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REVIEW

Why are vaccines against many human viral diseases still unavailable; an historic perspective?

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Abstract

The number of new and improved human viral vaccines licensed in recent years contrasts sharply with what could be termed the golden era (1955-1990) when vaccines against polio-, measles, mumps, rubella, and hepatitis B viruses first became available. Here, we attempt to explain why vaccines, mainly against viruses other than human immunodeficiency virus and hepatitis C virus, are still unavailable. They include human herpesviruses other than varicella-zoster virus, respiratory syncytial and most other respiratory, enteric and arthropod-borne viruses. Improved oral poliovirus vaccines are also urgently required. Their unavailability is attributable to regulatory/economic factors and the properties of individual viruses, but also to an absence of relevant animal models and ethical problems for the conduct of clinical of trials in pediatric and other critical populations. All are portents of likely difficulties for the licensing of effective vaccines against emerging pathogens, such as avian influenza, Ebola, and Zika viruses.

KEYWORDS
human, immunity, vaccine development, veterinary, viruses

1 | INTRODUCTION

Utinam tam facile vera invenire possem, quam falsa convincere

(I wish I could find the discovery of truth as easy as the exposure of error)

Cicero: *de natura deorum, Liber I*

Over the past 40 years the rate of development of newly licensed human viral vaccines, compared with veterinary vaccines, has been disappointingly slow. Viral vaccines licensed for human immunization and distribution in the United States and other countries in 2019 (https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts//ucm093833.htm) are mainly used for the prevention of pediatric diseases. Polyvalent ProQuad vaccines (against measles, mumps, rubella, and varicella viruses) were originally licensed as monovalent vaccines and the same is true for pentavalent hepatitis B (HBV) vaccines that also include the protective antigens of three common bacterial vaccines (diphtheria, tetanus, acellular pertussis) and inactivated poliovirus vaccine (IPV) and used extensively in developing countries.

Despite much early optimism throughout the 1980s, benefits from the use of molecular recombinant DNA (rDNA) technologies in human viral vaccine development were perceived in 1991 to be underwhelming.[1] The single exception was the then recently approved HBV vaccine, prepared from virus-like particles (VLPs) after expression in yeast. Fast-forward to 2019, traditional approaches have, in the meantime, resulted in several new and improved vaccines. However, after 28 years, human papillomavirus (HPV) vaccines, also prepared as VLPs in yeast,[2] and an influenza vaccine consisting of baculovirus-expressed hemagglutinin (Flublok)[3] are the only other examples of newly licensed human vaccines that were developed *entirely* by the use of rDNA technologies, in contrast to the much larger number of rDNA veterinary viral vaccines that were licensed over the same period.[4] The development of HPV vaccines, in particular, provide a powerful example of the potential for recombinant DNA technologies in vaccine development. This is not to deny the critical molecular role of reverse genetics in the development and manufacture of some human vaccines and others under development.[5-7]

Reasons, why vaccines against human immunodeficiency viruses (HIV) are unavailable, are complex and have been comprehensively dealt with elsewhere.[8] However, despite monumental efforts over 30 years, vaccines for the prevention and/or prophylaxis of infection by HIV still appear to be some years away. It now appears that much early misplaced optimism can be attributed to the fruits of the pre-molecular era (1955-1990) and should have been tempered by a realization that, for reasons still largely unknown, immune responses to animal lentivirus infections very rarely result in long-term reductions in viral load and accompanying pathogenesis.[9] The same is true of several members of the genera Hepacivirus and Pestivirus of the family Flaviviridae, as represented by HCV and several veterinary viruses that produce chronic infections. The absence of much-needed vaccines against herpes simplex virus types -1 and -2 (HSV-1, -2), respiratory syncytial viruses (RSV), most other respiratory and arthropod-borne viruses (except Japanese encephalitis and, until very recently, dengue viruses) and genetically stable live-attenuated oral poliovirus vaccines (OPVs) is attributable to the properties of individual viruses and to the deficiencies of currently used animal models of human pathogenesis, notably mice.[10] It is also partly due to a continuing and inevitable disconnect with veterinary virology where the relevance of animal models is rarely an issue. These factors, together with the difficulties and prohibitive costs of conducting Phase III clinical trials on vaccines against newly emergent pathogens,[11] have made modern human vaccine development and manufacture in many countries a marginally profitable enterprise that can only be sustained with major inputs from government. Regulatory concerns expressed, sometimes years after the initial registration, are especially a problem and specific examples are given below. The predictable consequences, at least in Western countries, are a declining skill base in older technologies, such as the development and the large-scale application of diploid and continuous cell lines for use in the preparation of vaccine viruses and antigens, and a concomitant increase in risk aversion.

