Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection (2017) 74, S2–S9

BIA
British Infection Association

www.elsevierhealth.com/journals/jinf

Whither vaccines?

Charlene M.C. Rodrigues [a,*], Marta V. Pinto [b], Manish Sadarangani [b,c], Stanley A. Plotkin [d]

[a] *Department of Zoology, University of Oxford, Oxford, UK*
[b] *Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, UK*
[c] *Vaccine Evaluation Center, BC Children's Hospital Research Institute, University of British Columbia, Vancouver BC, Canada*
[d] *Department of Pediatrics, University of Pennsylvania, Philadelphia, USA*

Available online 23 June 2017

KEYWORDS
Vaccinology;
Influenza;
Pertussis;
HIV;
Rotavirus;
Dengue;
CMV;
RSV;
Structural biology;
Host immunity

Summary Currently used vaccines have had major effects on eliminating common infections, largely by duplicating the immune responses induced by natural infections. Now vaccinology faces more complex problems, such as waning antibody, immunosenescence, evasion of immunity by the pathogen, deviation of immunity by the microbiome, induction of inhibitory responses, and complexity of the antigens required for protection. Fortunately, vaccine development is now incorporating knowledge from immunology, structural biology, systems biology and synthetic chemistry to meet these challenges. In addition, international organisations are developing new funding and licensing pathways for vaccines aimed at pathogens with epidemic potential that emerge from tropical areas.

Introduction

Vaccination has been described as the single most important health intervention in human history. From the first smallpox vaccine conceived by Jenner in 1798[1] developing the idea of attenuation to the reverse vaccinology derived capsular group B meningococcal protein vaccines,[2] the requirement for protective, long-lasting host immune responses to vaccine antigens that are safe to handle and administer with broad coverage has been the aim. To deliver these requirements, the next generation of vaccine development is drawing on major scientific advances in microbiology, structural biology, immunology and most recently molecular biology. However, despite the huge success of vaccinology to date, many challenges remain involving scientific, financial, political and social domains. Here we discuss a range of these with examples impacting on child health.

* Corresponding author. Department of Zoology, University of Oxford, Oxford, UK. Tel.: +44 (0)1865 281061.
E-mail addresses: charlene.rodrigues@gtc.ox.ac.uk (C.M.C. Rodrigues), marta.valentepinto@paediatrics.ox.ac.uk (M.V. Pinto), msadarangani@cfri.ca (M. Sadarangani), stanley.plotkin@vaxconsult.com (S.A. Plotkin).

Specific vaccination challenges

Pathogen variability: Influenza vaccines

Seasonal influenza epidemic infections are commonly caused by influenza types A and B. Vaccination continues to be one of the most efficacious ways to prevent disease, reducing morbidity and mortality particularly in vulnerable groups.[3,4] Despite this, vaccine efficacy is considered only moderate and dependent on variables including vaccine type, match between circulating strains and vaccine strains and target age groups.[5] Influenza virus is considered highly variable and can evade the immune response in previously vaccinated populations.[6] Current vaccines are designed to induce the production of specific antibodies against the viral membrane surface proteins haemagglutinin (HA) of two influenza A strains and one or two influenza B strains.[3,6] Variation within the influenza virus, due to antigenic shift and drift results in the accumulation of amino acid replacements in the HA epitopes,[3,7] leading to mismatches between the vaccine strains and circulating strains, independently of all the predictions made each year by the World Health Organization (WHO).

Both live and inactivated influenza vaccines are available, but the effectiveness of these vaccines in the most vulnerable groups is suboptimal.[8] In children, inactivated vaccines also have a reduced effectiveness when compared with live attenuated vaccine in some studies but not in others.[5,9] Strategies to improve influenza vaccine efficacy include adding a second lineage of type B and a high HA dose, are currently applied to licensed vaccine.[6] Other strategies are also described and tested in different phase trials. Inactivated influenza vaccine with MF-59 as an adjuvant has been licensed in Canada in young children and the elderly,[10,11] with studies showing increased immunogenicity of the vaccine in both groups.[12,13]

Alternative options include adding TLR adjuvants like AS01 or flagellin, adding conserved epitopes from the viral nucleoprotein, the M2e or stalk of HA could yield improved vaccine efficacy.[14] Genetic vaccines, DNA and RNA based, can also contribute to produce efficacious influenza vaccines, for example, alphavirus-based H7N9 RNA vaccine have shown promising results,[6] although not yet commercially available.

Short effector memory: Pertussis vaccines

Since the introduction of pertussis vaccines in the 1940–50s, the incidence of pertussis has decreased dramatically in countries where the vaccine is given routinely. The WHO estimated 16 million pertussis cases in 2008, 95% of those occurring in developing countries.[15] Despite the availability of effective prophylactic vaccines against pertussis, there has been a rise in the incidence of disease, with epidemics throughout the world in the last decade.[16] Different reasons have been hypothesized for the outbreaks: waning immunity after vaccination, greater awareness and disease reporting, increased virulence of the bacteria and circulation of new strains.[17] The true burden of disease in industrialised countries may be underestimated with an increased incidence in previously vaccinated populations including older children and the elderly.[18]

Table 1 Estimated vaccine efficacy after administration of any brand of Tetanus, diphtheria and acellular Pertussis vaccine (TdaP) to Wisconsin residents between 1998–2000.[19]

Years after TdaP	Vaccine efficacy % (95% CI)
1	75.3 (55.2–86.5)
2	68.2 (60.9–74.1)
3	34.5 (19.9–46.4)
≥4	11.9 (−11.1 to 30.1)

