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Edward Jenner’s documented inoculation of cowpox, which caused mild disease to protect humans against the virulent, often fatal disease caused by smallpox virus set the stage for a game-changing strategy to save many more lives than any other medical procedure. Jenner coined the term vaccination derived from vacca (Latin for cow) to distinguish it from variolation, the previously used procedure which inoculated a weakened preparation of the smallpox virus (variola). The development of modern vaccines invariably is a lengthy and expensive process and involves discovering the components to include, their production, pre-clinical and clinical testing for safety, efficacy as well as longevity of the induced protection in the target population. The COVID-19 pandemic justified an unprecedented level of collaboration to develop and fast-track the emergency approval of vaccines with strong evidence of protection against SARS-CoV-2. The two approved COVID-19 mRNA vaccines proved remarkably effective at preventing serious disease and at reducing transmission rates [1,2]. While the pandemic has been ameliorated in many places, a number of issues still need to be resolved. Additional items to address include the need for biomarkers, the duration of protection, determining whether protection is diminished against variant strains and the need to better understand the frequency and pathology of so-called long COVID. The two mRNA vaccines provided the first solid evidence that nucleic acid vaccines can be highly effective. In this instance, modified mRNA encoding the viral spike (S) protein (the receptor binding domain responsible for SARS CoV2 attachment and entry) into susceptible cells using a lipid nanoparticle-based delivery vehicle has demonstrated the viability of this vaccine strategy [3–6]. The success of these vaccines was widely unanticipated due to the previous history that was marked by the serial failure of nucleic acid vaccines.

This review discusses the reasons why mRNA based vaccines have been so successful against COVID-19 and some of their potential shortcomings. We also speculate about viral diseases in humans and domestic animals that may be amenable to prevention by mRNA vaccines. As some currently available vaccines have common (usually transient) adverse events or have only low to moderate efficacy, mRNA vaccines may provide superior alternative

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in some instances. mRNA vaccines are relatively easy to prepare, adaptable to any viral pathogen once its genomic sequence is determined. Using this technology, it is possible to induce responses to multiple antigens from the same pathogen, to cover multiple strains of the same virus, and even induce responses against more than one pathogen. Consequently, mRNA vaccines promise to provide a safe and effective strategy for the prevention of any number of diseases, including those that have thus far eluded prevention via other vaccine strategies. We also project that mRNA strategies may be valuable as therapeutic vaccines that can balance the immune response to avoid or minimise tissue damage.

1. Brief history of successful mRNA vaccine development

The idea of using genetic material as vaccines emerged in the early 90s with most of the attention focused on using DNA vaccines [7]. Using mRNA to induce protective immunity against pathogens received minimal investigation and the approach had many limitations that precluded any practical use at that time. The problems included unacceptable toxicity and immunopathogenic responses due to the recognition of RNA by innate immune receptors, as well as difficulties with preparation and stability of the vaccine components. Furthermore, delivery of the naked RNA to antigen presenting cells (APCs) constituted a major bottleneck in large-scale rollout programs [4,8]. Early studies with mRNA in test animals showed them to be powerful stimulants of innate immunity particularly the interferon response and this also accounted for the unacceptable toxicity. Indeed, part of the success of the current mRNA vaccines against COVID-19 includes an initial interferon response that helps counteract any existing or yet to be acquired viral infection, a common scenario during the rampant spread of the SARS-CoV2 infection [9–11]. Soon after the isolation of the virus that caused the Coronavirus pandemic, the virus was sequenced and the effort to develop effective vaccines began. In the US, the so-called ‘Warp speed’ mission was headed up by Moncef Slaoui, an experienced vaccinologist and several vaccine strategies were selected for evaluation. These included two mRNA candidate vaccines both of which were provisionally licensed and recommended for emergency use in less than a year – an unprecedented speed for the approval of vaccines [11]. Both mRNA vaccines were over 90% effective at protecting against clinical disease and also succeeded in reducing the transmission rate. The latter effect is less clear in the context of the Omicron variants, which have R0 transmission rate values of 13, ranking them among the most transmissible viruses known to science [12]. The adenovirus vectored COVID vaccine was also effective and approved for emergency use, but its efficacy was substantially lower than that observed for the mRNA vaccines. They became the vaccines of choice for use in the US and in some other countries. One caveat to the generally favourable overview of the COVID mRNA vaccines is that protective efficacy wanes rather quickly, and adults in the US are now advised to receive two booster doses beyond the two doses given initially. Moreover, persons who have been vaccinated and boosted twice remain susceptible to re-infections that are transmissible and that occasionally cause typically mild disease.

There have been several technological breakthroughs that contributed to the success of mRNA vaccines. The first was the removal of toxic double stranded RNA contaminants, which improved the safety of the vaccines. Another was the substitution of the uracil nucleotide with a methylated derivative such as 1-methyl-pseudouridine, a modification that both enhanced the translation of the spike protein and dampened the associated hyper-inflammatory response [4,13,14]. Choosing RNA sequences of the viral CoV2 spike protein that encoded two prolines at critical sites resulting in a product in the prefusion form that favoured the generation of neutralizing antibodies [15]. Other refinements included the use of nanoparticles composed of lipids that optimised the stability and delivery of the immunogenic sequence into endosomes to favour optimal antigen expression and immune induction [4,13,14]. A major reason for the success of vaccines against COVID-19 could be due to the predominant entry process involving the binding of the virus surface S protein with the cellular ACE2 receptor [16]. This interaction initiates the generation of fusion of the virion to the target cell (see Fig. 1). The mRNA vaccines incorporated the sequences of S protein from an initial isolate and many variants have arisen since then. The vaccines remain effective, but effectiveness against the latest variants are less protective. A reasonable case has been made to reformulate the vaccine to target the strains that are circulating. Recombination events in the coronavirus genomes are also known to be an important contributor to coronavirus evolution [17]. In addition, the coronavirus spike protein gene includes a hypermutation region possibly leading to additional means of attachment and entry. In fact, the S protein is known to also interact with several other host surface proteins, but the physiological relevance of such interactions on pathogenicity remains to be established [17]. Some of those putative entry receptors are well represented on immune cells, possibly enabling coronaviruses to extend its infectivity to components of the immune system with possible clinical consequences [17–20]. The success of the current mRNA vaccines is largely attributed to their induction of neutralizing antibodies, a helper T cell-dependent process [21]. All of this suggests that, despite the initial success of mRNA vaccines, there may be clouds on the horizon that will make frequent vaccine reformulation necessary. Thus far no other mRNA vaccines have been licensed and recommended for use in humans. This is perhaps not surprising since the urgency of developing the COVID vaccines would have interrupted the pursuit of other vaccines. In addition, since other vaccines are not being produced to address a public health emergency, vaccine development will be expected to comply with the usual protocols typically requiring at least 5 years. Given the success of the COVID vaccines, additional successes seem likely however long it takes.

