral byproducts that are normally deemed as toxic by host cells, triggering the activation of the interferon system, which in turn plays a crucial role in signalling to more central areas of the host immune system that an infection has occurred. In other words, a delayed activation of the interferon system may bring serious consequences with regards to the quality and precision of the overall immune activation against the infectious agent. Likewise, the phenomenon of "transient immune silencing" may constitute a severe type of transient immunocompromisation due to the fact that viral agents replicate and become distributed without considerable restriction, resulting often in the overwhelming of the immune responses when an activation process actually occurs. Such immune overwhelming occurs because immune activation often occurs only by the time the viral load is markedly high in quantity and/or widely distributed throughout host tissues and organs in such cases (Carp T., 2024). Type III Interferons were often particularly found to pronouncedly stimulate the production of antiviral immune responses and reduce the extent of pathogenic inflammatory activation. Some pathogens target exocytosed Interferon-Stimulated Gene products in a direct

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response against their ability to lyse specific viral proteins, such as non-structural proteins 1 and 2, that evolved in their initial response against first-line immunity, thereby highlighting the core evolutionary battle section, despite the central importance of adaptive immune components, such as primary dendritic cells, monocytes, M1 and M2 macrophages, B- and T-lymphocytes, alongside IgA, IgM and IgG antibodies. Microbial evasion is known to be visibly associated with the development of disrupted ratios of M1 to M2 macrophage activation, often implicating the first as aberrantly activated and the latter as weak. It could be that, the more advanced level of induced first-line immune escape, the more disrupted the M1/M2 macrophage ratio often is. Specifically, it was recently discovered that RABV induces M1 macrophage polarisation and, instead of the initial expectations that there would be the M0 state of neutralisation reached, an experimental study showed that several states of M1, M2a and M2c polarisation were detected post RABV-infection, with overall a unique macrophage polarisation extent, potentially marking the advanced level of virus-induced immune escape. Importantly, multiple viral infective pathways were detected to have significant implications upon the interferon system, which implies that RABV has direct ways of inhibiting major antiviral immune responses. Rather than inhibiting the activation of M1 and M2 macrophages, RABV prevalently agonises it, resulting in an effect upon the activity of antiviral genes, such as IFIT, OAS, TRIM and APOBEC3A. Furthermore, a recent paper implicating in-vitro and in-vivo studies has demonstrated that the activation of Interferon-Stimulated Gene 15 (ISG15), which leads to the translation of the Ubiquitin-Like Modifier Activating Enzyme 7 (UBA7), restricts the extent RABV replicates and is distributed (Zhao P. et al., 2017). Nonetheless, it was shown that the virus prevalently stimulates the synthesis of Type I Interferons rather than inhibiting it, which could indicate a next-level type of immune evasion. Moreover, the fact that the RABV glycoprotein, or in short known as RABV-G, is the main factor of virulence and the main element inducing first-line immune evasion simultaneously, further indicates that it is the tremendously advanced level of viral immune evasion that causes over 99.9% of the symptomatic patients to experience an irreversible progress of the disease. Hence, this study could indicate the allowance of RABV for delayed immune responses to operate freely, as its load would already be advancing through a new group of cells and silencing their antiviral genes by the time cells that had been infected a longer time beforehand would have their antiviral genes activated after the induced delay (Embregts C. et al, 2023).

It is perhaps interesting to note the poetic traits of human immunity, the foundational role played by its peripheral side, which marks innate immunity (Carp T., 2023). In the determination of a precise resolution of the big image of human immunity, it may also be important to mention the existence of a strong relationship of co-dependence and of interdependence between major innate and adaptive immune components. In the case of RABV, due to the negative-sense nature of the viral ribonucleic acid, the number of expressed viral proteins is restricted. Nevertheless, the viral genome was found to translate glycoproteins for the purpose of inhibiting Type I Interferon-based immune responses, and the therapeutic approach of RNA interference has been taken into consideration to aid host cells in their process of IFN I signalling (Denizot M. et al., 2012). Furthermore, it has been suggested that small drug-like molecular agonists could be developed to stimulate the activity of RLRs, as well as of the Interferon Regulatory Factor 3 (IRF3), which in turn would lead to the production, exocytosis and transmission of Type I IFNs to cells containing various ISGs that are responsible with the production of major antiviral cytokines. IRF3 is known to play a major role in the adequate synthesis of Type I IFNs and produce a broad extent of viral load clearance. Overall, such an approach could result in a tissular activation of innate immune mechanisms responsible for the induction of a sharp decrease of the local viral load, and this could very likely apply to the rabies viral disease (Pattahbi S. et al., 2015). Furthermore, lambda-interferon has been shown to manage RABV infection and restrict encephalic inflammation following the onset of rabies, by promoting the activation of the JAK-STAT pathway, by enhancing the activation and expression of ISGs and by stimulating the expression of the blood-brain barrier (BBB)-bound ZO-1 protein, which is implicated in the maintenance of the integrity of tight junctions and overall, in the restriction of encephalic inflammation. RABV specimens containing IFN-lambda2- and IFN-lambda3-encoding genes were found to reduce the production rate of pro-inflammatory cytokines in infected astrocytes and