Adverse reactions such as high fever, severe irritability, hypotonic hyporesponsive episodes and severe local skin reactions were described after administration of the whole cell vaccine (wP). This led to its replacement in developed countries with an acellular, less reactogenic vaccine (aP), first developed in Japan in the 1980s. Several studies have shown that immunity after immunisation with pertussis vaccines, especially after aP, can wane rapidly. A study by Koepke *et al.* in Wisconsin described the progressive reduction of aP vaccine efficacy throughout the years (Table 1).[19]

The immune mechanism induced by each vaccine may be responsible for these differences. Natural infection or immunisation with wP induces stimulation of T cell immunity, in particular IFN-γ-secreting CD4+ T cells (Th1 and Th17 cells), whereas aP vaccines induce strong antibody responses and Th2-type responses.[20] The baboon model has contributed both to our understanding and the relevance of these immune mechanisms.[21] Following natural infection or vaccination with wP or aP, their immune responses are similar to those observed in humans.[22] There is evidence that the Th17 responses induce clearance of *B. pertussis* from mucosal surfaces, thereby reducing colonisation and transmission.[23] The lack of Th17 activity following aP vaccination may account for the ongoing disease transmission and resurgence in countries widely using the aP vaccine.

In order to improve aP vaccine efficacy, possible strategies have been described including the introduction of strains that contain the *ptx*P3 promoter. These new circulating strains induce higher host production of pertussis toxin (PT), providing a possible selective advantage.[24,25] The use of new adjuvants, which may or may not be associated with aluminium, can skew the immune response and stimulate a balanced Th1/Th17 response, like CpG oligonucleotides, AS04 (combination of monophosphoryl lipid A and aluminium) and TLR-2 agonists.[20] Interventions such as using modified bacteria virulence factors like adenylate cyclase and tracheal cytotoxin,[26,27] a live nasal attenuated pertussis vaccine (BPZE1)[28] or the use of recombinant pertussis DNA vaccine[29] are also being studied.

Obtaining the right functional response: HIV vaccines

There have been various effective methods employed for HIV prevention, but many challenges remain to the development of a successful vaccine. There is uncertainty about what the protective functional response is since there is no natural recovery from infection, although long-term survival is

possible.[30] Broadly neutralizing antibodies develop, but occur late in natural infection[30] and a successful vaccine would need to induce a more rapid response. Dissemination from the mucosal site of infection takes place within 24–72 hours, and any protective response would therefore have to rapidly neutralize the virus at the mucosal surface or kill all HIV-infected cells throughout the body. As with many vaccines, a HIV vaccine would have to overcome strain variation – there are currently 9 genetically distinct clades (subtypes) of HIV-1 and significant variation within clades also occurs.[31] Previous trials have adopted a variety of approaches to obtain the right functional response. Early trials focused on eliciting neutralizing antibodies targeting the gp120 envelope protein.[32] Subsequent trials have utilized viral vectors to deliver HIV antigens, including adenovirus-5 and canarypox virus.[32] These have aimed to induce protective cellular immune responses, and have included prime-boost strategies using DNA encoding HIV proteins followed by recombinant proteins. Only one study has demonstrated protective vaccine efficacy, combining a canarypox vector vaccine prime with booster injections of a gp120 subunit vaccine in Thailand.[33] There was 31% reduction in HIV acquisition due to induction of IgG3 antibodies directed against the V1V2 region of gp120 mediating antibody-dependent cellular cytotoxicity (ADCC),[34,35] although efficacy was high early after vaccination and declined over time. The importance of such non-neutralizing antibodies has been shown with other pathogens, most notably influenza where infection and inactivated vaccine induce ADCC (with strain cross-reactive antibodies.[36,37] Future strategies for HIV will focus on the induction of broadly neutralizing antibodies via use of envelope trimer structures, which are more immunogenic than monomers, use of better vectors or adjuvants to improve on the induction of ADCC observed in the Thailand trial and induction of effector CD8+ memory T cells directed against non-canonical epitopes using persistent viral vectors. This latter strategy was effective in clearance of simian immunodeficiency virus in rhesus macaques, using a CMV vector, and this may be a useful strategy for HIV and other chronic infections.[38]

Population specific challenges: Rotavirus vaccines

The highest burden of severe, symptomatic rotavirus disease is in 3 to 24 month old children.[39] The impact on childhood mortality is significant in developing countries, whilst there is much morbidity in industrialised countries. Longitudinal studies identified that children naturally infected with rotavirus developed adaptive immunity to prevent primary infection and to protect against re-infection, in particular those causing severe disease.[40–42] Therefore, the principle of administering oral live attenuated rotavirus strains was developed. Currently, monovalent human G1 vaccine Rotarix® and pentavalent human-bovine re-assortment vaccine Rotateq® are the most widely used rotavirus vaccines globally.

The efficacy of these vaccines is very high in industrialised countries.[43,44] Since their introduction in the U.S. in 2006, there has a been a reduction in hospitalisation and emergency department presentations, all-cause diarrhoea attendance, and rotavirus hospitalisation in unvaccinated individuals, suggesting some degree of indirect protection.[43,45] However, lower efficacy has been well documented in many developing countries, clearly noting that vaccine efficacy has an inverse relationship with childhood mortality.[46] The reasons for this are unclear, proposed explanations include the presence of transplacental and breast milk derived IgA having a neutralising effect, concomitant poor nutrition or chronic helminth infections suppressing immune response.[47,48] However, the microbiota is increasingly implicated in immunological development and modulation of infant mucosal immunity, but the mechanisms remain poorly understood, although microbial interference and zinc deficiency appear the most important.