2. Could mRNA vaccines be the logical first responder approach against emerging and re-emerging pathogens?

In the past, the types of vaccines launched to control newly emerging pathogens were usually inactivated virus preparations, attenuated live vaccines or vectors that expressed one or more viral components. Development of these vaccines was very time consuming and expensive. Henceforth, mRNA vaccines may be the most preferred approach to developing vaccines against emerging and re-emerging diseases, at least for those pathogens that pose a serious public health threat. The example of the COVID-19 mRNA vaccines have certainly demonstrated the feasibility of this approach. Dozens of mRNA vaccines are currently in various stages of evaluation for many different pathogens [8]. Some pathogens for which other vaccines have repeatedly failed have sophisticated mechanisms for evading immunity, and carefully designed mRNA vaccines might be able to overcome these obstacles. Studies thus far have failed to identify any immune evasion tactics for the virus other than impairing the type 1 interferon response [22–25]. In addition, SARS-CoV2 does not usually establish a persistent infection, although recent studies have revealed the presence of fragmented viral components in some organs, e.g., in the gastrointestinal tract [22,26–30]. While not infectious, there is speculation that the viral fragments might be involved in the still cryptic phenomenon of long COVID [26–28]. The prospect of
achieving the success obtained with mRNA vaccines against COVID-19 with other pathogens would be reduced if pathogens have multiple means of entering cells, although this could be addressed by producing multivalent mRNA vaccines containing message for two or more viral proteins.

Apart from COVID-19 and some other coronaviruses, there have been several emergent viruses that have become significant pathogens of humans. These include some new strains of influenza A virus (IAV) and several infections that have emerged or become more relevant as pathogens in tropical and sub-tropical areas. These agents include Nipah virus (NV) in South East Asia, Dengue virus (DENV) in many regions, Chikungunya virus (CHIKV), Zika virus (ZV) in Brazil, Japanese encephalitis virus (JEE), Crimean-Congo haemorrhagic fever. Ebola virus had spread to humans from primates in tropical Africa on several occasions as have a number of less well characterised viruses such as severe fever with thrombocytopenia virus and Chandipura virus emerging in parts of India [31–35]. There are also many animal viruses for which effective vaccines are needed. None may be more relevant to the world food supply than African swine fever virus which recently devastated the swine population of China where pork is a major component of the diet [36,37]. Vaccines using mRNA could provide solutions for some of these viruses; that said, vaccine development is a costly investment, and therefore will generally be pursued if the prospective market is large. Table 1 lists some of the viruses of human and animals against which mRNA vaccines are either being pursued or could be developed.