2 | CONTEMPORARY HUMAN VIRAL VACCINE MANUFACTURE

Research on human vaccines these days is usually undertaken by smaller commercial enterprises associated with research institutes. By contrast, commercial manufacture in first world countries is confined to a decreasing number of very large multinational companies (Bigpharma) and institutional facilities. The decision in 2015 by Novartis, to exit human vaccine manufacture illustrates the point. Many smaller manufacturers, including State Serum Institutes, have been subsumed by Bigpharma which, in earlier times, was able to oversee all aspects of vaccine development from basic research to clinical appraisal.

The decreasing numbers of commercial manufacturers have been driven, in part, by the need to achieve economies of scale in the manufacture and by an increase in testing requirements before vaccine release that have been underpinned by codes of good manufacturing, laboratory, and clinical practice (GMP, GLP, and GCP). These codes in large measure have resulted from the willingness of US courts in earlier years to sanction significant claims against manufacturers, some for negligence but others now attributable to gaps in our knowledge of molecular aspects of viral replication and immunology, unknown at the time of the initial registration. The role of government in underwriting liability for manufacturers in national immunization programs has markedly increased since the decision by the US government to mandate the swine influenza vaccine program of 1976. Most human viral vaccine manufacture in developing countries is undertaken locally. For veterinary viral vaccines, the economic consequences of failure in all countries are very small by comparison.

The most successful period for pediatric viral vaccine development (1955-1990) followed advances in the cultivation of many human and animal viruses in primary cell cultures, notably monkey kidney and chicken embryo fibroblast cultures. Associated developments in the late 1970s early 1980s were the accreditation of human diploid fibroblast cell lines and continuous epithelial lines (most notably the Vero and MDCK lines) as substrates for the growth of vaccine viruses. Accreditation of both types of cell culture signified a major shift in thinking by regulators as to their oncogenic potential.[12] Until then, continuous cell lines had been used for the preparation of veterinary viral vaccines and shown to be safe and efficacious for animals but disallowed for use in the preparation of human vaccines. Without these changes, it is doubtful whether many highly successful vaccines against poliovirus, measles, mumps, and rubella would still be available today. This is especially true for OPVs prepared from primary monkey kidney epithelial (MK) cultures until the early 1980s. MK cultures allow high poliovirus yields but had long been recognized as problematic for use in vaccine manufacture for reasons of cost, the diminishing availability of primates and the ubiquitous presence in cultures of contaminating simian viruses. Targeted vaccination programs have largely eliminated poliomyelitis, a commonplace childhood disease in developed countries until the late 50s. Despite some unforeseen difficulties with their expanded use in developing countries and molecular evidence of genetic instability following human passage, OPVs continue to have a critical role in the prevention of poliomyelitis.[13]

3 | CHARACTERISTICS OF PEDIATRIC VIRAL VACCINES IN USE TODAY THAT WERE LICENSED BETWEEN 1955 and 1990

Most traditional vaccines continue to be at the forefront of pediatric disease control and alternatives are unlikely to become available any time soon. Features of these vaccines that have allowed the development of such successful *preventive medicines* include:

- the use of live attenuated viruses, except for inactivated poliovirus, hepatitis A and B, human papilloma and most influenza vaccines. Highly effective live vaccines against smallpox and yellow fever viruses were developed in the 19th and early 20th centuries; between 1960 and 1980 critically important live pediatric vaccines for the prevention of viral measles, mumps, and rubella became available.

- the component viruses of all live vaccines have been attenuated largely by empirical (nonmolecular) means, including the simple, widely-used and historically validated practice of multiple passages in cell culture or embryonated eggs, and the use of seed-lot systems for defining passage number. Despite this, the basis of attenuation for many live vaccines is still poorly understood.
- all produce acceptably low levels of vaccine-associated side-effects and for the most part an overwhelming clinical and economic benefit in favor of their continuing widespread use.