Disturbance of the resident microbiota with antibiotics in mice results in reduced susceptibility to poliovirus infection and reduced infectivity of rotavirus.[49,50] Interestingly, treatment with ampicillin and neomycin before and during oral rotavirus inoculation produced a longer duration and higher titres of serum and faecal IgA compared with untreated controls.[49] These intriguing findings raise many further questions about the impact of microbiome composition on mucosal immunity and methods of immunomodulation (e.g. probiotics, antibiotics) that could improve vaccine efficacy, in particular in developing countries with high mortality from rotavirus infection.

Uncertain correlates of protection: Dengue vaccines

The difficulty in development of a successful dengue vaccine lies in the pathogenesis of dengue hemorrhagic fever, the most severe dengue syndrome. There are four dengue virus serotypes,[1–4] and infection results in the development of long-lasting serotype-specific antibodies[51,52] which contribute to protection. While infection results in lifelong protection against the infecting serotype, there is only temporary cross-protection to other serotypes. Waning of cross-neutralizing antibodies later renders an individual more susceptible to severe dengue in the event of infection with a serotype different to the previous infection.[53–57] There is currently only one licensed dengue vaccine, CYD-TDV, which has been registered for use in endemic areas in individuals aged between 9 and 45 years. CYD-TDV is a tetravalent vaccine which includes four live, recombinant chimeric viruses with the prM (membrane) and E (envelope) proteins from each dengue virus serotype inserted separately into the yellow fever 17D genome. The vaccine has been evaluated in clinical trials in Asia and South America in over 30,000 participants, and had an overall efficacy of 57–61%,[58,59] inducing antibody to each serotype. Efficacy varied depending on serotype, with highest efficacy against serotypes 3 (74–78%) and 4 (75–78%), and lowest against serotype 2 (35–42%). A number of explanations for the differing serotype-specific efficacies have been suggested, including different exposure doses with different serotypes, differing levels of antibodies needed for protection against different serotypes, a difference in the pathogenesis of serotype 2 (such as more rapid infection of monocytes or poor replication) or an alteration in the conformation of the envelope protein in the chimeric vaccine for serotype 2.[60] The major safety concern was a finding that children aged 2–5 years had an increased risk of hospitalization when more than two years post-vaccination, which may

be due to an antibody enhancement effect similar to that seen with wild type infections with different serotypes. Vaccination is recommended by the SAGE committee of WHO beginning at 9 years of age and only in those countries where the population is likely to have been infected by one or more dengue serotypes by that age. Future studies will clarify if there is any danger to seronegatives, particularly children.

Understanding host immunity: Human cytomegalovirus vaccines

Human cytomegalovirus (CMV) is a DNA Herpesvirus. The prevalence of seropositivity in the adult population is high and the virus is responsible for lifelong latent infections. Despite high seropositivity, CMV congenital infections due to primary infection or reinfection are a reality and no effective preventive strategies are currently available. In the infected population, reactivation normally occurs in the immunocompromised host and is associated with severe outcomes.[61]

In whole organ or stem cell transplant recipients, CMV infection is considered one of the most common with associated acute infections and long term indirect effects, that could contribute to allograft injury and subsequent graft loss.[62] The implementation of a CMV vaccine could decrease the disease burden with a significant economic impact.[63]

Several vaccine candidates are under development. One of the first CMV vaccines, widely tested in humans, particularly in renal transplant patients, was developed by Stanley Plotkin's laboratory, one of the authors of this paper.[64] It contained the Towne strain, and reduced severe disease in seronegative renal transplant patients, although the results were not considered significant for infection.[65] However, the vaccine was not successful in protecting seronegative mothers from acquiring infection from their young children.[66] More recently sub-unit vaccines have been developed. Glycoprotein B (gB) antibodies are specific and present in all CMV seropositive individuals.[67] They are currently used in new vaccine design and include the gB/MF59 adjuvant, gB/Canarypox vector and DNA vaccines gB/pp65/IE1 Trivalent DNA vaccine and gB/pp65 Bivalent DNA vaccine.[68,69] Other CMV vaccine candidates include: replication-defective CMV strains, soluble pentamer proteins, replication-defective adenovirus vectors, alphavirus replicons, dense bodies and self-replicating RNA vaccines.[70]

The importance of structural biology: Respiratory syncytial virus vaccines

Respiratory syncytial virus (RSV) is the most important cause of infant hospitalisation in developed countries and globally is responsible for the most deaths in infants after malaria, clearly there is an urgent need for effective vaccine development.[71] In the 1960s, formalin-inactivated RSV (FI-RSV) vaccines did not protect from primary RSV infection, and paradoxically resulted in more severe clinical disease, in particular in young vaccinated children.[72,73]