3. Could the mRNA vaccine be a superior vaccine to replace existing vaccine platforms used in humans?

A number of human viral infections are effectively controlled by existing childhood vaccines including measles, mumps and rubella. These are all effective live attenuated vaccines and provide durable immunity. It is unlikely that an mRNA platform will ever replace them, although in recent decades there have been numerous mumps outbreaks among previously vaccinated young adults [38]. Children also receive highly effective subunit vaccines for hepatitis B (HBV) and rotavirus (RV). It also seems doubtful if an mRNA vaccine would have any advantage over the RV subunit vaccines, although an mRNA vaccine against RV might circumvent safety issues such as those documented in ‘Rotashield’ live attenuated vaccine, which was discontinued due to its rather low risk of intussusception in children [39,40]. The HBV vaccine is a subunit vaccine with the recombinant surface antigen (HBsAg) as the immunogen. The mRNA format could replace this, but it is highly unlikely to do so since the current vaccine is given to newborns and there is reluctance to use the anti-COVID-19 vaccine even in two-year-old children [41,42]. The trivalent oral polio virus (PV) vaccine has been replaced by inactivated PV vaccine in most countries, including the US. This was done because the type 2 PV strain is capable of reverting to wild type virus that may be shed by the immunized person and transmitted to susceptible persons. Since PV is now on the endangered species list because of its endemicity in two countries and current vaccines are very effective,
| Virus                        | Potential target antigen(s)                      | Prospects as mRNA vaccine                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Comment/s                                                                                                                                                                                                                      |
|-----------------------------|------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Human viruses               |                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                              |
| Influenza A Virus (IAV)     | Hemagglutinin, Neuraminidase, Nucleoprotein, M2 protein | mRNA vaccines for influenza A have been designed to include sequences that, at least in animals, induce a broader response that is cross-protective against heterologous influenza strains. If this strategy can be brought to fruition for humans it may obviate the need for annual reformulation of the vaccine                                                                                                                                          | • The current strategy of reformulating the influenza vaccine annually based on epidemiological evidence for the dominant strains circulating in other parts of the world is a bit hit or miss, and the vaccines frequently display a broad range of effectiveness. Development of a universal vaccine has been a long sought-after goal. |
| Dengue Virus (DenV)         | prM and Envelope (E) protein                   | Dengvaxia, a viral vectored vaccine provides considerable protection but causes in some individuals ADE. A multivalent mRNA vaccine would be useful.                                                                                                                                                                                                                                                                                                                                                                                                   | • The development of an effective vaccine for DENV has been complicated by the unusual immunology-related pathogenesis of the viruses referred to as ADE. Quadrivalent vaccines have been produced and are in use in some endemic areas, but an mRNA vaccine comprising all four serotypes could conceivably be more effective, eliciting a more comprehensive T and B cell response and possibly a response against viral components that do not mediate ADE. |
| Chikungunya virus           | Envelope proteins (E1 and E2)                 | No vaccine is available                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                              |
| ZIKA virus                  | Envelope and NS1 protein                      | No vaccine is available                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                              |
| Japanese encephalitis virus (JEEV) | Envelope (E) protein                         | An inactivated vaccine is available                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                              |
| Rotavirus                   | Outer capsid glycoproteins                    | A live attenuated vaccine is available that could rarely cause intussusception. A potent recombinant subunit vaccine is available.                                                                                                                                                                                                                                                                                                                                                                                                                                   | • A potent mRNA vaccine could have less side effects such as intussusception. • mRNA vaccine could be used either for priming or boosting those who received subunit as the primary vaccine • Developing an mRNA vaccine may not be a lucrative option for industries given the success of available vaccines in eradicating the virus from most countries. |
| Hepatitis B virus (HBV)     | HBsAg                                         | A potent recombinant subunit vaccine is available.                                                                                                                                                                                                                                                                                                                                                                                                                                       | • Effective antivirals are available but an mRNA vaccine would be desirable that could also serve as a therapeutic vaccine. |
| Poliovirus (PV)             | Capsid proteins (VP1-4)                       | Trivalent polio vaccine and inactivated vaccines are available. Reversion of the virus in some vaccinated individuals reported. Trivalent vaccine also caused immune interference therefore supporting the efforts in developing mRNA vaccine.                                                                                                                                                                                                                                                                   | • An effective antiviral is available but is cost ineffective. • A vaccine could offer prophylaxis against subsequent reinfection. • As with all human herpesviruses, permanent latent infection is established on first encounter. HSV frequently reactivates, which might be expected to bolster protection in fact fail to prevent either reactivation or reinfection. |
| Human immunodeficiency virus (HIV) | Glycoprotein 140, Envelope protein          | No vaccine is available despite extensive efforts made                                                                                                                                                                                                                                                                                                                                                                                                                                   | • As with all human herpesviruses, permanent latent infection is established on first encounter. HSV frequently reactivates, which might be expected to bolster protection in fact fail to prevent either reactivation or reinfection. |
| Hepatitis C virus (HCV)     | Non-structural proteins (NS4A and B), Core protein | Multivalent vaccine targeting key viral proteins could provide protective immunity                                                                                                                                                                                                                                                                                                                                                                                                                                                     | • An effective antiviral is available but is cost ineffective. • A vaccine could offer prophylaxis against subsequent reinfection. |
| Herpes simplex virus (HSV)  | Glycoprotein B, Glycoprotein D                | Development of an HSV mRNA vaccine that incorporates carefully selected proteins could result in a vaccine that bypasses the multiple immune evasion mechanisms and elicits effective T and B cell responses                                                                                                                                                                                                                                                                                                                                 | • No vaccine developed for HSV prevention has been successful. |
| Varicella zoster virus (VZV) | Glycoprotein E                                | An mRNA vaccine for the prevention of shingles is likely to be feasible. An extremely effective vaccine is currently licensed using recombinantly produce glycoprotein E, but it is significantly reactogenic likely due to the adjuvant system.                                                                                                                                                                                                                                                                                                                        | • An effective vaccine could reduce reactivation and thereby reduces transmission. • There is at best limited incentive to produce a new vaccine to replace the currently effective chickenpox vaccine; the recombinant adjuvanted shingles vaccine — while extraordinarily effective at preventing shingles — it is also highly reactogenic. |
| Virus Type | Target Protein | Additional Notes |
|------------|----------------|------------------|
| Human cytomegalovirus (HCMV) | Glycoprotein B | There is general consensus that any strategy for vaccine development needs to include at least several HCMV target proteins. A multivalent mRNA vaccine could prove effective, particularly if it engages both B and T cell responses and is able to bypass the many immune evasion mechanisms employed by viable HCMV. |
| Epstein Bar virus (EBV) | Glycoprotein B | As with VZV, it is conceivable that an mRNA vaccine for EBV could be developed in a monovalent format targeting glycoprotein B, which includes both B and T cell epitopes. |
| Respiratory syncytial virus (RSV) | F protein, G protein | Long history of ineffective candidate vaccines; first vaccine actually caused pathology. A multivalent mRNA vaccine targeting both B and T cell epitopes could be efficacious. |
| Animal viruses | | |
| Marek’s disease virus (MDV) | Glycoprotein B, Glycoprotein D | MDV is a highly contagious tumorigenic herpesvirus. Injectable vaccines are available. They prevent disease but do not prevent infection or transmission. There is considerable incentive to develop vaccines that can address at least some of these limitations. |
| Infectious bursal disease (IBD) | Non-structural protein (VP2) | An attenuated vaccine is used via mucosal routes (eye or in drinking water). A viral vectored vaccine wherein VP2 was expressed using a herpesvirus backbone was generated. mRNA approach could nonetheless add value to immunization protocols. |
| Chicken infectious anemia (CIA) | Capsid protein (VP1) | A live vaccine is available but an mRNA-based vaccine would be useful. |
| Equine Herpesvirus Type 1 and Type 4 (EHV-1 and EHV-4) | Glycoprotein E | A vaccine for EHV-1 and EHV-4 must be given soon after birth in the face of maternal immunity to avoid early infection. Ability to extend vaccination to an mRNA strategy will depend on the ability to provide efficient protection. |
| African swine virus (ASF) | p54 and p30 | Inactivated and live attenuated vaccine is available. Inactivated vaccines do not work efficiently. Therefore, mRNA vaccine could be developed. |
| Feline Calicivirus (FCV) | Capsid proteins | |

- While gE has proven adequate to prevent shingles for multiple years, it may be possible to expand the efficacy by additionally targeting dominant T cell epitopes.
- There may be some incentive to produce an mRNA vaccine using a less inflammatory adjuvant system with equivalent or improved effectiveness.
- A vaccine to prevent the development of congenital HCMV disease has been a priority for decades; no vaccine has thus far proven effective. At least 15 candidates are currently being evaluated, including an mRNA vaccine.
- Associated with several types of cancer such as Burkett’s lymphoma and nasopharyngial carcinoma.
- Associated with multiple sclerosis.
- Developing an effective vaccine possibly using mRNA approach is desirable.
- Large obstacle is need to administer during 1st year of life, when competing maternal antibodies may be present.
- Multiple vaccines evaluated over half a century have all failed to elicit protective immunity.
- The mRNA strategy would be the first to engage a comprehensive cell-mediated response in addition to a humoral response.
- As with most vaccines for use in agriculturally important animals, any vaccine must be inexpensive and, ideally, capable of being administered efficiently, e.g., in the water supply.
- A vaccine for MDV must be given soon after chicks emerge.
- Ability to extend vaccination to an mRNA strategy will depend on the ability to provide the vaccine through the mucosal route.
- This virus causes apoptosis of B cells.
- CIAV can transmit in otherwise healthy birds to due to contaminated vaccines. Therefore, immunization of 9 –12 weeks old susceptible birds against the CIAV is desirable to save losses from EHV-1 (as its close relative EHV-4) is a moderately contagious disease that can have dramatic outcomes, including abortion and the so-called equine herpesvirus myelonecephalopathy (EHM).
- Currently available vaccines have questionable efficacy. There is considerable incentive to develop vaccines that can address at these limitations.
- ASF causes huge economic losses and hampers food supply. (continued on next page)
| Virus | Potential target antigen/s | Prospects as mRNA vaccine | Comment/s |
|-------|---------------------------|---------------------------|-----------|
| Inactivated or recombinant vaccine are available (containing the capsid protein) but adjuvants used are implicated in a rare but serious adverse effect of feline vaccines, feline fibrosarcoma and avoidance of adjuvants is desirable. A potential mRNA vaccine could therefore be developed. | **FCV** is a highly contagious and evolving virus in cat populations and is particularly prevalent in shelters and in circumstance of high cat density. | • FCV is a highly contagious and evolving virus in cat populations and is particularly prevalent in shelters and in circumstance of high cat density. |
| Feline Panleukopenia Virus | Capsid protein (VP2) | Modified live virus or inactivated are available. Adjuvant used has been implicated in a rare but serious adverse effect of feline vaccines, feline fibrosarcoma. | • It causes an ulcerative disease that causes lesions in the oronasal cavity and more recent strains are of higher virulence. |
| Feline Immunodeficiency Virus and Feline Leukemia Virus | Viral core antigens (p24, p40) | Inactivated or vectored preparations are used as vaccine both of which are adjuvanted. The use of adjuvants has been implicated in a rare but serious adverse effect of feline vaccines, feline fibrosarcoma. As the envelope glycoprotein(s) are deemed protective, an mRNA approach targeting the major immunogen is desirable. | • The two independent virus diseases are caused by retroviruses and cause immunodeficiency (FIV) or leukosis (FeLV). • FeLV is more prevalent and current vaccines are not sufficiently protective. |
| Rabbit Hemorrhagic Disease Virus (RHDV) | Capsid protein (VP60) | RHDV virus cannot be propagated in cultured cells. Inactivated vaccines were generated by infecting experimental animals. Establishment of efficacious vaccines based on recombinant technology or an mRNA vaccine is highly desirable. | • RHD is a highly transmissible and often lethal disease of rabbits and no treatment options are available. • Comprehensive vaccination must be used to protect against the disease that is notifiable in many countries around the globe. |
no pharmaceutical firm is likely to invest in an mRNA platform-based PV vaccine.

