Oral mRNA Vaccines Against Infectious Diseases- A Bacterial Perspective [Invited]

Vijayakumar Jawalagatti†‡, Perumalraja Kirthika†‡ and John Hwa Lee*

Department of Veterinary Public Health, College of Veterinary Medicine, Jeonbuk National University, Iksan, South Korea

The mRNA vaccines from Pfizer/BioNTech and Moderna were granted emergency approval in record time in the history of vaccinology and played an instrumental role in limiting the pandemic caused by SARS-CoV-2. The success of these vaccines resulted from over 3 decades of research from many scientists. However, the development of orally administrable mRNA vaccine development is surprisingly underexplored. Our group specializing in Salmonella-based vaccines explored the possibility of oral mRNA vaccine development. Oral delivery was made possible by the exploitation of the Semliki Forest viral replicon and Salmonella vehicle for transgene amplification and gene delivery, respectively. Herein we highlight the prospect of developing oral replicon-based mRNA vaccines against infectious diseases based on our recent primary studies on SARS-CoV-2. Further, we discuss the potential advantages and limitations of bacterial gene delivery.

Keywords: bacterial delivery, alphaviral replicon, mRNA vaccine, oral, mucosal vaccine, SARS-CoV-2, infectious diseases

INTRODUCTION

Edward Jenner’s innovative contribution played a pivotal role in the ultimate eradication of smallpox and served as the harbinger of vaccination. This was followed by the works of Louis Pasteur, who spearheaded the development of live-attenuated cholera vaccine and inactivated anthrax vaccine in humans in 1897 and 1904, respectively. The field of vaccine research soon became popular, and vaccines were developed against a plethora of infectious diseases of medical and veterinary importance. First-generation traditional vaccines based on the use of live, live-attenuated, and inactivated organisms were instrumental in the control of measles, polio, rubella, mumps, classical swine fever, and many other diseases, and responsible for the eradication of smallpox in humans and rinderpest in cattle. Although live and live-attenuated vaccines are effective, they may pose significant health risks to vaccinated individuals, including the development of disease, transmission to healthy individuals, and reversion to a virulent form in individuals with compromised immune system (1–5). All this changed with the advent of molecular biology and recombinant DNA technology, which paved the way for the development of safer vaccines. However, DNA vaccines did not achieve their expected clinical success owing to limited or poor immunogenicity (6, 7). Technological refinements were made to improve DNA vaccine efficacy (8–15), but the risk of mutagenesis induced by exogenous DNA integration has limited their use in humans (16–19). This has led to a renewed interest in the use of RNA in vaccines and therapeutics.
Synthetic RNA vaccines fall into two main categories: non-replicating and self-amplifying mRNA vaccines. The non-replicating mRNA vaccine is a straightforward approach wherein administered mRNA is directly translated in the cytoplasm of transfected cells to produce immunogenic proteins. The extent of non-replicating mRNA vaccine-induced antigen expression is proportional to the number of transfected cells and thus, requires the injection of a large dose of mRNA. This can be overcome by the use of self-amplifying RNA replicons from alphaviruses, such as Sindbis virus (20), Semliki Forest virus (SFV) (21), and Venezuelan equine encephalitis virus (VEE) (22). Different vector systems, namely replication-competent viral particles, replication-deficient viral particles, and DNA-launched-mRNA vector approaches, have been exploited for transgene expression (reviewed in 23, 24). DNA-launched-mRNA vectors were engineered by deleting the structural genes from the genome and replacing them with the target genes (21, 25). The resulting vector backbone with non-structural proteins (nsp1–4) forms a replicase complex that drives efficient transgene expression by a self-amplifying mechanism (21, 24). The mRNA vaccines developed to combat SARS-CoV-2 constitute the first success story in the long history of mRNA vaccine development. Nonetheless, oral delivery of an mRNA vaccine has surprisingly not been exploited. In this article, we highlight a strategy for the development of oral replicon-based mRNA vaccines by taking cues from our recent publications and discussing the advantages of Salmonella-mediated oral gene delivery.