This FI-RSV vaccine failed due to alterations in the fusion (F) protein during formalin treatment that resulted in an imbalanced host response with immune complex deposition and Th2 skewing.[74] The F glycoprotein remains the main antigenic target for RSV vaccine development, due to its role in host cell viral entry, conservation across both A and B subtype and the ability of anti-F passive monoclonal antibodies (palivizumab) to prevent severe disease in RSV naïve infants.[75] In human sera, the neutralising activity of Pre-F (metastable) epitopes is much greater than Post-F (stable), requiring an intricate understanding of the atomic-level protein structure in order to stabilise this for conversion into vaccine candidates. Using Pre-F-specific monoclonal antibodies (D25, 5C4, AM22) to bind to Pre-F, the crystal structure was visualised, identifying novel antigenic sites not available on Post-F due to conformational changes occurring on membrane fusion to allow viral entry (Figure 1).[76] Optimal stabilisation of Pre-F was achieved by insertion of cysteine residues to form disulphide bonds and cavity filling mutations, resulting in a stable Pre-F DS-Cav1 which induced potent neutralising antibodies in mice and macaques.[76] These molecular approaches are rapidly being applied to synthetic vaccine development for the identification of suitable candidate antigens, and the understanding of the B cell repertoire[77] leading to structural antigen modifications.[78] The efficacy of current Pre-F vaccines in clinical trials is yet to be demonstrated.

Many RSV vaccines are in clinical trials, but due to the previous safety concerns and impact of passive maternal antibody on vaccine efficacy, direct protection of infants is not the priority. Alternative vaccine strategies involve immunisation of pregnant women to passively transfer neutralising antibodies to protect the infant up to 3–4 months of age, immunisation of older children to protect them and to disrupt transmission, and immunisation of the elderly to provide direct protection to this vulnerable group.[75] Other approaches showing promise in clinical trials include extended half-life monoclonal antibodies, directed against prefusion F proteins. These potentially provide passive immunity through one intramuscular dose per season, for healthy and high-risk infants in their first RSV season.[79]

Broader issues in vaccinology

Additional paths in the future of vaccinology

There are several aspects relating to vaccine design and understanding vaccine-induced immunity which will be important to investigate in the coming years. A number of new technologies are being developed in pre-clinical studies to improve future vaccines, such as DNA plasmids with or without electroporation,[80] self-amplifying mRNA molecules[81] and vaccines which are better able to activate dendritic cells. The non-specific effects of vaccines are far from being understood and may have consequences which reach beyond the field of vaccinology if vaccine antigens are able to shape the immune system in early life.[82]. The reduction in speed and cost of genetic sequencing means that it is will become easier to use genetic information from vaccinated individuals to better predict immunogenicity and reactogenicity of vaccines, which will aid future vaccine design. Persistence of vaccine-induced protection remains an issue, with almost all vaccines requiring multiple doses.