Other viral vaccines given to children and teens include Human papilloma virus (HPV), IAV and for selected areas, Dengue virus (DENV). In the case of IAV, most mRNA vaccines against IAV being tested include sequence of the stalk of haemagglutinin [43]. Such vaccines in preclinical trials in mice induced neutralizing antibodies against both the homologous (H1N1) and heterologous (H5N1) strains and offered protection to the animals challenged with a 10 times the lethal dose (LD50) dose [44]. In the case of vaccines against IAV, there is an ongoing search for a universal vaccine and if this proves successful, the mRNA vaccine could prove to be a valuable new approach for influenza. Prospective vaccines may need to include multiple components which is readily feasible with mRNA formulations. These components could include cross-protective HA encoding mRNA sequences as well as those encoding other protective immunogens such as the neuraminidase (NA), nucleoprotein (NP) as well as the M2 protein of the virus. An influenza mRNA preparation evaluated in a mouse model induced robust neutralizing antibody responses and protected against extraordinarily high challenge doses (500 × LD50) [43]. However, the use of a mRNA vaccine against IAV in resource-poor societies would be problematic because of its high production cost and the requirement for cold storage. Furthermore, IAV vaccines do not constitute part of routine immunization protocols in several developing countries.

Another viral disease of humans that will benefit from a more effective vaccine is DENV, which has four different serotypes. It has been known for some time that infection with one serotype of the virus, followed by subsequent infection with a second serotype, can lead to dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). These conditions are most commonly observed in children under 10, and can be fatal. The adverse effect is attributed to a phenomenon referred to as antibody dependent enhancement (ADE). One explanation for ADE is that low affinity antibodies resulting from the primary infection fail to neutralize the subsequent infection by a heterologous viral serotype, but instead facilitate infection of cells such as monocytes, macrophages that in turn overproduce inflammatory cytokines and molecules such as VEGF involved in vascular leakage and the ensuing clinical problems [45,46]. The ADE depends upon Fc receptor-mediated events and it is conceivable that mRNA vaccines could be designed to induce antibodies with higher affinities against all four serotypes [46]. Thus, monovalent DENV vaccines have been problematic mainly because of the potential of inducing ADE. Induction of a non-neutralizing antibody response and possibly the less well characterised T cells that recognize the heterologous serotype of the virus with a lower affinity can result in a condition similar to DHF/DSS [47]. Dengvaxia is the only available viral vectored vaccine. The dengue virus prM and envelope protein encoding genes represent each of the four dengue serotypes have been inserted into a yellow fever vaccine genome [48–51]. Thus, the vaccine simultaneously protects against all four serotypes. The vaccine has been approved in the US for American Samoa, Virgin Islands, Puerto Rico, and other dengue endemic US territories. The vaccine was discontinued for use in DENV naïve children because approximately 15% of them encountered DHF/DSS during subsequent exposures with the virus. An mRNA vaccine strategy could induce more complete and robust protective immunity including neutralizing and high avidity antibodies as well as comprehensive T cell response. This is because such a vaccine predominantly acts by inducing an initial IFN response that could efficiently help B cells differentiate into IgG antibody forming cells.

Zika virus (ZIKV) is closely related to DENV, sharing more than 50% homology at the amino acid level [52]. As such, persons previously infected with ZIKV may have circulating antibodies that could induce ADE in persons infected with DENV. Evidence for this phenomenon is limited and inconsistent [53,54]. Vaccines are under development using an engineered ZIKV immunogen that does not elicit antibodies capable of triggering ADE. The application of this type of designer antigen would be easily adaptable to an mRNA format. The mRNA candidate for ZIKV vaccine preparation included prM-E protein since neutralizing antibodies were induced against it [55]. The vaccine induced neutralizing antibodies as much as 100-fold higher in magnitudes when compared to those generated by an inactivated virus or the DNA vaccine injected in non-human primates [55]. While mRNA vaccines might be useful for those viruses where conventional approaches have yielded equivocal results, it is also worth noting that concern about ZIKV is limited, making it a lower priority for vaccine development.

4. Could the mRNA vaccine platform provide success where other approaches have failed in humans?

As mentioned previously, there are a number of serious viral pathogens for which vaccine development has met with serial disappointment. The most relevant of these are Human Immunodeficiency Virus (HIV), Respiratory Syncytial Virus (RSV), Hepatitis C Virus (HCV) and the human herpesviruses, including, such as Herpes Simplex Virus (HSV), Human Cytomegalovirus (HCMV) and Epstein–Barr Virus (EBV). In spite of enormous cost and effort, we lack an effective prophylactic vaccine against HIV and there is no reason to imagine that the mRNA platform will achieve success either. This is because HIV replication is very rapid and highly error-prone [56]. Developing a subunit vaccine using a single protein or its encoding mRNA sequence is rather daunting as it may be represented by dozens of dominant variants in an infected person. Secondly, HIV targets and kills CD4+ T cells, the key orchestrators for multiple components of the immune system. However, this has become less of a concern with the availability of highly active antiretroviral therapy, which largely restores normal levels of CD4+ T cells. Thirdly, a DNA copy of HIV virial genome is produced in infected cells that integrates in the host cell genomes and such cellular sanctuaries are called latent reservoirs. A case for therapeutic vaccines probably in the form of mRNA can still be made for managing HIV.