These highly successful vaccines were first licensed in a more permissive regulatory environment than exists today and attempts to license several vaccines, now widely in use, would these days have presented significant difficulties. They include OPVs, which have been essential for the near-global elimination of paralytic poliomyelitis under WHO-sponsored programs over the past four decades. Their development during the 1950 60 seconds took place in the almost complete absence of molecular data as to the genetic stability of individual vaccine viruses. The subsequent application of contemporary molecular technologies has shown that, for OPVs, genetic instability associated with neurovirulence occurs following human passage.[14] The neurovirulence risk can be overcome by the replacement of OPVs with IPVs in vaccination programs. However, IPVs require higher antigen doses and the costs of manufacture are higher because of the need for increased safety testing. The introduction of IPVs throughout the developing world is likely to be gradual and, because type II nonvaccine viruses have not been detected in susceptible populations for several years, recent OPVs have been modified to exclude Type II vaccine viruses as an additional safety measure.

The development of live measles vaccines is another case in point. These vaccines developed in the early 1960s and were initially licensed before recognition of subacute sclerosing panencephalitis (SSPE). This rare immune-based complication of measles infection was subsequently shown to be associated with chronic infections of the CNS. If SSPE had then been widely recognized at the time, the developers of live measles vaccines could have faced potentially insurmountable hurdles to prove that mass administration would not be associated with increases in the incidence of SSPE. Given that satisfactory in vitro markers for SSPE still do not exist, such a requirement would have placed a near-impossible burden upon manufacturers, despite extensive evidence of safety in clinical trials. These vaccines have been responsible for the virtual elimination of measles in developed countries and have made enormous inroads into its control in developing countries.[15] Unlike inactivated polio vaccines, inactivated vaccines designed to protect against measles, mumps and rubella viruses are ineffective and, alarmingly in the case of measles, vaccination followed by the natural challenge is associated with immunopathologic disease.[16]

The development of experimental vaccines for neonates also presents unique immunological and ethical difficulties. Respiratory syncytial virus (RSV) infections are the cause of over 30% mortality in infants less than 1 year of age.[16] Early attempts to use formalin-inactivated virus in an experimental vaccine not only failed to protect against infection but sensitized the recipients to severe adverse (Arthus) type III hypersensitivity reactions that resulted from subsequent natural challenge.[17]

Indeed, the outcome of those earlier studies have influenced subsequent attempts for the development of effective vaccines against RSV, which include the use of intranasally-administered live attenuated and, more recently, subunit complementary DNA and vector-based vaccines. In spite of an urgent need, no licensed vaccines against RSV or other pediatric respiratory viral pathogens, such as human paramyxoviruses 1 to 4, are available.

Thus a combination of economic factors, combined with altered regulatory environments and the still empirical nature of modern vaccine *discovery* are major complicating factors in successful vaccine development. It should also be noted that for the guidance of vaccine development and initial registration, the contribution of modern immunology has been minimal. It has however provided such a wealth of subsequent data on mechanisms of action. Thus it is likely that a deep understanding of knowledge of innate and adaptive immunity may be necessary to avoid adverse reactions in general and as a guide to the development of effective vaccines against more challenging organisms in intractable infections.

4 | PASSIVE IMMUNE THERAPY

Passive immune therapy (PIT) has been a historically successful approach to the prevention or treatment of some intoxications or viral infections.[18] The use of immune gamma globulins has been largely abandoned because of the risk of anaphylaxis or blood-borne virus transmission and currently, their use is not feasible for population-based coverage. However, the emergence of monoclonal antibody (mAb) therapy in inflammation and cancer has raised the possibility that PIT may be useful in the acute management of viral infections as alternatives to traditional vaccines for use in infants, such as RSV whose development has been problematic. The mAb, Palivizumab, has been licensed for clinical use in the prevention of RSV infection. Combinations of up to three therapeutic mAbs have also been considered for development as a possible PIT in Ebola virus infections.[19,20] A related development in the use of postexposure prophylaxis, involves the use of combination passive and active immunization which is widely used for the treatment of verified rabies infections.[21]

It has become apparent that anticancer antibodies act, at least in part, by harnessing effector responses of the innate immune system (antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) or complement activation.[21] Most interestingly, they can also induce active immunity to tumor cells.[22] The induction of adaptive immunity associated with mAb therapy involves cooperation between innate and adaptive immune systems and is probably related to the long-recognized and potent capacity of antibody-antigen (immune) complexes to alter immune responses. This includes the induction of immunological memory, which has been recognized for over three decades.[22-24]