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microglial cells (Li Y. et al., 2020). IFN-lambda was also found to stimulate the recovery of patients from central nervous system-related autoimmune responses (Manivagasam S. et al., 2022). Due to the high extent of viral self-camouflaging, it seems that the only approach that would substantially decrease the mortality rate of the disease would be to develop a counter immune "self-camouflaging" method, which would also be known as "fighting off the disease without fighting at all". This status would, of course, be temporary and, in an accordance as high as possible with the safety of a silent host immunity. The most complicated situation would be in patients with underlying health conditions and/or with family history of immunological, oncological and genetic disease. This scenario would only highlight the stage of immunological evolution mankind is at, where self-defence becomes a necessary method to survive advanced microbial evolution.

Nonetheless, there is a slight possibility for a high quality offensive against advanced pathogens during this process of immune "self-camouflage". Due to the fact that oral and nasal immunisation with UV-neutralised RABV copies was made possible for the animal species of foxes and mice respectively, there could be a novel research method of combining a low dose of glycosylated alpha-, beta- and lambda-interferons with the neutralised viral copies and/or with anti-RABV and/or PRV IgA immunoglobulins, depending on the viral agent, due to the proximity of the nasal cavity to the encephalon, as well as to the existence of pores that make connections between the nasal cavity and the encephalic neuronal network more facile. IgA immunoglobulins represent key elements of mucosal immunity and, although they are responsible for the development of local immune responses and memory and aim to train such immune compartments to tackle the first stages of viral replication and distribution, also by creating strong links between early innate immune activation and an extent of adaptive immune activation adequate to the extent of infection and viral distribution, its area of operation could be highly relevant to the context of immunopathogenesis and it may be that a local immune training as such will aid in the prevention of the viral distribution beyond the blood-brain barrier, as well as beyond the entry point between the spinal cord and the encephalon. Studies also showed that the existing vaccines against rabies target the RABV-G protein specifically, meaning that it brings a broader extent of support for the host's innate immunity. Nevertheless, it was often shown that the developed immune responses only had short-term efficacy due to the heterogeneous nature of the viral protein. A group of potential neutralising antibodies against RABV-G were named RVA122 and observed afterward to interact with a quaternary epitope situated at the superior pole of the glycoprotein in order to link domains to each other and keep the glycoprotein's protomers in the inactive pre-fusion state. When RABV-G becomes activated, it undergoes a trimerization process that involves the peripheral interaction between its central alpha-helix and adjacent loops, and not the expected interactions that would take place between the central alpha-helix and the fusion loops that are located at the inferior side of the G protein, which ultimately offered information of an increased resolution with regards to the needed steps to improve currently-available vaccines (Callaway H. M. et al., 2022). Moreover, given the efficacy of clinical trials involving the intracerebral vaccination of mice against rabies, as well as the development of nonvirulent RABV copies containing an induced loss-of-function mutation in the 333th codon of the glycoprotein (the new codon specifies arginine instead of glutamic acid), there could be an immunisation approach developed for latter stages of the asymptomatic progress of RABV migration toward the encephalon that would be into the cerebrospinal fluid (CSF) (Lafay F. et al., 1994). The disadvantage of the current context of immunisation and therapeutic research is that no plant, animal or human gene has been discovered to produce proteins that exhibit anti-RABV-G activity, making the process of anti-rabies medical research complex amongst the overall research procedures particularly due to this reason.