A highly effective antiviral drug cocktail is also available that is curative for HIV; however, it is prohibitively expensive for those without insurance coverage in the developed world and is effectively out of reach for people in developing countries. Consequently, development of an effective vaccine that would protect against infection with all 6 genotypes of HCV would provide a relatively inexpensive alternative for HCV prevention. Several vaccine approaches and their correlates of protection have been evaluated, particularly in the era when the chimpanzee animal model was still in use to quantify immunity to HCV [57–59]. Beyond the need for an effective prophylactic vaccine to prevent infection, vaccines for HCV are also needed to boost protective immunity in persons that have gone through successful antiviral therapy since they are susceptible to reinfection. A multivalent vaccine using mRNA technology is certainly worth exploring, particularly since valuable information on in vivo correlates of protection are available in a non-human primate model [57]. Another human viral pathogen that lacks an effective vaccine is RSV, which accounts for more than 60,000 hospitalizations annually in the US alone. It can also cause serious, sometimes life-threatening disease in children and the elderly in nursing homes. Children often suffer repeated infections indicating that natural virus infection induces inadequate levels of immunity. Combined with the age at which the vaccine must be administered, this is not
an encouraging sign that vaccines will succeed. Many attempts have been made in the last 50 years to develop effective vaccines, but none so far have been successful; moreover, the disaster with formalin-inactivated vaccine mentioned earlier continues to drive considerable caution as vaccinees showed enhanced susceptibility to subsequent virus infection [60–62]. This situation was studied in animal model systems where inactivated vaccines against RSV were shown to set the stage for exaggerated lung pathology following challenge with live virus. The disease pattern was attributed to a Th2 immunopathological reaction, while protection was correlated with Th1 responses in such model systems [63,64]. Recent studies from the Graham laboratory demonstrated that, if the fusion (F) protein of RSV is manipulated to adopt the prefusion configuration, then the protein was more effective at inducing protective neutralizing antibodies than the native fusion-active configuration [65–67]. These findings guided the selection of sequences to include in one of the highly effective mRNA vaccines used against COVID-19 [8]. It should be feasible to select immunogenic sequences of the prefusion F protein and incorporate them into a mRNA vaccine that induces protective neutralizing responses. If necessary, additional mRNAs encoding RSV epitopes that induce protective CD4+ and CD8+ T cell responses would be expected to expand protective immunity against RSV.

For decades, many investigators have tried to develop effective vaccines to protect against or diminish the consequences of being infected with human herpesviruses. The story as it relates to the alphaherpesvirus HSV has been particularly perplexing since we have an effective vaccine against another alphaherpesvirus, VZV [68]. With HSV, just about any vaccine format tested in rodent models has been remarkably effective. However, all candidates that have succeeded in models and subsequently tested in a double-blind fashion in humans have failed. No HSV vaccine has been licensed. This issue was recently discussed in detail in a review with several reasons advocated to explain the antithetical outcome stories for HSV and VZV [69]. Possibly one or a number of mRNAs encoding different HSV components could achieve success where other approaches have failed. This needs to be evaluated and most likely is underway since the HSV vaccine field is replete with optimists. While we have major reservations about the likely success of mRNA vaccines used prophylactically against HSV, we have enthusiasm for the platform’s potential to change the consequences of recurrent HSV lesions in latently infected persons. This issue is further explained in a later section.

As mentioned, we do have a successful prophylactic live-attenuated vaccine against chickenpox caused by VZV. There could be a case made to replace the attenuated vaccine with the mRNA platform since the attenuated vaccine can occasionally be the cause of shingles even in children, which could not happen with an mRNA vaccine. With VZV we also use an effective vaccine (Shingrix) which prevents more than 90% of herpes zoster for 5 years or longer [69–71]. Shingles occurs due to reactivation of VZV from a latent infection in peripheral nerve ganglia, or very occasionally reactivation of the live virus vaccine given earlier in life. We now consider that the immune function that stops the reactivation is an anti-gE antibody mediated event in addition to T cells [72]. A shortcoming of the Shingrix recombinant gE vaccine is that it is very reactogenic, largely due to the potent adjuvant system. It should be possible to develop mRNA vaccines, possibly with a less inflammatory adjuvant, that effectively reduce the risk of shingles and has fewer transient side effects. However, such a vaccine is more likely to induce protective response in individuals previously exposed to the virus. Curiously, the vehicle currently used in the effective anti-COVID-19 vaccine is similar to that used in the current Shingrix vaccine.

For a long time, infectious disease experts, particularly vaccine pioneer Stanley Plotkin, have argued the need for a vaccine against HCMV. Starting with the Plotkin Towne vaccine in the early 1970s, many candidates have been advocated and tested one of which includes an mRNA vaccine currently being pursued by Moderna, who gave us one of the valuable COVID-19 mRNA vaccines. Most of the healthy population would not need a vaccine, but two groups would greatly benefit. One group includes pregnant women especially those who are HCMV seronegative since congenital or neonatal infection can cause cognitive impairment and hearing loss. The second group is the immunocompromised individuals either because of receiving immunosuppressive drugs to facilitate transplants or those with acquired infection induced diseases such as HIV-AIDS. Many vaccine platforms have been tested and attempts made to find correlates of immunity and the HCMV components that should be targeted [73–81]. At least 15 candidate HCMV vaccines are under evaluation at the present time. These include replication defective virus, gB subunit, multivalent subunit, pentameric complex, triplex, peptide, plasmid DNA, gB virus-like particle, dense body and mRNA vaccines [81,82]. While no CMV vaccine has so far proven to be effective, there is consensus that multiple HCMV antigens need to be incorporated. The Moderna mRNA candidate vaccine includes messages for at least three HCMV proteins and therefore could turn out to be the preferred vaccine if approved.

The other common herpesvirus that lacks a vaccine, but not vaccine enthusiasts, is EBV, a cause of infectious mononucleosis and as well as some cancers and perhaps some autoimmune diseases. Recently, for example, a strong case was made for EBV being a possible cause of multiple sclerosis [83–86]. In most cultures, the prevalence of EBV infection is very high and the majority become infected as children with usually no consequences. Both antibodies and CD8+ T cells confer protection to the host following EBV infection. Similar results were obtained in the mouse model wherein gB specific CD8+ T cells controlled the infection caused by MHV68 [130]. In humans, cross-reactive epitopes occurring in the EBV protein, BMLF1, expanded T cell clones especially in individuals previously infected with IAV [87,88]. Such an enormous expansion of T cells resulted in immunopathological manifestation of infectious mononucleosis. If a vaccine is to be used perhaps in the mRNA format the best candidates could include sequence encoding the viral proteins such as glycoprotein B that harbours both T and B cells epitopes. Similar to the viral S protein of SARS-CoV2, several EBV proteins including gB harbours polybasic furin cleavage sites [89–91]. It was recently shown that antibodies targeting epitopes encompassing the polybasic residues in furin cleavage site could neutralise not only SARS-CoV2 but several other viruses with such sites [92]. Therefore, eliciting antibodies in addition to T cell response by mRNA vaccine could represent a promising option in managing EBV.