Whether such vaccinal effects will result from the use of anti-RSV or other antiviral mAbs remains to be seen but it is clear that passive

antibody therapy can induce active and durable adaptive immune responses. Thus, as a general consideration, it does not require a great stretch of the imagination to believe that passive antibody therapy of infectious disease, either as a monotherapy in established infections or in combination with the use of conventional inactivated vaccines or other vaccine candidates, may result in sterilizing responses to otherwise difficult infectious agents. Indeed, experimental evidence for this is beginning to emerge, albeit slowly.[25,26] Perhaps a radical rethink on how to approach immune responses/immunity may offer transformational change.

5 | IMPROVEMENTS TO EXISTING HUMAN VIRAL VACCINES SINCE 1991

Continuing improvements have been made to measles, mumps, and rubella vaccine viruses, which fortunately are antigenically relatively stable. They include replacement with strains that are more immunogenic and/or are associated with fewer adverse reactions. Over 40 years licensed US measles vaccines have been prepared from the original Edmonston to the currently used Moraten (previously known as the Edmonston-Enders) strain.[27] These changes, unfortunately, have not extended to the replacement of OPVs. Such issues that figure high in today's highly regulated vaccine market have in the recent past been a severe economic disincentive to any of the few remaining poliovirus vaccine manufacturers in developed countries even mildly interested in OPV strain replacement. However, the situation may change with the development of codon-pair bias de-optimization methodologies that allow the possible use of redundant underrepresented codon pairs without introducing changes to the amino acid sequences of virion proteins of OPVs or other live vaccine viruses.[28] The key question is whether the introduced mutations result in changes to viral immunogenicity, which can only be determined from large, expensive clinical trials. Great improvements in disease control have resulted from improved vaccination regimes and the use of trivalent measles-mumps-rubella (MMR) and quadrivalent measles-mumps-rubella-varicella (MMRV) vaccines. However, it should be noted that high levels of maternal antibodies arising from natural infection compromise the efficiency of vaccination in infants. In developed countries, the universal use of MMR/MMRV vaccines for infants was only possible after the virtual elimination of endemic measles by vaccination. Before that, vaccination was undertaken at 15 to 18 months when levels of the maternal antibody had declined, and several months after the administration of the first dose of other pediatric viral vaccines. Conversely, in the developing world protection afforded by measles vaccines is lower in populations where the levels of maternal antibody are high.[29]

6 | NEW LIVE VIRUS VACCINES LICENSED SINCE 1991

Although improvements to *traditional* vaccines have been the mainstay of vaccine development into the 1990s, a limited number of similarly attenuated human vaccines have been registered since then, which include:

- Oral rotavirus vaccines.
- Intranasal live attenuated influenza vaccines (LAIVs).
- Subcutaneous varicella-zoster virus (VZV) vaccines.
- An oral adenovirus vaccine, containing type 4 and 7 viruses and licensed for limited institutional use, such as the military, but not discussed here.

6.1 | Rotavirus vaccines

A tetravalent reassortant oral vaccine derived from a simian rotavirus donor strain with human surface antigen genes was licensed in 1998 about 25 years after the etiology of rotaviruses, the most significant of human gastrointestinal pathogens, had been established. Following large clinical trials in both developed and developing countries, Food and Drug Administration (FDA) approval was granted for its use in infants. However and despite the medical need and encouraging clinical data the vaccine was withdrawn when subsequent data indicated a small increase in intussusception.[30]

Fortunately two other vaccines, one a pentavalent human-bovine reassortant vaccine (RotaTeq®) prepared using the previously tested bovine rotavirus strain (WC3) as a vector and the other, a monovalent vaccine (Rotarix®) prepared from the attenuated P1A G1 human strain by multiple cell culture passage, were licensed in 2006 which have had a profound impact on the prevention of rotavirus infections in infants, especially in the developing world.[31]