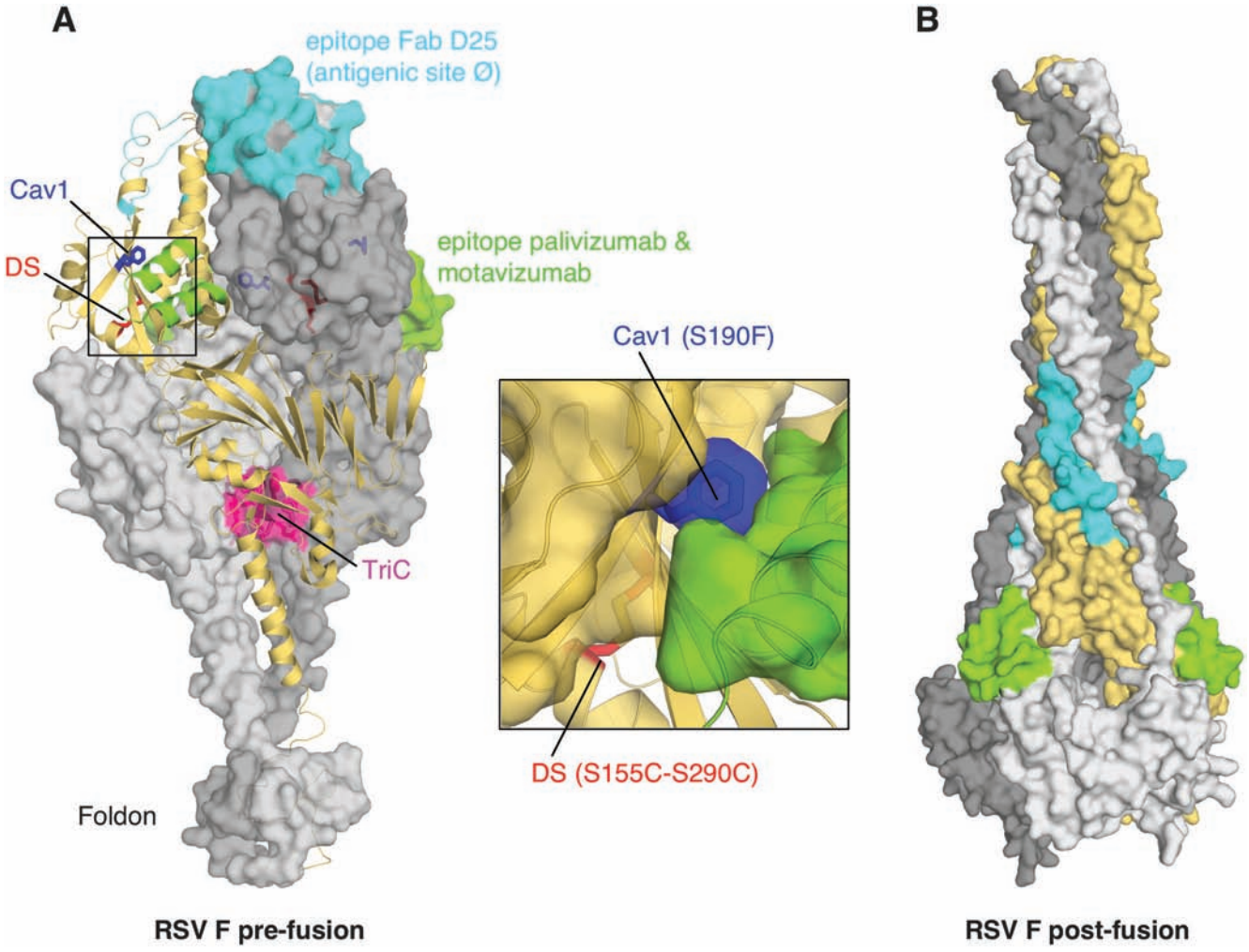

Figure 1 A. Pre-fusion respiratory syncytial virus (RSV) F protein stabilised at three sites with insertion of cysteine residues to form disulphide bonds and cavity filling mutations (coloured in blue, green and pink). The regions of binding to monoclonal antibodies D25, palivizumab and motavizumab are coloured cyan and green respectively. The central panel shows the DS-Cav1 cavity filling mutation. B. Post-fusion F protein showing the monoclonal antibody binding sites. The trimeric structure of the protein is demonstrated in both images by light grey, dark grey and yellow. Figure created by E. Malito, reproduced unchanged under CC BY 4.0.[84]

This may be improved in future by better understanding the role of follicular T cells in induction of protective responses, with IL-7 possibly having a role in improving persistence of protection.[83] Most pathogens have multiple virulence antigens which could be included in vaccines, and understanding the natural immune response to these, and the difference in responses between individuals during infection will aid antigen selection. Identification of conserved epitopes may help design vaccines with 'universal' protection against a pathogen, although this has been difficult to achieve to date. Some vaccines still do not have a defined correlate of protection, and newer systems biology approaches may be able to characterize these, which are likely to be complex and dependent on a number of factors rather than a single cut-off value in an antibody assay. Better adjuvants are required to improve responses in immature immune systems (such as infants and the immunocompromised), specifically stimulate selective responses (such as Th1, Th2, Th17) and generate T cell immunity in the absence of vaccine replication.

Societal challenges

Financial constraints on pharmaceutical companies may result in reduced vaccine development and production. With national public health bodies not stipulating their priorities to industry developers, combined with the time frame for conception to licensure, there is an inevitable delay and lack of cohesion. There is a further lack of enthusiasm and economic incentive for the investment into vaccines for emerging diseases such as flaviviruses (Zika, West Nile), haemorrhagic fevers (Ebola, Marburg, Lassa) or coronaviruses (MERS, SARS). However, the expansion of the Developing Countries Vaccine Manufacturing Network to encourage sustainable and affordable vaccine production and the recent changes in patent protection for drug manufacture in developing regions may help to ameliorate some of these issues.

Collective responsibility in response to recent large-scale global epidemics, led to the establishment of the Coalition for Epidemic Preparation and Innovation in January 2016.

This multi-faceted partnership involving government, industry, civil bodies and foundations will focus on vaccine development for potential epidemic infections. The challenges lie in prioritising infections, organising the development and manufacturing processes and obtaining sustainable funding arrangements from governments and foundations.

Conclusion

For the host, vaccinations must be safe, with minimal side effects, must provoke immune responses with specificity and memory to induce long-lasting protection which can ideally be quantified. The context in which the vaccinee resides may impact on the ability to respond to the vaccine antigens because of demographic or environmental conditions. Vaccine antigens should be able to capture the diversity of the pathogenic organism, have well-defined structures and methods of enhancing recognition by the host and be ultimately easily reproducible for mass production. Host-pathogen interactions at the molecular and cellular level will play an important role in the development of future vaccines. We continue to aspire to developing vaccines with excellent immunogenicity in the extremes of age, to protect against the diverse array of pathogenic organisms, and which ultimately should be available to all populations.

Conflicts of interest

CMCR and MVP have no conflicts of interest. MS has been an investigator on grants awarded from Pfizer, but has received no personal payment. SP is a paid consultant to most major vaccine manufacturers and to a number of biotechnology companies working on vaccine development.