5. Could mRNA vaccines replace existing vaccine platforms used in domestic and/or exotic animals?

Many different types of animals require vaccines. These include food animals such as chickens and turkeys as well as swine and ruminants. Those species are reared mainly for production of human food (eggs, meat and milk) but also wool and hides for the leather and textile industries. Other groups include companion or recreational animals, mainly dogs and cats, but also horses and an increasing number of exotic pet species most of which are held in zoos, but increasingly also in private households. All of these animals need to receive multiple vaccines particularly when they are housed in large, concentrated groups as is the norm with modern...
agriculture in many countries. Moreover, since modern ways of production involve rapid turnover and narrow profit margins, the vaccines that are used need to be inexpensive and easy to store and administer. With poultry for example, vaccines may be administered in the drinking water or as aerosols in confined housing. Accordingly, it is doubtful if mRNA preparations can be engineered to be more effective and less costly than currently used vaccine platforms, but this issue merits exploration. However, should it prove possible to achieve good immunogenicity with mRNA vaccines given to mucosal surfaces as one unconfirmed report seems to indicate [21], the case for using such vaccines would be greatly encouraged. Some poultry vaccines, particularly those used in production of meat chickens and turkeys, are administered using specialized equipment into very large numbers of 18-day embryonating eggs. These vaccines include those preventing Marek's disease, an oncogenic disease caused by an avian herpesvirus, infectious bursal disease (IBD) caused by a Birnavirus, chicken infectious anemia (CIA) caused by a Circovirus and Newcastle disease (ND) that is an infection with a Paramyxovirus and also referred to as atypical fowl plague. It is conceivable that the mRNA platform could replace one or more of the vaccines currently used. One prime candidate would be a vaccine against CIAV infection as this virus is hard to grow and encodes only one structural (nucleocapsid) protein against which immunity has to be mounted [93,94].

In swine and ruminants, many effective anti-viral vaccines are in routine use and one vaccine used to counteract swine influenza virus (SIV) uses the mRNA platform, being in fact the first mRNA vaccine licensed for use in humans or animals [95]. The vaccine is far more complex than the effective COVID-19 vaccines and uses a Venezuelan Equine Encephalitis Virus (VEEV) replicon that is packaged with the SIV hemagglutinin into a VEEV-like particle. It is not yet evident if this mRNA platform is superior or has any other advantages over alternative vaccines, but the vaccine has been on the market since 2019.

It is conceivable that the mRNA/replicon vaccine format could work for other farm animal viral pathogens including pathogens that are difficult or impossible to propagate in vitro or for which high levels of biosafety are mandatory for production of conventional vaccines. Examples worth evaluating are vaccines against the calcivirus Rabbit Hemorrhagic Disease Virus (RHDV), Foot-and-Mouth-Disease Virus (FMDV) in ruminants and pigs as well as Classical Swine Fever Virus (CSFV) in swine.

Companion animals, particularly dogs and cats, have multiple viral infections against which we routinely vaccinate. High cost and storage problems are less important factors, and there could be a place for replacing some of the existing vaccines with mRNA-based products. A potential candidate for such control could be canine distemper virus (CDV), a cousin of measles virus. With CDV, although it is often not necessary, it is common to revaccinate, but the vaccination boost may be inhibited by residual immunity [96]. Such immunity would not be expected to interfere with a nucleic acid-based vaccine, so using a mRNA platform is a logical approach worthy of evaluation. Another potential candidate viral disease for an mRNA vaccine as a primary or more likely a booster vaccine is canine parvovirus type 2 (CPV-2) a now world-wide devastating diarrhea-causing disease that appeared about 50 years ago and is just now wreaking havoc again in the US (Michigan). Similar to the case of CDV, CPV-2 vaccination is performed very early in life and booster vaccinations in short intervals are necessary especially in kennels holding larger number of dogs and puppies.

Cats also receive several vaccines against viral diseases, some of which are not considered highly effective. These include vaccines against Feline Leukemia Virus infection and the cat enteric coronavirus (FeCoV) that occasionally mutates and then causes a lethal peritonitis (FIP) following a mutation in the spike glycoprotein [97]. Past attempts at vaccinating against FIP have been unsuccessful, with some vaccine formulations making animals even more likely to develop FIP [98,99]. Given the success of mRNA vaccines against the human coronavirus SARS-CoV-2, a strong case can be made to explore the mRNA approach against FIP.

Horses are another species that are vaccinated against several viral pathogens, some with notable success and in need of improvement. Thus, in many countries, horses are vaccinated biannually against Equine Influenza Virus (EIV), Equine Herpesvirus Type 1 and Type 4 (EHV-1 and EHV-4). Horses in the Americas also receive vaccines against three alphaviruses that can cause encephalomyelitides (Venezuelan, Western and Eastern Equine Encephalitis – VEE, WEE, EEE) as well as a vaccine against West Nile Virus (WNV). The vaccines against the three alphaviruses are quite satisfactory, but the vaccine against particularly herpesviruses far less so. It is well possible that mRNA formulations would not fare any better. Curiously, the first (and so far the only) registered nucleic acid-based vaccine was developed to protect against WNV [100,101]. This vaccine encoded the prM and E genes of WNV and was resisted by the horse community since standard vaccines were working well and were less expensive than the DNA vaccine. It could be noted a mRNA vaccine would also perform well, but unaffordable and inexpensive might not be welcome especially as the disease is relatively uncommon and there is no horse-to-horse transmission.

In conclusion, there appears to be few if any compelling reasons to develop mRNA vaccines against viral diseases that affect food and companion animals. mRNA vaccines would be an alternative should they clear the hurdle of relatively easy mass administration, i.e., if they were applicable via (drinking) water, aerosol or, preferably, in ovo inoculation in the case of poultry. While swine vaccines are still applied by “traditional” intramuscular or subcutaneous injection, the trend of poultry vaccines will likely be followed. Proof-of-concept that mRNA vaccines are applicable locally was recently provided [102], and there are a number of antigens that would become immediate candidates for mRNA-based vaccines. Although there are fewer considerations with respect to non-traditional applications in bovines, equines, dogs or cats, budgetary considerations remain in all these cases and vaccines must certainly be more cost-effective than in humans.

Hence, a case for replacing any of the currently used vaccines with those based on an mRNA platform may appear unlikely because of cost and administration difficulties. In addition, especially mass application in non-clinical settings would require a more temperature-resistant vaccine formulation, which may be overcome if alternative delivery systems and/or adjuvants were explored. As mentioned earlier, it is remarkable but maybe unsurprising that the first mRNA vaccine was licensed for swine. It is an injectable VEEV replicon expressing SIV hemagglutinin (HA) and delivered after packaging into a VEEV virus-like particle [103]. The vaccine is efficacious and in use for almost five years now. While VLP delivery may not work or be advisable in all cases, recent developments in nanoparticle delivery of nucleic acids by various routes, including mucosal application, will almost certainly result in the development and registration of more veterinary vaccines based on mRNA/replicon technology.