Initially, Dr. Louis Pasteur discovered a method in which the human body would be exposed for repeated times to a version of the rabies virus that is inactive, and also antibodies that had been specialised against the virus would be collected and administered to the patients. The patients who were exposed to the disease first had to undergo around thirty doses of the vaccine in the abdomen. As time went by, the number of the required doses decreased substantially and, recently, patients also no longer require to have the anti-rabies immunisation administered in their abdomen, but only

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into their deltoid muscle. The currently available vaccines for humans are not completely effective against certain variants of RABV in 100% of the cases and have slightly increased risks for the development of significant adverse reactions, particularly in people with specific immunological or oncogenic co-morbidities. For example, it was estimated that 1 in 10,000 vaccine recipients develop a transient immune condition known as anaphylactic shock, which involves the sudden development of a systemic immune activation, and which may pose significant health risks in the long run. It is possible that vaccine recipients with autoimmune conditions would be at least slightly predisposed to this condition. It is important to also note that anaphylactic shocks have a mortality rate of under 1% and that all potential cases of exposure should be dealt with in an absolutely serious manner, as there is no cure available for the disease. There are three available pathways of rabies vaccination for humans; the utilisation of inactivated RABV copies either in chick embryo cells, rhesus monkey foetal cells or human diploid cells. The first pathway comprises the approach with the least bioethical implications, as life begins at the moment of fertilisation according to the conclusive evidence from all main branches of Science, and the utilisation of cells derived either from aborted human foetuses or rhesus monkey foetuses implicates the utilisation of cells from living organisms that had their life taken from them without the consideration for the Universal flow of interest to keep living humans and animals alive at all costs. Living humans and animals do not have any less value of a living being, as long as the "winner" living sperm has reached the living oocyte. Some scientists and clinicians argue that the used cells are not aborted cells, but that they were only derived from aborted foetal lung cells, but it is important to add that the offspring cells replicated based upon the genetic and protein models of the aborted parent cells, and this violates the moral vision of many patients throughout the world, just as the main books of the world's most followed religions argue that "the root of good branches cannot be rotten", and this analysis is aimed at the practice of the widespread usage of cells derived from aborted foetal tissue and not the foetal tissue itself. Even if the condition of sickness apply, the life of the foetus and of the mother should be protected equally and the utilisation of cells from sick living organisms poses increased risks of health implications for the treated individuals. Perhaps, the locally imperfect conditions for human vaccinations should also be counted in the argument that the domain of vaccinology may need to be updated considerably. Overall, the urgency for the development of safe and effective immunising, prophylactic and possibly therapeutic drugs should be maintained at a high level, with the ultimate goal of developing and rolling out facile and cheap medical approaches for the entire world, given the fact that more than half of the world's population still cannot benefit from medical protection that is financially accessible.

Given the current level of evidence available on all public health databases, there could be a probability of a significantly effective early therapeutic approach against rabies, alongside the existing vaccines. The timeline of the approach could include the following; the administration of strong innate immune stimulators and modulators, the administration of low to medium doses of Zinc ionophores, such as quinine or chloroquine, to temporarily inactivate the immune system, the allowance of the viral load to further spread without a significant immune reaction, the administration of human recombinant IFN I, II and III with a glycosylation site on the N- or O-terminus, alongside IFN-treated natural and adaptive lymphocytes, which could be deemed as "super-lymphocytes", and also of primary dendritic cells, due to their increased intelligence against the highly developed methods of first-line immune evasion by RABV, as well as the intravenous administration of high doses of immunostimulatory/modulatory minerals, such as Vitamins B3, 6, 9 and 12, Vitamin C, Vitamin D3 and Zinc, alongside others. For example, a repeated dosage of 100,000 IU of Vitamin D3 could be administered for the purpose of contribution to prophylaxis and to early therapy. Recombinant interferons with glycosylation sites were shown to have a higher efficacy rate in mounting early antiviral responses, and this may be due to the interaction between the glycosyl group of the IFNs with the glucose molecules that are present on the cellular surface, thereby facilitating the binding of the IFNs to the IFNAR1/2, IFNGR1/2 or IFNLR1/IL10R2 receptors respectively for each class of IFNs. Glycosylated IFNs I and III, and primary dendritic cells may be in a relationship of reciprocal immunomodulatory support, given that dendritic cells produce and are