References

1. Baxby D. Edward Jenner's inquiry after 200 years. *BMJ* 1999;**318**(7180):390.
2. Giuliani MM, Adu-Bobie J, Comanducci M, Arico B, Savino S, Santini L, et al. A universal vaccine for serogroup B meningococcus. *Proc Natl Acad Sci U S A* 2006;**103**(29):10834–9.
3. Pica N, Palese P. Toward a Universal Influenza Virus Vaccine: Prospects and Challenges. *Annu Rev Med* 2013;**64**(1):189–202.
4. Manzoli L, Ioannidis JPA, Flacco ME, De Vito C, Villari P. Effectiveness and harms of seasonal and pandemic influenza vaccines in children, adults and elderly. *Hum Vaccin Immunother* 2012;**8**(7):851–62.
5. DiazGranados CA, Denis M, Plotkin S. Seasonal influenza vaccine efficacy and its determinants in children and non-elderly adults: A systematic review with meta-analyses of controlled trials. *Vaccine* 2012;**31**(1):49–57.
6. Schotsaert M, García-Sastre A. Influenza Vaccines: A Moving Interdisciplinary Field. *Viruses* 2014;**6**(10):3809–26.
7. Hensley SE, Das SR, Bailey AL, Schmidt LM, Hickman HD, Jayaraman A, et al. Hemagglutinin Receptor Binding Avidity Drives Influenza A Virus Antigenic Drift. *Science* 2009;326(5953):734–6.
8. McElhaney JE, Coler RN, Baldwin SL. Immunologic correlates of protection and potential role for adjuvants to improve influenza vaccines in older adults. *Expert Rev Vaccines* 2013;**12**(7):759–66.
9. Ambrose CS, Levin MJ, Belshe RB. The relative efficacy of trivalent live attenuated and inactivated influenza vaccines in children and adults. *Influenza Other Respir Viruses* 2010;**5**(2):67–75.
10. Novartis Vaccines and Diagnostics Inc. FLUAD® (Influenza Vaccine, Surface Antigen, Inactivated, Adjuvanted with MF59C.1), Product Monograph. 2014.
11. National Advisory Committee on Immunization. Canadian Immunization Guide Chapter on Influenza and Statement on Seasonal Influenza Vaccine for 2015–2016. Canada: Public Health Agency of Canada, 2015.
12. Zedda L, Forleo-Neto E, Vertruyen A, Raes M, Marchant A, Jansen W, et al. Dissecting the Immune Response to MF59-adjuvanted and Nonadjuvanted Seasonal Influenza Vaccines in Children Less Than Three Years of Age. *Pediatr Infect Dis J;* 2015;**34**(1):73–8.
13. Frey SE, Reyes MRA-DL, Reynales H, Bermal NN, Nicolay U, Narasimhan V, et al. Comparison of the safety and immunogenicity of an MF59®-adjuvanted with a non-adjuvanted seasonal influenza vaccine in elderly subjects. *Vaccine* 2014;**32**(39):5027–34.
14. Plotkin SA. Increasing Complexity of Vaccine Development. *J Infect Dis* 2015;**212**(suppl 1):S12–S6.
15. World Health Organisation. Pertussis vaccines: WHO position paper. *Wkly Epidemiol Rec* 2010;**40**(85):385–400.
16. Kmietowicz Z. Pertussis cases rise 10-fold among older children and adults in England and Wales. *BMJ* 2012;**345**(jul23 1):e5008–e.
17. de Greeff SC, de Melker HE, van Gageldonk PGM, Schellekens JFP, van der Klis FRM, Mollema L, et al. Seroprevalence of Pertussis in the Netherlands: Evidence for Increased Circulation of Bordetella pertussis. *PLoS One* 2010;**5**(12):e14183.
18. Lee Grace M, Lett S, Schauer S, LeBaron C, Murphy Trudy V, Rusinak D, et al. Societal Costs and Morbidity of Pertussis in Adolescents and Adults. *Clin Infect Dis* 2004;**39**(11):1572–80.
19. Koepke R, Eickhoff JC, Ayele RA, Petit AB, Schauer SL, Hopfensperger DJ, et al. Estimating the Effectiveness of Tetanus-Diphtheria-Acellular Pertussis Vaccine (Tdap) for Preventing Pertussis: Evidence of Rapidly Waning Immunity and Difference in Effectiveness by Tdap Brand. *J Infect Dis* 2014;**210**(6):942–53.
20. Allen AC, Mills KHG. Improved pertussis vaccines based on adjuvants that induce cell-mediated immunity. *Expert Rev Vaccines* 2014;**13**(10):1253–64.
21. Warfel JM, Merkel TJ. The baboon model of pertussis: effective use and lessons for pertussis vaccines. *Expert Rev Vaccines* 2014;**13**(10):1241–52.
22. Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. *Proc Natl Acad Sci U S A* 2014;**111**(2):787–92.
23. Rathore JS, Wang Y. Protective role of Th17 cells in pulmonary infection. *Vaccine* 2016;**34**(13):1504–14.
24. Lam C, Octavia S, Bahrame Z, Sintchenko V, Gilbert GL, Lan R. Selection and emergence of pertussis toxin promoter ptxP3 allele in the evolution of Bordetella pertussis. Infect Genet Evol 2012;**12**(2):492–5.
25. Sealey KL, Harris SR, Fry NK, Hurst LD, Gorringe AR, Parkhill J, et al. Genomic Analysis of Isolates From the United Kingdom 2012 Pertussis Outbreak Reveals That Vaccine Antigen Genes Are Unusually Fast Evolving. *J Infect Dis* 2014;**212**(2):294–301.
26. Sebo P, Osicka R, Masin J. Adenylate cyclase toxin-hemolysin relevance for pertussis vaccines. *Expert Rev Vaccines* 2014;**13**(10):1215–27.
27. Fedele G, Bianco M, Ausiello CM. The Virulence Factors of Bordetella pertussis: Talented Modulators of Host Immune Response. *Arch Immunol Ther Exp* 2013;**61**(6):445–57.
28. Thorstensson R, Trollfors B, Al-Tawil N, Jahnmatz M, Bergström J, Ljungman M, et al. A Phase I Clinical Study of a Live Attenuated Bordetella pertussis Vaccine – BPZE1; A Single