mRNA VACCINES: A BRIEF HISTORICAL BACKGROUND

The vaccines developed against SARS-CoV-2 by Pfizer/BioNTech and Moderna constitute the first success stories in mRNA vaccine history. Although the delivery of mRNA wrapped in cationic liposomes was shown to produce proteins in human cells in 1989 (26), the potential of mRNA as a vaccine has yet to be exploited. During these past 3 decades, many scientists studied mRNA, and collective scientific advances enabled the production of the first successful mRNA vaccine in record time (Figure 1A). Some of the most important inventions to the adaptation of mRNA vaccination were the chemical modification of mRNA and lipid nanoparticles for delivery. Without lipid nanoparticle encapsulation, administered mRNA would be detected by the immune system and probably degraded by RNases. Of note, mRNA was shown to elicit TLR3-mediated immune activation of dendritic cells (DCs) (30), and bacterial RNA can prime DCs for higher IL-12 secretion (31). Replacing
uridine with pseudouridine, the chemical modification that diminished immune recognition of administered mRNA, paved the way for mRNA treatments (32). The encapsulation of mRNA by lipid nanoparticles (LNPs) provided an effective and safe delivery platform (reviewed in 33). The discovery of increased protein expression and potent antibody responses to the SARS-CoV-2 spike protein in its stabilized prefusion conformation (34) is vital to the efficacy of mRNA vaccines. The developments and progress in mRNA vaccines against infectious diseases have been reviewed elsewhere (35, 36).

**mRNA DELIVERY TECHNOLOGIES: PROGRESS AND LIMITATIONS**

The poor uptake of mRNA by cells is associated with the rapid degradation of naked mRNA by extracellular RNases (37–41). Developments of efficient mRNA delivery platforms have been fruitful in the last decade. From advancements in transfection reagents and liposomes to nanoparticles and nanoemulsions, in vivo antigen presentation and the immune response to mRNA-based vaccines have recently improved (42–51). The aforementioned mRNA complexing strategies have been shown to affect mRNA stability during storage (52). Thus, a continuous supply of raw materials is crucial for the uninterrupted production of mRNA vaccines. Such requirements can prove challenging at times when the demand is high (52–57). Additionally, substituting rare codons with frequently used synonymous codons and introducing modified nucleosides have been shown to enhance mRNA translation and stability in the context of vaccination (reviewed in 38). A major disadvantage associated with base modifications is that they may result in altered mRNA secondary structure, which may influence translation and protein folding. These alterations may, in turn, prove detrimental to efficacy (58–60). One of the major drawbacks with in vitro transcribed RNA is the presence of dsRNAs that trigger the innate immune response and reduces the vaccine efficacy. Advancement such as cellulose-based purification was shown to remove the dsRNA byproducts leading to the lower type I interferon response and improving the efficacy of a self-amplifying mRNA vaccine against Zika virus (61). Continuous efforts have been made to minimize the drawbacks associated with mRNA vaccines, enabling an array of these vaccines to enter phase IIb clinical trials (38, 62–67). Most of the current mRNA vaccines against infectious disease are administered using the conventional delivery routes, namely intramuscular, subcutaneous, intradermal, or intranodal routes. Most of these routes of administration require injection and specific conditions for storage and transport. Furthermore, the concerns associated with the stability of these vaccines and the addition of adjuvants to enhance immunogenicity increase the cost of production and pose toxicity threats (68, 69). Despite the success of mRNA vaccines in controlling infectious diseases, the limitations associated with their production and administration demonstrate the need to develop better and safer routes of administration for mRNA vaccines (70, 71).