There are other areas where mRNA vaccines in the veterinary field may be superior to current vaccines. Next to virus infections where efficacious vaccines may be lacking, there are some general considerations and conditions that must be met or would be conducive to developing mRNA/replicon vaccines. The differentiation of vaccinated from infected animals (DIVA principle) is of paramount importance in the control of many of the most devastating virus infections in livestock, including Foot-and-Mouth
Disease (FMD) in cattle, small ruminants and swine; classical swine fever in pigs, avian influenza in commercial and exotic fowl, alphavirus (VEEV, WEEV, EEEV) and flavivirus (WNV, Japanese encephalitis virus) infections in equines and swine to only name a few. While the DIVA principle can be followed with all types of vaccines, it is arguably most readily achievable with subunit and nucleic acid-based vaccines. In a similar vein, many of the aforementioned and other virus infections are controlled by standard inactivated vaccines. Given the high biosafety and biosecurity considerations for generation of the virus base for subsequent inactivation, which generally must occur under BSL-3 or even BSL-4 (e.g., FMDV) conditions, vaccine production would undoubtedly be made more cost-effective and safer with the production of mRNA/repliconcatem vaccines. The lower production (facility) cost may even offset the higher unit price for vaccines, at least in some instances such as the equine alphaviruses. Also, there are still several veterinary viruses that are hard or impossible to grow at all or at efficiencies required for vaccine production. Those include the calicivirus Rabbit Hemorrhagic Disease Virus (RHDV) or CIAV. Given the immunogenicity of the RHDV and CIAV capsid protein that seem to suffice for the induction of protective immune responses, mRNA/repliconcatem vaccines may be a viable alternative.

6. Could mRNA vaccines provide a way to protect against animal viral infections that currently lack effective vaccines?

Like human virus vaccines, some veterinary virus vaccines are not efficacious in their current formulations, in fact some were shown to be disease enhancing [99]. Notable examples include RSV infections in cattle [104,105] and, interestingly, FIP that we already mentioned [99]. Both these viral diseases may be controllable by the use of mRNA vaccines expressing the envelope proteins F (fusion) and/or G (glycoprotein) in the case of RSV or the spike protein of FeCoV. Perhaps equally important is the issue of adjuvants, particularly in animals reared for human consumption. The avoidance of conventional adjuvants maybe a driver for the use of mRNA/repliconcatem vaccines in livestock but the problematic nature of adjuvants is also a big issue in cats. As many of the important feline virus pathogens (FelV, FIV, FPV, Feline Calicivirus and others) are in the category of one/few antigens needed to create an efficacious vaccine, nanoparticle delivery systems may offer the added advantage of fewer adverse effects. Cats sometimes develop vaccine (adjuvant)-related fibrosarcomas that are the lead cause for amputation of the extremities [106]. As such, the use of the modern technology may be an active contribution to animal welfare.

Could the mRNA platform come to the rescue for protection against other animal virus diseases? Although vaccines are in frequent use against some animal herpesvirus infections, their efficacy is often questionable. One good example is EHV-1 (see above), a varicellovirus that is closely related to VZV, the human herpesvirus where we do have effective vaccines that protect against primary disease (chickenpox) and disease following reactivation (shingles or herpes zoster). As discussed in a previous section, there could be a case for exploring the use of the mRNA platform to prevent shingles since it would not be blocked by preexisting immunity. Such a vaccine should be explored to boost immunity against EHV-1 so as to minimize the sequelae of outbreaks of the respiratory, neurological or abortigenic diseases that often occur in affected horses. Based on the encouraging experience with the Shingrix subunit vaccine and given similar epidemiology (nearly all equines are infected with EHV-1 and its close relative EHV-4 within the first months of life) [107,108], boosting of existing immunity by virus infection with an mRNA vaccine seems to be a tempting approach that should be explored.

7. Can mRNA vaccines be engineered and used successfully as therapeutic vaccines?

Viruses that defy immune removal and persist in the body are apt to interact with one or more components of immunity resulting in some form of tissue damage. The classical example studied by the viral immunological elite has been Lymphocytic choriomeningitis virus (LCMV) infection of mice. No virus infection in humans or domesticated animals has a pathogenesis quite like LCMV, but that model has revealed much of our basic understanding of virus/host interaction, pathogenesis and fundamental immunology. It revealed, for example that when a virus persists some aspects of the immune response act to mediate an inflammatory reaction that results in tissue damage [109–111]. We have also learned that whereas some aspects of immunity are proinflammatory others serve to regulate such responses and diminish the extent of tissue damage [109,110]. Since the different components of immune activity have different induction, activation and survival requirements, it should be feasible to rebalance the nature of the immune response from one that contributes to tissue damage to a pattern that does not. One situation that illustrates this scenario is recurrent lesions caused by HSV which can occur at dermal sites, the eye and sometimes, with often dire consequences, in the CNS [68,112]. Following primary infection, HSV invariably persists lifelong in the peripheral nervous system in a non-replicating state termed latency [68]. However, latency periodically breaks down in some neurons with the virus either causing an inflammatory lesion at the peripheral site or being silently shed from that site. The lesions that occur are considered to be at least in part immunopathologically orchestrated by proinflammatory T cells [113,114]. Also, it is known from experimental studies in model systems that the extent of lesions can be limited or even prevented by cellular and molecular mediators such as regulatory T cells, myeloid derived suppressor cells, certain subtypes of macrophages or by the activity of cytokines such anti-IL-10, TGF-β as well as by several other host molecules such as resolvins, protectins and galecinins [112,114]. Accordingly, a case can be made to use strategies that will succeed in diminishing the proinflammatory aspects of immunity and expanding the anti-inflammatory components. Elsewhere, we have discussed various ways of achieving this objective [112,115]. One such way could be to use vaccines that rebalance the participation of different aspects of immunity. This objective might be achievable with the mRNA platform since this could be designed to include multiple objectives. For example, in some instances the epitopes recognized by some T cell subtypes, such as proinflammatory Th1 cells, could differ from subsets such as Treg that play an anti-inflammatory role [116]. The mRNA platform could also be designed to express molecules required for recruitment and activation of T cell subsets which themselves could differ for the various components of the inflammatory response. The therapeutic mRNA cocktail might also include molecules that have anti-inflammatory activity such as some cytokine, although this would be only a temporary manoeuvre. In the case of HSV induced lesions in humans, the primary immune defense system that controls the infection is considered to be tissue resident memory T cells, that are principally antigen specific CD8+ T cells [117,118]. This population could be recruited, expanded and activated with the larger more potent population being able to more rapidly abort the infection minimizing the chance of any overt lesions. Thus, the mRNA vaccine cocktail could contain multiple components to achieve these events. One harbinger is that it may be necessary to administer the vaccine at local sites, but this issue needs to be fully explored. Alas there is a dearth of appropriate animal models to evaluate these therapeutic vaccine ideas.
Some of other viruses responsible for high infection rates in the population or those against which drugs are available, could constitute the ideal candidates against which therapeutic mRNA vaccines would be valuable to develop. Examples include HIV and HCV. Pre-existing immunity would have less likely impact to limit the efficacy of such mRNA vaccines. Furthermore, if approved for use such vaccines could either help significantly reduce or completely eliminate the use of drugs and the associated cost for managing such chronic infections.