Centre, Double-Blind, Placebo-Controlled, Dose-Escalating Study of BPZE1 Given Intranasally to Healthy Adult Male Volunteers. *PLoS One* 2014;**9**(1):e83449.
29. Li Q-t, Zhu Y-z, Chu J-y, Dong K, He P, Feng C-y, et al. Granulocyte-macrophage colony-stimulating factor DNA prime-protein boost strategy to enhance efficacy of a recombinant pertussis DNA vaccine. *Acta Pharmacol Sin* 2006;**27**(11):1487–94.
30. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet* 2014;**384**(9939):258–71.
31. Lihana RW, Ssemwanga D, Abimiku A, Ndembi N. Update on HIV-1 diversity in Africa: a decade in review. *AIDS Rev* 2012;**14**(2):83–100.
32. Kim JH, Excler JL, Michael NL. Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. *Annu Rev Med* 2015;**66**:423–37.
33. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* 2009;**361**(23):2209–20.
34. Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, Williams WT, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci Transl Med* 2014;**6**(228):228ra39.
35. Excler JL, Ake J, Robb ML, Kim JH, Plotkin SA. Nonneutralizing functional antibodies: a new "old" paradigm for HIV vaccines. *Clin Vaccine Immunol* 2014;**21**(8):1023–36.
36. Jegaskanda S, Weinfurter JT, Friedrich TC, Kent SJ. Antibody-Dependent Cellular Cytotoxicity Is Associated with Control of Pandemic H1N1 Influenza Virus Infection of Macaques. *J Virol* 2013;**87**(10):5512–22.
37. Jegaskanda S, Luke C, Hickman HD, Sangster MY, Wieland-Alter WF, McBride JM, et al. Generation and Protective Ability of Influenza Virus-Specific Antibody-Dependent Cellular Cytotoxicity in Humans Elicited by Vaccination, Natural Infection, and Experimental Challenge. *J Infect Dis* 2016;**214**(6):945–52.
38. Hansen SG, Piatak M, Jr., Ventura AB, Hughes CM, Gilbride RM, Ford JC, et al. Immune clearance of highly pathogenic SIV infection. *Nature* 2013;**502**(7469):100–4.
39. Dennehy PH. Rotavirus vaccines: an overview. *Clin Microbiol Rev* 2008;**21**(1):198–208.
40. Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. *N Engl J Med* 1983;**309**(2):72–6.
41. Fischer TK, Valentiner-Branth P, Steinsland H, Perch M, Santos G, Aaby P, et al. Protective immunity after natural rotavirus infection: a community cohort study of newborn children in Guinea-Bissau, west Africa. *J Infect Dis* 2002;**186**(5):593–7.
42. Velazquez FR, Matson DO, Calva JJ, Guerrero L, Morrow AL, Carter-Campbell S, et al. Rotavirus infections in infants as protection against subsequent infections. *N Engl J Med* 1996;**335**(14):1022–8.
43. Rha B, Tate JE, Payne DC, Cortese MM, Lopman BA, Curns AT, et al. Effectiveness and impact of rotavirus vaccines in the United States – 2006–2012. *Expert Rev Vaccines* 2014;**13**(3):365–76.
44. Cortes JE, Curns AT, Tate JE, Cortese MM, Patel MM, Zhou F, et al. Rotavirus vaccine and health care utilization for diarrhea in U.S. children. *N Engl J Med* 2011;**365**(12):1108–17.
45. Giaquinto C, Dominiak-Felden G, Van Damme P, Myint TT, Maldonado YA, Spoulou V, et al. Summary of effectiveness and impact of rotavirus vaccination with the oral pentavalent rotavirus vaccine: a systematic review of the experience in industrialized countries. *Hum Vaccin* 2011;**7**(7):734–48.
46. World Health Organisation. Rotavirus vaccines: an update. Weekly Epidemiological Record. 2009;**84**:553–40.
47. Babji S, Kang G. Rotavirus vaccination in developing countries. *Curr Opin Virol* 2012;**2**(4):443–8.
48. Cooper PJ, Chico ME, Losonsky G, Sandoval C, Espinel I, Sridhara R, et al. Albendazole treatment of children with ascariasis enhances the vibriocidal antibody response to the live attenuated oral cholera vaccine CVD 103-HgR. *J Infect Dis* 2000;**182**(4):1199–206.
49. Uchiyama R, Chassaing B, Zhang B, Gewirtz AT. Antibiotic treatment suppresses rotavirus infection and enhances specific humoral immunity. *J Infect Dis* 2014;**210**(2):171–82.
50. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, et al. Intestinal microbiota promote enteric virus replication and systemic pathogenesis. *Science* 2011;**334**(6053):249–52.
51. Rodrigo WW, Block OK, Lane C, Sukupolvi-Petty S, Goncalvez AP, Johnson S, et al. Dengue virus neutralization is modulated by IgG antibody subclass and Fcgamma receptor subtype. *Virology* 2009;**394**(2):175–82.
52. Wu RS, Chan KR, Tan HC, Chow A, Allen JC, Jr., Ooi EE. Neutralization of dengue virus in the presence of Fc receptor-mediated phagocytosis distinguishes serotype-specific from cross-neutralizing antibodies. *Antiviral Res* 2012;**96**(3):340–3.
53. Montoya M, Gresh L, Mercado JC, Williams KL, Vargas MJ, Gutierrez G, et al. Symptomatic versus inapparent outcome in repeat dengue virus infections is influenced by the time interval between infections and study year. *PLoS Negl Trop Dis* 2013;**7**(8):e2357.
54. Reich NG, Shrestha S, King AA, Rohani P, Lessler J, Kalayanarooj S, et al. Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. *J R Soc Interface* 2013;**10**(86):20130414.
55. Endy TP, Yoon IK, Mammen MP. Prospective cohort studies of dengue viral transmission and severity of disease. *Curr Top Microbiol Immunol* 2010;**338**:1–13.
56. Graham RR, Juffrie M, Tan R, Hayes CG, Laksono I, Ma'roef C, et al. A prospective seroepidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia I. studies in 1995–1996. *Am J Trop Med Hyg* 1999;**61**(3):412–9.
57. Thein S, Aung MM, Shwe TN, Aye M, Zaw A, Aye K, et al. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg* 1997;**56**(5):566–72.
58. Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 2014;**384**(9951):1358–65.
59. Villar L, Dayan GH, Arredondo-Garcia JL, Rivera DM, Cunha R, Deseda C, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med* 2015;**372**(2):113–23.
60. de Alwis R, Bangs DJ, Angelo MA, Cerpas C, Fernando A, Sidney J, et al. Immunodominant Dengue Virus-Specific CD8+ T Cell Responses Are Associated with a Memory PD-1+ Phenotype. *J Virol* 2016;**90**(9):4771–9.
61. Navarro D. Expanding role of cytomegalovirus as a human pathogen. *J Med Virol* 2016;**88**(7):1103–12.
62. Baron C, Forconi C, Lebranchu Y. Revisiting the effects of CMV on long-term transplant outcome. *Curr Opin Organ Transplant* 2010;**15**(4):492–8.
63. Modlin JF, Arvin AM, Fast P, Myers M, Plotkin S, Rabinovich R. Vaccine Development to Prevent Cytomegalovirus Disease: Report from the National Vaccine Advisory Committee. *Clin Infect Dis* 2004;**39**(2):233–9.
64. Plotkin S, Friedman H, Fleisher G, Dafoe D, Grossman R, Lynn Smiley M, et al. Towne-vaccine-induced prevention of cytomegalovirus disease after renal transplants. *Lancet* 1984;**323**(8376):528–30.
65. Plotkin SA, Higgins R, Kurtz JB, Morris PJ, Campbell DA, Shope TC, et al. Multicenter trial of towne strain attenuated