**IS IT POSSIBLE TO ORALLY DELIVER mRNA VACCINES?**

Despite three decades of history supporting mRNA vaccine development and the successful rollout of mRNA vaccines during the COVID-19 pandemic, the possibility of oral delivery has surprisingly been underexplored (71). This could be attributed to the highly unstable nature of mRNA and the gut posing a significant barrier for mRNA delivery. However, some of the oral antigen delivery strategies such as yeast ghosts, microencapsulated antigens and microbial adhesions have been developed to overcome the harsh conditions in the gut (reviewed in 72). But they suffer from major limitation of poor intestinal epithelial barrier crossing and have not been explored to deliver mRNA (72). Further, lipid-based approaches such as liposomes, bilosomes and immunostimulating complexes (ISCOMs) also provide with a potential delivery vehicle for oral biologic delivery (reviewed in 71). The oral delivery of mRNA vaccines is possible due to the exploitation of an alphaviral replicon and Salmonella bactofection for mRNA amplification and gene delivery, respectively. Our group specialized in the development of Salmonella-based vaccines against diseases of veterinary and medical importance (73–79), exploited this platform for the development of an oral mRNA vaccine. Further, we exploited the Semiliki Forest virus replicon for mRNA amplification (23, 24). We made several modifications to the original vector backbone (pSFV3) to enable transcription in host cells and plasmid maintenance in bacteria (Figure 1B) (25). The SP6 promoter was replaced with the Cytomegalovirus (CMV) promoter to enable transcription by mammalian RNA polymerase. The replacement of the ampicillin selection marker with the aspartate-semialdehyde dehydrogenase (asd) auxotrophic marker allows for antibiotic-free plasmid maintenance and delivery (80). The Salmonella strains used for gene delivery carry a deletion in the asd gene, creating balanced-lethal host-vector systems. Diaminopimelic acid (DAP), the product of asd, is a vital component of the bacterial cell wall, and asd mutants will not survive unless DAP is supplemented in growth media or the asd gene is complemented from a plasmid vector. Thus, asd serves as a powerful antibiotic-independent selection maker for bacterial delivery. This DNA-launched-mRNA vector design was exploited for the Salmonella-enabled oral delivery of a replicon-based mRNA vaccine against SARS-CoV-2 (25, 81, 82). The detailed mechanism of vector delivery, transgene amplification, and the generation of an immune response upon oral administration of Salmonella carrying the SFV replicon vector encoding vaccine immunogens is furnished in Figure 2. The findings demonstrate the possibility and potential of bacteria-mediated gene delivery for the development of oral replicon-based mRNA vaccines against infectious diseases.

**ADVANTAGES AND LIMITATIONS OF Salmonella-MEDIATED ORAL GENE DELIVERY**

The delivery of vaccines through the oral route can elicit a potent mucosal response considering the extensive presence of
gut-associated lymphoid tissues (GALT). The bacterial species, *Salmonella* has the ability to interact with immune cells in Payer's patch, leading to efficient induction of the mucosal response (83, 84). Mucosal vaccines play a pivotal role in limiting infections caused by digestive and respiratory pathogens. Moreover, gut bacteria can influence SIgA production in the lungs through CD103+ DCs (85). In agreement, we and others have documented the elicitation of mucosal response in respiratory sites by oral *Salmonella*-based vaccine administration (82, 86). Further, *Salmonella* can translocate through M cells in the intestine and reach organs such as the liver and spleen, eliciting a systemic response as well.
(87–89). One of the most important advantages of *Salmonella* is its innate ability to invade and proliferate in professional antigen-presenting cells (APCs), such as dendritic cells (DCs) (90) and macrophages (91), during which it directly delivers the DNA cargo to these cells. As antigens must be formed within the APC or cross-presented to an APC to elicit a cellular response (92), gene delivery and antigen expression within APCs result in robust cellular immunity along with the induction of a potent humoral response. Moreover, vaccine production can be easily scaled up, and a high number of doses can be prepared rapidly at an inexpensive rate. Importantly, bacteria-mediated vaccine delivery does not require additional adjuvants or delivery systems, which further cuts down the cost of manufacturing and limits the frequency of vaccine-associated adverse events (68, 69). Most important of all, the availability of licensed oral *Salmonella* Typhi vaccines provides the possibility of direct translation to humans. Further, the availability of a licensed live-attenuated *Vibrio cholerae* vaccine (Vaxchora; https://www.fda.gov/media/98688/download) provides with additional bacterial vector to develop vaccines against diseases of medical importance. The fact that *Salmonella* can be lyophilized permits a thermostable way to dispatch the vaccines and represents progress towards needle-free mass oral immunizations. Collectively, the data suggest the highly prospective nature of exploiting bacteria to develop oral mRNA vaccines with the ability to elicit potent systemic and mucosal immune responses. The intranasal delivery could also be exploited to develop potent mucosal mRNA vaccines. However, as the vaccine uses live-attenuated bacterium poses a significant safety and regulatory hurdle. The intranasal route is more suitable and safer for delivery of mRNA through polymeric delivery systems. The advantage of oral vaccine over an intranasal vaccine would be superior patient compliance and easy mass administration. Therefore, bacteria-mediated delivery of mRNA vaccines for mucosal vaccine development would be feasible when administered orally rather than intranasally.