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activated by the IFNs of such types. Moreover, dendritic cell-based immunisation and immunotherapy have been suggested to be effective against solid tumours (Păunescu V., 2004) and HIV-induced AIDS (Espinar-Buitrago M., 2022), indicating the rather powerful effects of primary dendritic cells in shaping the host innate and adaptive immune systems alike according to the most recent stages of microbial adaptation. Zinc ionophores represent drug-like compounds that enhance the process of ion channel-mediated endocytosis of Zinc, which is known for its major role in immunomodulation and the induction of microbial lysis. Interestingly, chloroquine is an antimalarial drug that is also known as a major immunosuppressant, meaning that, whilst some therapeutic drugs stimulate the production of immune responses whilst increasing the process of clearing the microbial load, other drugs inhibit existing immune responses whilst enhancing microbial clearance, and this highlights the complexity of immunity as a whole. A relatively decreased dosage of corticosteroids could be combined with the chloroquine immunosuppressant drugs to enhance a transient reduction of the immune reactions during important stages of viral encephalitis. Also, a number of antiviral drugs have been tested for both Rabies and Pseudorabies viruses (RABV and PRV respectively), and some studies suggest that certain broad-spectrum antiviral drugs, such as Umifenovir, Favipiravir, Ribavirin and low-dose Ivermectin play a considerable role in the prevention of the disease by means of inducing viral clearance in major areas of the peripheral and central nervous system. One study suggested that Favipiravir could play a role in viral clearance as major as anti-RABV immunoglobulins. Given that the disease is viral in nature and any potential secondary infections that would usually be caused by bacteria or fungi would only contribute to an increased morbidity, antibiotics or antibacterial agents, whilst useful against such opportunistic infections are most likely ineffective with regards to a decrease of the mortality rate. Nonetheless, it was discovered that the Staphylococcus enterotoxin C2 plays a significant role as a vaccine adjuvant in mice, leading to the production of anti-RABV IgG antibodies that are more specific in nature and also to further production of gamma-interferons and interleukin-4 (IL-4), alongside an increased RABV-G-specific proliferation of splenocytes (Yao S. et al., 2018). Furthermore, surgery could become a viable option if the virus causes uncontrollable harm in the encephalon, whether directly or indirectly, as a direct method of treatment with UVB and UVC radiation on the surface of the encephalon would become necessary to destroy the viral load and stimulate host immunity to restrict and cease its damaging reaction against vital components of the central nervous system, which is known to coordinate all bodily functions, thereby to have substantial implications on the general integrity and functioning of the human body. It could be possible to project that an immunomodulatory process as such would decrease the mortality rate of the disease, from over 99.9%, all the way to 10% or less, provided that the methods of neuronal surgery are precise up to date in relation to the current state of medical progress. The method of targeting the virus using its own physical frequency, based on the models developed by Dr. Rife, could be researched further, as the high mortality rate of the disease may be raising a bioethical urgency to develop safe methods of precise biophysical targeting of the microbe. Also, there are circulating hypotheses that the activity of listening to sounds and music at the frequency of 432Hz, that does not contain negative suggestive messages and/or tunes, and that had been widely used in the world before 1938, could be a method that contributes to the development of a sharper and more flexible immune system long-term, as it is believed that the 432Hz frequency resonates better with the Earth's frequency that is estimated to be between 7.8 and 8Hz. Whilst it is likely not a determining factor, it may represent a valid contribution to the overall imagery of a persistent, successful strive toward the prevail of the human body in front of such a damaging zoonotic disease. More scientific evidence needs to be collected to confirm or infirm the validity of such hypotheses.

Furthermore, there may be a hypothesis concerning some possible slight updates to the existing anti-RABV vaccines, to perhaps stimulate the host immune systems to outcompete such viruses, by particularly starting their approach from the interferon system, rather than to focus more particularly the adaptive immune activation and buildup of memory only. In order to develop such vaccine candidates, the viral genome could be sequenced and only the gene encoding the proteins causative of pathophysiology, including the RABV-G, the RABV-M and the RABV-P proteins, could be either

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completely silenced or eliminated from the viral genome, and such an approach could further stimulate an evolutionary push for more sensitised activation processes of the host interferon system, which would probably help build a broader and firmer "bridge" between the first-line and third-line immune systems. Such an approach should include a low concentration of Type I and/or Type III recombinant interferon proteins in such cases, to ensure proper connection between the immune lines during exposure to the lysed viral copies. In other words, there could be a transformation from a "national road" to a "motorway" between the periphery and the central areas of the host immune system, effectively bringing the required materials for a united immune system and aiding it in outcompeting advanced evolutionary methods of virally-induced immune evasion. At the same time, it is highly important to acknowledge that substantially more evidence needs to be collected to get such a hypothesis practically tested, given also the fact that the rabies disease is deemed as an infectious disease bringing high clinical consequences throughout the world (Carp T., 2024). Henceforth, a primary focus of immunological and vaccine-based research would be the buildup and distribution of the concept called "a United Immune System", and such a concept would substantiate once the practical means will have been reached, and on a broader scale as well.