virus vaccine in seronegative renal transplant recipients. *Transplantation* 1994;**58**(11):1176–8.
66. Adler SP, Starr SE, Plotkin SA, Hempfling SH, Buis J, Manning ML, et al. Immunity Induced By Primary Human Cytomegalovirus Infection Protects Against Secondary Infection Among Women Of Childbearing Age. *J Infect Dis* 1995;**171**(1):26–32.
67. Kirchmeier M, Fluckiger AC, Soare C, Bozic J, Ontsouka B, Ahmed T, et al. Enveloped Virus-Like Particle Expression of Human Cytomegalovirus Glycoprotein B Antigen Induces Antibodies with Potent and Broad Neutralizing Activity. *Clin Vaccine Immunol* 2013;**21**(2):174–80.
68. Griffiths P, Plotkin S, Mocarski E, Pass R, Schleiss M, Krause P, et al. Desirability and feasibility of a vaccine against cytomegalovirus. *Vaccine* 2013;**31**:B197–B203.
69. Schleiss MR. Cytomegalovirus Vaccine Development. Current Topics in Microbiology and Immunology: Springer Science + Business Media; 2008. p. 361–82.
70. Plotkin S. The history of vaccination against cytomegalovirus. *Med Microbiol Immunol* 2015;**204**(3):247–54.
71. Drysdale SB, Sande CJ, Green CA, Pollard AJ. RSV vaccine use – the missing data. *Expert Rev Vaccines* 2016;**15**(2):149–52.
72. Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J Epidemiol* 1969;**89**(4):405–21.
73. Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K, et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 1969;**89**(4):422–34.
74. Polack FP, Teng MN, Collins PL, Prince GA, Exner M, Regele H, et al. A role for immune complexes in enhanced respiratory syncytial virus disease. *J Exp Med* 2002;**196**(6):859–65.
75. Graham BS, Modjarrad K, McLellan JS. Novel antigens for RSV vaccines. *Curr Opin Immunol* 2015;**35**:30–8.
76. McLellan JS, Chen M, Leung S, Graepel KW, Du X, Yang Y, et al. Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. *Science* 2013;**340**(6136):1113–7.
77. Rappuoli R, Bottomley MJ, D'Oro U, Finco O, De Gregorio E. Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design. *J Exp Med* 2016;**213**(4):469–81.
78. Jones LH. Recent advances in the molecular design of synthetic vaccines. *Nat Chem* 2015;**7**(12):952–60.
79. Mejias A, Garcia-Maurino C, Rodriguez-Fernandez R, Peeples ME, Ramilo O. Development and clinical applications of novel antibodies for prevention and treatment of respiratory syncytial virus infection. *Vaccine* 2017;**35**(3):496–502.
80. Tregoning JS, Kinnear E. Using Plasmids as DNA Vaccines for Infectious Diseases. *Microbiol Spectr* 2014;**2**(6).
81. Ulmer JB, Geall AJ. Recent innovations in mRNA vaccines. *Curr Opin Immunol* 2016;**41**:18–22.
82. Goodridge HS, Ahmed SS, Curtis N, Kollmann TR, Levy O, Netea MG, et al. Harnessing the beneficial heterologous effects of vaccination. *Nat Rev Immunol* 2016;**16**(6):392–400.
83. Kang MC, Choi DH, Choi YW, Park SJ, Namkoong H, Park KS, et al. Intranasal Introduction of Fc-Fused Interleukin-7 Provides Long-Lasting Prophylaxis against Lethal Influenza Virus Infection. *J Virol* 2016;**90**(5):2273–84.
84. Malito E, Carfi A, Bottomley MJ. Protein Crystallography in Vaccine Research and Development. *Int J Mol Sci* 2015;**16**(6):13106–40.