One of the major limitations of live-attenuated bacteria is safety. However, the availability of tested and proven licensed vaccines provides safer delivery options. Furthermore, well-established tools to modify the bacterial genome provide an opportunity to create safer mutants (93). Another major limitation of using live-attenuated organisms for gene delivery is a hindrance from pre-existing immunity that can seriously affect vaccine efficacy (94, 95). Both SIgA and IgG could contribute to the pre-existing immunity against *Salmonella*. Nevertheless, this limitation could be overcome by deleting the O-antigen ligase (*rfai*) or any other gene(s) from the bacterial genome that mask the bacteria from detection by the immune system (77). However, several studies have shown the positive influence of pre-existing immunity and recorded stronger immune responses against the delivered antigen by *Salmonella* vectors (reviewed in 96). Thus, the effect of pre-existing immunity on heterologous antigen delivery is likely negligible or less variable. Of note, the effect of pre-existing immunity on viral vectors is more pronounced than on bacterial vectors (96).

### ORAL REPLICON-BASED mRNA VACCINE AGAINST SARS-COV-2

Our proof-of-principle studies using SARS-CoV-2 (25, 81, 82) provide evidence for the development of oral replicon-based mRNA vaccines against infectious diseases. *Salmonella* is an ideal bacterial vector owing to its unique ability to target GALT upon oral administration, resulting in both systemic and mucosal immune responses in vaccinated individuals. The possibility of oral delivery was partly enabled by creating a DNA-launched-mRNA design of the SFV replicon that essentially drives gene expression by a self-amplifying mRNA mechanism (25). Although the research on RNA vaccines and therapeutics spans over 3 decades, the possibility of oral mRNA vaccine delivery was yet to be explored. To the best of our knowledge, our studies are the first to demonstrate oral replicon-based mRNA vaccine delivery. To this end, we designed a multivalent SFV replicon-based vaccine targeting receptor-binding domain (RBD), heptad repeat domain (HR), membrane glycoprotein (M), and epitopes of nsp13 and employed *Salmonella* Typhimurium for gene delivery (25). The administration of the vaccine was highly safe in mice and hamsters inoculated both orally and intramuscularly (25, 82). The vaccine elicited potent Th1-dominated humoral and cellular immune responses in mice against all the target antigens, suggesting efficient antigen production and presentation (25, 82). Furthermore, RBD expressed after *Salmonella* delivery was confirmed to be antigenically intact in macrophage-like cells (82). We recorded the difference in mucosal immune response induction between oral and intramuscular routes of vaccine administration, highlighting the feasibility of exploiting oral administration for mucosal vaccine development (82). Most importantly, the vaccine protected hamsters against live SARS-CoV-2, and complete protection was elicited by oral immunization against viral replication and lung disease (82). Moreover, a robust cross-protection against the B.1.617.2 delta variant was evidenced following oral immunization in hamsters (82) and mice (81). The fact that an intranasal vaccine durably protected against SARS-CoV-2 variants (97, 98) and dimeric IgA had superior neutralizing activity (99) underscore the efficacy of the mucosal response exerted by oral vaccines in protection against rapidly replicating variants.