8. Are mRNA vaccines the most logical ones to use to boost immunity?

Although even in long-lived species like humans immunity to some virus infections appears to be lifelong, this is not true for all infections and in such cases there is a need to boost immunity by revaccinating. This procedure is used most frequently for IAV, although in this case the annually suggested revaccination is as much needed to combat new variants as it is to reverse declined levels of immunity. A potential issue with revaccinations is that its efficacy may be impaired by interference by residual usually antibody-mediated immunity that can partially or even completely block the response to the vaccine boost. This interference is more an issue with some types of vaccine than others with live attenuated vaccines being the most susceptible. In veterinary medicine, for example there is often a need to revaccinate against CDV, a cousin virus of measles, but pre-existing immunity can interfere with the response [96]. This pre-existing immune inhibition should not be an issue with genetic vaccines particularly the mRNA vaccines since they will not be inhibited by pre-existing antibodies and only express proteins after the genetic material has entered cells and been expressed for processing to stimulate T cells. The extra-cellular release of such protein could still interfere with the pre-existing antibodies before memory B cells could be efficiently recalled. The experience with COVID–19 mRNA vaccines has shown that levels of immunity are increased after revaccination although parallel studies comparing response to other types of vaccines made have not been done. We feel the mRNA vaccines could be the most logical to use to boost immunity against IAV, although its cost and need for cold storage could prove problematic for many societies.

There is one common virus infection of humans for which it is now common to employ a vaccine boost. This is the vaccine against VZV designed to prevent the occurrence of shingles. This syndrome occurs when VZV latency, established in peripheral nerve ganglia as a consequence of usually childhood infection with chickenpox (VZV), terminates from one or more ganglia giving rise to painful inflammatory lesions at surface sites. Unlike recurrent lesions that result from HSV, vaccines have been remarkably effective at preventing shingles, particularly a subunit vaccine that includes lipids similar to those used in one of the anti-COVID mRNA vaccines. Because the efficacy of the current subunit vaccine is subject to inhibition by pre-existing immunity, a case could be made to replace the subunit vaccine with the mRNA formulation, but this change maybe unlikely to occur anytime soon since the current formulation is achieving remarkable success.

9. Can mRNA vaccine be engineered and used successfully as mucosal vaccines?

One of the long cherished goals of vaccinologists is to develop needle-free effective vaccines which can induce protection at the portal of pathogen entry as well as help reduce the extent of vaccine hesitancy. The induced response at the mucosal sites could serve as a local facility to efficiently control the virus locally. Induced humoral response comprising predominantly of IgA isotype and the tissue homing antigen-specific T cells contribute to the local control. Efforts have been made to generate mucosal vaccines but when tested in large scale trials met with limited success. There has been some concern that vaccines administered systemically may not provide effective immunity at mucosal sites, a problem that could be more common with inactivated vaccine formulations. This issue was a topic of active debate in the early years of the polio vaccine development. Thus the initial Salk vaccine was remarkably protective against the virus causing paralytic polio but it did not fully protect against local replication of poliovirus in the gastrointestinal tract [119–122]. The subsequently developed live attenuated vaccine given orally was far more protective against local replication and also prevented lesions in the CNS. This could be due to the viral protein interacting with the cellular receptors expressed by the modified epithelial cells in gut, known as M cells. Underneath M cells at the submucosal sites, the cellular and molecular mediators are abundantly present that can help elicit potent response [123,124]. Live-attenuated vaccines against IAV given via the mucosal route are advocated to provide more protection than do inactivated preparations given systemically [125–127]. The currently administered mRNA vaccines given systemically already achieved remarkable success, but it is becoming evident that recovery from natural infection may be providing higher levels of protection particularly at the portal of viral entry. Conceivably, this could be explained by the natural infection providing protection at both the mucosal entry sites as was as systemically. Currently we do not know if mRNA vaccines when given mucosally will provide better protection at mucosal sites than if given systemically as is currently practised. This issue should be further investigated with the likelihood that modifications to the currently used formulations may need to be developed. Recently, it was shown that the prime boost approach induced an efficient mucosal immunity against SARS-CoV2 [128,129]. Interestingly, in this study the priming was done with mRNA vaccine using a systemic injection while the boost was achieved using the adenovirus based vaccine expressing the viral S protein given intranasally. Therefore, a strong case is made to boost immunity locally at mucosal sites to achieve the pathogen control at entry for a systemic infection sets in.

10. Conclusions and speculations

Nucleic acid vaccines were greeted initially with skepticism. They were conceived in the late 1980’s and proof-of-principle applications demonstrated in the 1990s, but not consummated for use in human vaccinology until 2020. Originally, the DNA format was the favourite child and there is one example of a licensed DNA vaccine in animals against West Nile Virus in horses. As we have all witnessed, however, the triumphant sibling turned out to be RNA, with mRNA vaccines staving to help control the world’s latest pandemic. In this review, we have aspired to make the case for a potentially more widespread application of this technology to vaccinology. The application of mRNA could also be extended beyond human diseases to pathogens that afflict mankind’s food and companion animals. Many veterinary vaccines in current use are marginally effective and some have deleterious side effects. The viral pathogens that have eluded effective vaccine strategies all have life cycle complexities that confound vaccine development. As such, realistically, some of those pathogens may not share the perfect marriage that existed between SARS-CoV-2 and mRNA vaccination. We provided several examples where mRNA deserve a serious look-see. As we indicated, there are a few instances where replacing an existing efficacious vaccine with a mRNA vaccine may be worth exploring. In general, however, we are advocating for the application of mRNA technology to serious diseases that have thus
far proven refractory to vaccine prevention. Vaccines against RSV and HCMV serve as prime example. We also hold high expectations for using mRNA vaccines to boost and broaden existing immunity to achieve marked reductions in disease severity or transmission rates for diseases that cannot be realistically amenable to preventing infection or achieving sterilizing immunity. Similarly, mRNA vaccines may be a welcome new tool to tackle pathogens in the animal kingdom where production of regular types of vaccines are difficult due to biosecurity concerns, such as Foot-and-Mouth-Disease Virus or Alphavirus encephalitides in horses. The coming era of mRNA vaccine promises to be exciting as well as rewarding.

Declaration of competing interest
The authors declare no conflict of financial interest.

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