The slight possibility of the immune system being the primary degrading factor of the infected tissues could suggest that the allowance of viruses to infect host neurons freely and in a transient manner does not necessarily result in the destruction of such cells, as long as the purpose of an approach as such would be the ultimate localization and neutralisation of the self-camouflaging viruses. This would suggest a comparison to a less known principle of battle during a complicated military invasion from a super-power, which could be deemed as "hide into the forest of defeat and defeat using the hidden, instinct-based self-defence approaches". In this case, the forest could represent the depths of the encephalic networks of neurons. In a similar manner, the innate immune system could be compared with the hidden instincts of self-defence mankind has diversely shown over the ages. Nevertheless, the gradual nature of the viral load decrease following the activation of inflammatory responses shows the complicated situation with regards to the struggle of host immunity to build the adequate and flexible antiviral responses. What adds to the problem is that, in the latter stages of the illness, the viral load was shown to be virtually constant, at a value just over 1 x 10^6 viral copies per cell count (Ojosnegros S. et al., 2010). This shows that the hyperactivated immune responses are virtually ineffective in defeating the viral infection, with the viral copies always being "one step ahead" of the immune responses, and suggests that at least the majority of the discussed potential therapeutic approaches should be used almost simultaneously to ensure that the infected host organism prevails against the virus, implying overall that a projected "immune restart" would have to occur in a fast-paced manner.

Conclusions

Whilst rabies is certainly situated among the most complicated and long-sustained infectious diseases that mankind has witnessed, the current state of scientific and medical progress, as well as the recent shift of attention toward the hidden "gem" of first-line immune pathways, it has now become possible that clinicians develop pharmaceutical approaches to considerably decrease the mortality rate of the disease whilst maintaining risks of drug-induced adverse events toward zero, via a specific inclusion of prophylactic and early therapeutic innate immune stimulation, to simultaneously stimulate and modulate the produced adaptive immune responses. The development of anti-rabies vaccines by Dr. Louis Pasteur certainly represents a milestone in the highly complex and often energy-draining process of microbiological research, as the discipline of vaccinology was discovered and implemented in human civilization during the midst of the modern era. As with all other mainstream processes that human life comprises, the discipline of vaccinology requires a continuous development to become up to date with the fresh demands of evolved microbial illnesses that locally threaten the integrity and wellbeing of human inhabitants. The administration of recombinant innate immune components, as well as adaptive lymphocytes with advanced innate immunity themselves could be combined with the administration of small drug-like agonist compounds that stimulate the activity of PRRs implicated in the detection of RABV copies, as well as

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the activation of the cGAS-STING pathway to ultimately stimulate a more systemic production of antiviral and anti-inflammatory cytokines. The advanced level of immune evasion displayed by the virus may imply that the areas of the immune system localised around the encephalon remain incapable of detecting the virus in cells where it induced the transient inhibition of their interferon system and the fact that the aberrant immune responses also remain behind the actual state of tissue infection during 99% of the cases may imply that the immune system at that stage does not have any control over the virus, despite the high intensity of its developed activity. As a result, the latter treatment method involving a restart of the immune system may be among the most feasible methods, potentially exceeding the 'Milwaukee' protocol substantially. Overall, it is likely that the research community needs to thoroughly apprehend the extent of complexity that human and mammal immunity displays, directly and subtly, before developing a "brainstorm" approach to control and possibly eradicate rabies in the end. It could be essential for all the diverse and unique research concepts in microbiology, immunology and vaccinology to be placed together for the purposes of comparison and joining of the research intellectual efforts to tackle the subtle manner the rabies-inducing viruses show in their process of pathogenesis and induction of severe disease morbidity. What is also critical is that all cases of animal rabies are detected and reported to the local health authorities so that members of the general public who were potentially exposed to the transmission of the virus are informed through the local and national news, and information should also include the physical details of the infected animal. Alternatively, local authorities could also use the mobile alert services to immediately inform locals and visitors of new cases of animal rabies, as many people often come across infectious animals and may not be aware of it. Whilst medical recommendations should follow robustly, health authorities and doctors should ensure that patients only undergo medical prevention and treatment by fully reaching their informed consent, and this includes the respect of their religious and moral views, given that the potentially high levels of fear and local cases of anxiety induced by the high mortality of the disease that is combined with the lack of the 100% certainty that the disease is not distributed through less commonly-known pathways may cause patients to take medical decisions without reaching the threshold level of informed consent. The purpose of such research is to filter safe and effective approaches from approaches with a given level of clinical uncertainty that were developed out of the feelings of necessity, in the possible process of discovery of a clinical method that would ultimately eradicate the infectious illness and place human immunity in a brighter and more elevated spot in relation to pathogenic agents with genetics-derived intelligence.

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