### CONCLUSIONS AND FUTURE DIRECTIONS

Our proof-of-principle studies have unraveled a novel method for the development of oral mRNA vaccines. The availability of some licensed live-attenuated bacterial vaccines increases the prospects of adopting such vaccines in the clinic. However, more studies using relevant bacterial species in suitable preclinical models are necessary to prove the hypothesis. Moreover, the possibility of other bacterial species, such as *Shigella*, could also be tested to optimize the choice of a bacterial vector.
AUTHOR CONTRIBUTIONS

VJ and PK: prepared the figures and wrote the article. JL: acquired funding and commented on the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Minor PD. Live Attenuated Vaccines: Historical Successes and Current Challenges. Virol (2015) 479:480-379–92. doi: 10.1016/j.virol.2015.03.032
2. Minor PD. The Polio-Eradication Programme and Issues of the End Game. J Gen Virol (2012) 93:457–74. doi: 10.1099/vir.0.036988-0
3. Marsden SA, Boulger LR, Magrath DJ, Reeve P, Schild GC, Taffs LF. Monkey Neurovirulence of Live, Attenuated (Sabin) Type 1 and Type II Poliovirus Vaccines. J Biol Stand (1980) 8:303–9. doi: 10.1016/S0092-1157(80)80008-4
4. Shaghati M, Parvaneh N, Ostad-Rahimi P, Fathi SM, Shahnahmooodi S, Abolhassani H, et al. Combined Immunodficiency Presenting With Vaccine-Associated Paralytic Poliomyelitis: A Case Report and Narrative Review of Literature. Immunol Invest (2014) 43:292–8. doi: 10.3109/08820139.2013.859156
5. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. IDSA Integrated Intramuscular Immunization. Hum Gene Ther (1999) 10:759–68. doi: 10.1089/104303499S018517
6. Nichols WW, Ledwith BJ, Magrath DI, Reeve P, Schild GC, Taffs LF. Monkey Neurovirulence of Live, Attenuated (Sabin) Type 1 and Type II Poliovirus Vaccines. J Biol Stand (1980) 8:303–9. doi: 10.1016/S0092-1157(80)80008-4
7. Cheung Y-K, Cheng SC-S, Sin FW-Y, Xie Y. Plasmid Encoding Subtype B Consensus-Based Envelope DNA Vaccine. Front Cell Infect IMMUNOL (2021) 13:411. doi: 10.3368/fcim.2021.00061
8. Vallianatou A, El-Shalakany A, El-Sayed M, Zayed L, Zayed M. Immunogenicity of Plasmid DNA Vaccine Delivered on Polyethylenimine (PEI) Functionalized PLGA Microparticles. j.jconrel.2006.04.006
9. Narum DL, Kumar S, Rogers WO, Fuhrmann SR, Liang H, Oakley M, et al. First Human Trial of a DNA-Based Vaccine for Treatment of Human Immunodeficiency Virus Type 1 Infection: Safety and Host Response. J Infect Dis (1998) 178:92–100. doi: 10.1086/515613
10. Yan J, Yoon H, Kumar S, Ramanathan MP, Troilo PJ, Wang X, Griffls TG, Pachioni SJ, Barhum AB, et al. Detection of Integration of Plasmid DNA Into Host Genomic DNA Following Intramuscular Injection and Electroporation. Gene Ther (2004) 11:711–21. doi: 10.1038/gt.3302213
11. Liu MA. DNA Vaccines: An Historical Perspective and View to the Future. Immunol Rev (2011) 239:622–8. doi: 10.1111/j.0909-9100.2010.00980.x
12. Xiong C, Levis R, Shen P, Schlesinger S, Rice CM, Huang HV. Sindbis Virus: An Efficient, Broad Host Range Vector for Gene Expression in Animal Cells. Science (80-) (1989) 243:1188–91. doi: 10.1126/science.2922607
13. Kaur R, Rauthan M, Vrati S. Immunogenicity in Mice of a Cationic Liposome-Mediated RNA Transfection. Proc Natl Acad Sci (1989) 86:6077–80. doi: 10.1073/pnas.86.16.6077
14. Kallid Y, Kikuchi K, Xu S, Weissman D, Cohen PA, Czerniecki BJ. Cutting Edge: Innate Immune System Discriminates Between RNA Containing Bacterial Versus Eukaryotic Structural Features That Prime for High-Level IL-12 Secretion by Dendritic Cells. J Immunol (2004) 172:3989–93. doi: 10.4049/jimmunol.172.7.3989
15. Kariko K, Buckstein M, Ni H, Weissman D. Suppression of RNA Recognition by Toll-Like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA. Immunity (2005) 23:165–75. doi: 10.1016/j.immuni.2005.06.008
16. Bushmann FD, Carrasco MJ, Alishetty S, Paige M, Alameh MG, Weissman D. Nanomaterial Delivery Systems for mRNA Vaccines. Vaccines (2021) 9:655. doi: 10.3390/vaccines906065
17. Pallesen J, Wang N, Corbett KS, Wrapp D, Kirchdoerfer RN, Turner HL, et al. Immunogenicity and Structures of a Rationally Designed Prefusion MERS-CoV Spike Antigen. Proc Natl Acad Sci (2017) 114:E7348–57. doi: 10.1073/pnas.1707301114
18. Bloom K, van den Berg F, Arbutnott P. Self-Amplifying RNA Vaccines for Infectious Diseases. Gene Ther (2021) 28:117–29. doi: 10.1038/s41434-020-00204-y