6.2 | LAIV and other nonrecombinant influenza vaccines

Greater recognition of influenza as a preventable disease of children has led to recommendations in some countries for annual vaccination of infants and children. Data largely from animal studies has consistently indicated that not only are live experimental vaccines most effective but that direct administration via the respiratory tract yields superior protection than that afforded by parenterally-administered inactivated vaccines.[32] A previous attempt to develop a novel intranasal inactivated vaccine using a mucoadhesive adjuvant was abandoned following the demonstrated but unknown association between vaccine administration and the symptoms of Bell's palsy.[33] However, live attenuated cold-adapted (ca) reassortant vaccines have been in use in the United States and Russian Federation for over a decade.[34] The initial 2002 approval in the United States restricted their use to 5 to 49-year-old recipients. Following receipt of further trial data, the age range for US vaccines was extended to 2 to 49 years in 2006. Live intranasally (i.n.) administered vaccines have been a significant component of US immunization programs since then. However, because of poor efficacy against more recent H1N1 swine pandemic viruses, use of the vaccine was temporarily suspended in the United States.[35]

In vivo tests of immunogenicity that measure differences between individual vaccine components are no longer required for influenza

vaccines. In the case of inactivated vaccines, potency is merely estimated by an in vitro antigen-binding assay to determine hemagglutinin (HA) antigen concentration.[36] Although not required for the licensing of the individual components of live vaccines, effectiveness can be measured in a mouse model according to the intranasal vaccination dose required to clear a standard challenge of the wild-type (*wt*) parental virus used to prepare 6:2 reassortants from the same attenuated donor strain and the vaccinating dose can be adjusted accordingly. In the case of earlier H1N1 reassortants, ~100× the infectious dose was required to achieve clearance from the lungs 3 days after challenge, in comparison with the dose required for early H3N2 reassortants.[37] Other issues that do arise concern the relevance of HAI antibody as a measure of the effectiveness of live influenza vaccines. Virus-specific secretary antibody responses, as determined by ELISPOT assays in the respiratory tract of mice, clearly indicate the superiority of live vaccines but, for ethical reasons, cannot be performed in humans. However, overall, despite recent experiences, ca live reassortant vaccines appear more effective in children than conventional trivalent inactivated vaccines, whose role in the prevention of influenza has been the subject of contention for many years.[38] Live vaccines were introduced for children in the UK in 2014 under their National Health Service.

Particular difficulties were faced by both regulatory authorities and vaccine manufacturers in updating influenza vaccines in 2017 and 2018 in both the Southern and Northern hemispheres, following unanticipated late changes to the hemagglutinin antigens of designated H3N2 viruses.[39] All other influenza vaccines, except the baculovirus-expressed recombinant vaccine (FluBlok), consist of inactivated subunit preparations containing the surface antigens of purified egg-grown H1N1 and H3N2 influenza A viruses and one or both lineages of recent influenza B viruses. All are administered by the intramuscular (IM) or intradermal (ID) routes. Some inactivated vaccines are adjuvanted. Now included in the list of FDA-approved vaccines is Flucelvax, an inactivated vaccine prepared from viruses grown in cultures of the MDCK cell line in place of embryonated eggs.

6.3 | VZV vaccines

Licensed by the FDA over 30 years after reports of the use of the live attenuated Oka strain as a subcutaneously (SC) administered the vaccine in Japan, [41] immunization against varicella vaccination is now an established arm of universal childhood vaccination. The Oka strain was further attenuated by Merck, and the renamed Oka-Merck strain has been used in the United States and other countries to prepare varicella vaccine (Varivax) after further adaptation to growth in cells of the WI-38 and MRC-5 human diploid lines. A 10-year review revealed that the vaccine is generally safe and well-tolerated.[40] Pediatric vaccines are administered in two doses, commencing at 18 months, with a further booster at the commencement of high school (12-13 years). For adolescents and adults who have not experienced childhood varicella infections, administration of two doses at an interval of 2 months is widely recommended. Recrudescent infections of childhood varicella infections (herpes zoster), constitute a significant disease burden for the elderly and, because of early priming experiences and the declining capacity of the elderly to mount protective immune responses, doses that are 14-fold higher than those used for the prevention of varicella in children are required.[41] The adult vaccine, now licensed as Zostavax is well tolerated and provides immunity against infection by herpes zoster virus and amelioration of the symptoms of postherpetic neuralgia.[42]