FUNDING

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2019R1A6A1A03033084).
73. Jawalagatti V, Lee JH. Salmonella Enterica Serovar Enteritidis Ghosts Carrying the Escherichia Coli Heat-Labile Enterotoxin B Subunit Are Capable of Inducing Enhanced Protective Immune Responses. *Clin Vaccine Immunol* (2014) 21:799–807. doi: 10.1128/CVI.00016-14

74. Jawalagatti V, Chaudhari AA, Jeon BW, Nandre RM, Lee JH. Characterization of a Novel Inactivated Salmonella Enterica Serovar Enteritidis Vaccine Candidate Generated Using a Modified C8577/A P R/Gene E Expression System. *Infect Immun* (2012) 80:1502–9. doi: 10.1128/IAI.00624-11

75. Kim HJ, Hajam IA, Lee JH. Oral Immunization With a Novel Attenuated Salmonella Typhimurium Encoding Influenza HA, M2e and NA Antigens Protects Chickens Against Heterologous H7N9 Infection. *Vet Res* (2018) 49:12. doi: 10.1186/s13567-018-0509-y

76. Lalsiamthara J, Kim JH, Lee JH. Engineering of a Rough Auxotrophic Mutant Salmonella Typhimurium for Effective Delivery. *Onco target* (2018) 9:25441–57. doi: 10.18632/oncotarget.25192

77. Kim B, Won G, Lee JH. Construction of an Inactivated Typhoid Vaccine Candidate Expressing Escherichia Coli Heat-Labile Enterotoxin B Subunit and Evaluation of Its Immunogenicity in a Murine Model. *J Med Microbiol* (2017) 66:1235–43. doi: 10.1099/jmm.0.000543

78. Lalsiamthara J, Lee JH. Brucella Lipopolysaccharide Reinforces Salmonella Delivering Brucella Immunogens Protects Mice Against Virulent Challenge. *Vet Microbiol* (2017) 205:84–91. doi: 10.1016/j.vetmic.2017.05.012

79. Nakayama K, Kelly SM, Curtiss R. Construction of an ASD+ Expression-Cloning Vector: Stable Maintenance and High Level Expression of Cloned Genes in a Salmonella Vaccine Strain. *Nat Biotechnol* (1988) 6:693–7. doi: 10.1038/nbt0688-693

80. Jawalagatti V, Kirthika P, Hewawaduge C, Yang M, Park J-Y, Oh B, et al. A Simplified SARS-CoV-2 Mouse Model Demonstrates Protection by an Oral Replicon-Based mRNA Vaccine. *Front Immunol* (2022) 13:eabf1555. doi: 10.1126/scitranslmed.abf1555

81. Jawalagatti V, Kirthika P, Hewawaduge C, Yang M, Park J-Y, Oh B, et al. Bacteria-Enabled Oral Delivery of a Replicon-Based mRNA Vaccine Candidate Protects Against Ancestral and Delta Variant SARS-CoV-2. *Mol Ther* (2022) 30. doi: 10.1016/j.mther.2022.01.042

82. Martinoli C, Chiavelli A, Rescigno M. Entry Route of Salmonella Typhimurium Initiates Murine Infection by Penetrating and Destroying the Specialized Epithelial M Cells of the Peyer’s Patch. *J Exp Med* (1994) 180:15–23. doi: 10.1084/jem.180.1.15

83. Penheiter KL, Mathur N, Giles D, Fahlen T, Jones BD. Non-Invasive Salmonella Typhimurium Mutants Are Avirulent Because of an Inability to Enter and Destroy M Cells of Ileal Peyer’s Patches. *Mol Microbiol* (1997) 24:697–709. doi: 10.1046/j.1365-2958.1997.3741745.x

84. Wick MJ. The Role of Dendritic Cells During Salmonella Infection. *Curr Opin Immunol* (2002) 14:437–43. doi: 10.1016/S0952-7915(02)00364-3

85. Gog JR, Murcia A, Osterman N, Restfli O, McKinley TJ, Sheppard M, et al. Dynamics of Salmonella Infection of Macrophages at the Single Cell Level. *J R Soc Interface* (2012) 9:2696–707. doi: 10.1098/rsif.2012.0163

86. Liu MA. A Comparison of Plasmid DNA and mRNA as Vaccine Technologies. *Vaccines* (2019) 7:37. doi: 10.3390/vaccines7020037

87. Datsenko KA, Wanner BL. One-Step Inactivation of Chromosomal Genes in Escherichia Coli K-12 Using PCR Products. *Proc Natl Acad Sci* (2000) 97 (12):6640–5. doi: 10.1073/pnas.120163297

88. Roberts M, Bacon A, Li J, Chatfield S. Prior Immunity to Homologous and Heterologous Salmonella Serotypes Suppresses Local and Systemic Anti-Fragment C. Antibody Responses and Protection From Tetanus Toxin in Mice Immunized With Salmonella Strains Expressing Fragment C. *Infect Immun* (1999) 67:3810–5. doi: 10.1021/167.8.3810-3815.1999

89. Mok DYL, Chan KR. The Effects of Pre-Existing Antibodies on Live-Attenuated Viral Vaccines. *Viruses* (2020) 12:520. doi: 10.3390/v12050520

90. Saxena M, Van TTH, Baird FJ, Coloe PJ, Smooker PM. Pre-Existing Immunity Against Vaccine Vectors – Friend or Foe? *Microbiology* (2013) 159:1–11. doi: 10.1099/mic.0.049601-0

91. Hassan AO, Shrihari S, Gorman MJ, Ying B, Yauw D, Raju S, et al. An Intranasal Vaccine Durably Protects Against SARS-CoV-2 Variants in Mice. *Cell Rep* (2021) 36:109452. doi: 10.1016/j.celrep.2021.109452

92. Hassan AO, Kafai NM, Dmitriev IP, Fox JM, Smith BK, Harvey IB, et al. A Single-Dose Intranasal ChAd Vaccine Protects Upper and Lower Respiratory Tracts Against SARS-CoV-2. *Cell* (2020) 183:169–184.e13. doi: 10.1016/j.cell.2020.08.026

93. Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Viant C, Gaebler C, et al. Enhanced SARS-CoV-2 Neutralization by Dimeric IgA. *Sci Transl Med* (2021) 13:eabf1555. doi: 10.1126/scitranslmed.abf1555