Latency has been a constraint in the development of attenuated live vaccines against HSV-1 and -2 but is much less of a problem with Varivax because of the limited capacity of the Oka vaccine viruses to spread, in comparison with wild-type epidemic strains.[43] However, like other VZVs, the Oka-Merck strain is labile, highly cell-associated and produces relatively low yields of infectious virus in cell culture. Lability can be overcome by sonic disruption of infected cells, followed by lyophilization and cold-storage. However, the reconstituted vaccine must be used within a relatively short time, which restricts its use in tropical countries. By comparison, polioviruses used in either OPVs or IPVs are highly stable, do not require lyophilization and can be stored at 4 to 6°C for long periods. They also produce yields of infectious virus in cell culture ~100× higher than VZV. Because of these constraints, much effort has been expended in recent years by GlaxoSmithKline to develop an adjuvanted, inactivated herpes zoster vaccine (Shingrix) with expressed surface glycoprotein E as the protective antigen. Early large clinical trials to determine protection have been encouraging.[44] While the potential advantages of such an approach are clear, earlier studies with the live Oka-Merck vaccine have indicated that cell-mediated immunity (CMI) is the prime determinant of protection against VZV[45] and the results of large clinical trials to determine CMI or other surrogate immunologic responses to the Shingrix vaccine are eagerly awaited.

Studies over many years with inactivated whole virus HSV-1 and -2 vaccines or where the active protective ingredient was expressed glycoprotein D have failed to identify responses essential for protection against genital herpesviruses.[46]

7 | FACTORS ANTITHETICAL TO HUMAN VIRAL VACCINE DEVELOPMENT

Recently imposed regulatory issues arising from greater insights into viral replication and pathogenesis have impacted significantly on viral vaccine manufacture in the developed world. Particular issues of concern are:

- Deficiencies in many animal models used for assessment of protection against human viral infections. In their absence, new vaccines, especially those designed for use in infants, face formidable regulatory obstacles.
- Use of inappropriate viruses to obtain predictive data in animal models (eg, mouse-adapted influenza and respiratory syncytial viruses; other viruses of uncertain passage history).
- Lack of recognition that, despite legitimate concerns as to the possibility of reversion to virulence, immunity to most human viral diseases is best achieved by live attenuated viruses (eg, herpes and

enteric viruses; RSV and respiratory viruses, including influenza; other viruses whose pathogenesis is complex and dependent on amplification in more than one target organ, such as measles and yellow fever viruses.

- The possibility that some live herpesvirus vaccine viruses will undergo latency during replication, followed by recrudescence and subsequent infection by pathogenic viral progeny (eg, HSV-1 and -2). However, for live veterinary herpesvirus vaccines, reported problems from recrudescence have been few, the best example being the herpesvirus of turkeys (HVT) that has been used for over 40 years for control of Marek's disease virus (MDV), an oncogenic avian herpesvirus and a cause of great economic loss in intensively raised chickens.[47] Until the mid-1990s, HVT was the first and only example of an effective vaccine in either animals or humans that could be used for the prevention of neoplastic viral disease. The genome of HVT has 70% to 80% nucleotide homology with pathogenic MDVs but is completely innocuous for chickens. The effectiveness of live human VSV and MDV and other veterinary vaccines suggests that it may be worth revisiting the development of live vaccines using more readily cultivable human herpesviruses.
- The inability of many human viruses to either grow in cell cultures (eg, HBV, human noroviruses) or produce yields of infectious progeny sufficiently high for use in vaccines (HIV, HCV; other herpesviruses). However, a recent report describes the propagation of human noroviruses in stem cells derived from human intestinal enteroid cultures,[48] which may have great significance for the development of effective vaccines.
- Poor antigenic responses to glycosylated surface antigens expressed in *Escherichia coli*, *Salmonellae*, or yeasts, all of which were first proposed in the early 1980s as alternatives to embryonated eggs in inactivated influenza vaccine manufacture.[49]
- Poor immunogenicity of peptide epitope vaccines based on defined regions associated with protection. Although their use in vaccines is attractive from the standpoint of standardization, peptides suffer from two major disadvantages. First, the B-cell antigenic sites of many proteins are conformational epitopes, consisting of discontinuous sequences of amino acids, and such conformations are difficult to recapitulate synthetically. Second, the immune responses of inbred mice, used in most studies, are genetically restricted according to the haplotype of the mouse.[50] The MHC diversity between individual mouse strains is limited and therefore immune responses in inbred mice are likely to be even more restricted than in an outbred human population. The greater MHC diversity in a human outbred population would probably result in a high proportion of nonresponders to individual peptides and necessitate the need for a large number of epitopes to the major histocompatibility complex. Furthermore, responses to peptides from influenza A virus matrix protein 2 (MP2), that has been proposed as a universal protective antigen or to peptides from the protective hemagglutinin antigen, have been shown to be very weak unless coupled to carriers, such as multiple antigenic peptide constructs (MAPs) or KLH, whose likely approval for large-scale human use is open to question.
- DNA vaccines prepared from genomic DNA or cDNA from RNA viruses and administered by the IM or SC routes, widely touted in the 1990s as measures for the ultimate control of viral diseases, especially influenza, hepatitis B and HIV. Unfortunately, the results from many early clinical trials were disappointing, with most DNA vaccines being shown to be poorly immunogenic, even when used with an adjuvant.[51] However, more recent reports on the protection afforded by Zika DNA vaccines in primates are encouraging.[52] For influenza cDNA vaccines, further regulatory issues arise concerning immunologic recall after earlier priming with heterotypic hemagglutinin antigens (the phenomenon of *original antigenic sin* [53,54]). However, up to and including 2019 no DNA vaccines have been licensed.

8 | ONGOING PUBLIC HEALTH CONCERNS AND VACCINE DEVELOPMENT

8.1 | Newly emergent human viral pathogens

Over the past 20 years great concerns have been expressed by the WHO and other bodies following disease outbreaks by avian influenza A subtypes H5N1, H7N9, H9N2, and H3N2v and the SARS and MERS coronaviruses, Ebola filoviruses and the Zika flaviviruses—all single-stranded enveloped RNA viruses from different families, some first described in developing countries. Infections by influenza A H5N1, SARS, MERS, and Ebola viruses can result in high rates of mortality (>60%). However, Zika viruses are especially a concern because of their ability to induce neural and other birth defects in the developing fetus, and to be transmitted sexually.

Pandemic influenza A viruses present a much greater long-term threat to public health than the other viral pathogens, because of their widespread presence in water birds—potential reservoirs of new pandemic strains and their capacity to spread rapidly and to undergo large and unpredictable changes in antigenicity and virulence. Such changes were responsible for the pandemic of 1918 to 19, considered to have been responsible for 50 to 100 million deaths, and the estimated costs of a similar pandemic have been estimated to be as much as 5% of the US GDP or three trillion dollars.[55] The SARS and MERS coronaviruses, first reported in 2002 and 2012 also have the potential to spread rapidly but in 2019 do not appear to be the global public health threats originally feared. Infections by Ebola viruses, first described in Zaire (now the Democratic Republic of the Congo) 1975 and other Sub-Saharan Countries, appear to be sporadic.

8.2 | Avian influenza vaccines and universal influenza vaccine development

Programs to develop effective vaccines against recently described viral pathogens have received much support over the past 5 to 15 years but only inactivated egg-grown avian influenza A vaccines prepared by largely traditional methods have so far been licensed. Early clinical trials with H5N1 avian influenza vaccines have not been

encouraging. Two adjuvanted doses of vaccine are required to induce HI antibody titers of 1:32 to 1:40 which are considered necessary for the protection against seasonal influenza viruses.[56] However, the protective antibody level required for vaccines most avian subtypes is unknown. Because of these limitations, much effort has been directed in recent years towards the development of so-called *universal* vaccines, the ultimate goal being the induction of long-term protection against both pandemic and nonpandemic influenza A viruses. Approaches to their development have included the use of:

- Expressed nonglycosylated M2 surface antigen or an M2e peptide fused with a bacterial protein. Some protection was demonstrated in mice where the response appears to be nonneutralizing and directed at target cells; early human trials were largely unsatisfactory.[57] However, better results have been achieved with HA2-based conformational mutants expressed in *E. coli*.[58]
- The variable stalk region of the HA and the identification of broadly neutralizing antibodies from different regions of the stalk, using so-called *headless* proteins as antigens.[59-66] Other groups have attempted to design vaccines containing small conserved regions of the stalk. However, it is not known why antibodies are not normally made to the stalk as a consequence of infections by influenza A viruses. Good cross-protection across H3N2 sub-types was shown from earlier studies in mice but cross-protection against H1N1 viruses was less impressive.
- Chimeric viruses prepared from high-yielding vectors with internal group-specific antigens. Vectors include vesicular stomatitis virus, adenoviruses, or poxviruses.[67-69] At issue is whether the long-term administration of common vector antigens inhibits specific responses to the inserted surface antigens of new pandemic viruses.
- Pseudotypes that do not produce infectious progeny, but induce satisfactory short-term protective responses in mice after i.n. administration, including antibody to both HA and NA surface antigens, CTL and resistance to challenge.[70,71] Importantly, they have been shown to be effective in heterotypic challenge experiments in ferrets whose pathogenesis is similar to that of humans.[72] Such an approach could provide short-term protection in the initial stages of a pandemic.

8.3 | Vaccines against Ebola, Dengue, and Zika viruses under development

Live attenuated vaccines are usually superior at inducing immunity to viruses involving multiple target organs (eg, yellow fever, measles, mumps, and rubella viruses) than nonreplicating inactivated vaccines. Approaches most favored for potential Ebola vaccine development include the use of VLPs or recombinant chimeric vaccines using VSV, human paramyxovirus-3 or replication-deficient adenoviruses as vectors. Of particular interest are recent reports of the successful use for ring vaccination of an experimental vesicular stomatitis virus - vectored Ebola vaccine in human trials under field conditions.[73,74]

A chimeric live quadrivalent vaccine (Dengvaxia®), using the 17-D YFV vaccine strain as the vector and the prM and E protein genes of DEN 1 to 4, has been developed by Sanofi-Pasteur as the first licensed vaccine against dengue viruses. These vaccines have been designed to induce responses to the four major dengue serotypes, in attempts to prevent the dengue hemorrhagic fever syndrome (DHF) due to immune antibody-dependent enhancement (ADF) following superinfection with heterotypic dengue viruses. However, early studies with Dengvaxia® have shown that protective responses in very young children, compared with older primed children and adults, were relatively poor.[75,76] Many aspects of immunologic protection against dengue infections are not well understood.

Three different types of Zika vaccine have been recently evaluated in primates and early clinical trials are underway.[77] They include purified inactive virus, plasmid DNA (referred to above) and single-shot rhesus adenovirus serotype 52-vectored vaccines. All were able to induce neutralizing antibodies sufficient for protection against subsequent Zika virus challenge. Another immunization strategy involves the delivery of nanoparticles containing viral mRNA to the host and the in vivo development of protective immune responses to Zika viruses. Early challenge studies in mice appear promising.[78]

Although ADE does not appear to be a problem following infection by heterotypic Zika and/or other flaviviruses, further challenges lie ahead. They include possible risks from the Guillain-Barré syndrome, which is an occasional feature of Zika infections.[79]

9 | CONCLUSIONS

This review indicates that fewer new human viral vaccines have been licensed in recent years than during the period 1955 to 1990 when vaccines against polio-, measles, mumps, rubella, and hepatitis B viruses for global use first became available. Recent successes include hepatitis A, either alone or in combination with HBV, herpes zoster, HPV and newer Japanese encephalitis vaccines, although their overall impact has been smaller. In a sense, all the *low-hanging fruit* has been picked and the licensing of effective vaccines against HIV, HCV, most herpesviruses and other enteric-, arthropod-, and most vector-borne viruses in the near-term seems unlikely.

Reasons for the unavailability of other urgently needed non-HIV vaccines are complex and directly related to the properties of individual viruses and associated economic considerations. To paraphrase Cicero, it is probably better to admit to this, than attempt to develop a unifying hypothesis that could be applied to all urgently needed vaccines. Clearly, an entirely profit-driven, nonpublically funded model for the rapid development of new vaccines by Bigpharma struggles to exist, and there appear to be very few signs of a re-visitation of the earlier model involving, in some countries, substantial government participation in both vaccine development and manufacture. History shows that government intervention can be a highly successful model. One example was the successful development and field testing of oral poliomyelitis vaccines in the former Soviet Union.[80] Similar government-sponsored programs of the period often go unrecognized but were essential to the development of vaccines in that *golden era*.

Despite the shortcomings of some older vaccines, we should be extremely thankful for what has been achieved. We are fortunate that control of critical diseases, such as poliomyelitis and measles, was accomplished at times much less litigious than the present and, perhaps counter-intuitively, when much less was known of molecular aspects of viral replication and pathogenesis than is known today. It also occurred in the absence of the present day anti-vaccination lobby that ignores the lessons of the past and especially the concept of vaccine-induced herd immunity. However, success continues to be achieved against a background of overwhelming public acceptance of the need to control the pediatric disease by vaccination.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

All authors contributed extensively to the work of writing and editing this manuscript.

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