Received: 27 October 2022 | Accepted: 11 November 2022

DOI: 10.1111/1751-7915.14181

MICROBIAL BIOTECHNOLOGY Applied Microbiology International

LILLIPUT

Do we need nasal vaccines against COVID 19 to suppress the transmission of infections?

Harald Brüssow

Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium

Correspondence
Harald Brüssow, Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, Belgium.
Email: haraldbruessow@yahoo.com

Abstract

Covid-19 vaccines have within the first year prevented about 14 million deaths but did not induce a strong mucosal immune response. Data from US, UK, Singapore and Israel showed a variable and mostly modest effects of vaccination on virus excretion during breakthrough infections. Contact studies showed decreased transmission of infection from vaccinated index cases, but the effect varied according to dominant virus type, with study type and the nature of the contact group and diminished with time after vaccination. Some researchers suspect that it is unlikely to stop the pandemic with injected vaccines alone. Promising animal experiments were conducted with mucosal vaccines. Mice nasally immunized with a chimpanzee adenovirus vector mounted a mucosal immune response, were protected against viral challenge after a single vaccine dose and suppressed nasal replication of the challenge virus. Phage T4 expressing SARS-CoV-2 spike and nucleocapsid induced a sterilizing lung immunity in nasally vaccinated mice. Also hamsters intranasally immunized with the prefusion-stabilized spike protein showed no infectious virus in nasal turbinates upon challenge. Other studies showed that intranasal vaccination with an adenovirus vaccine reduced but did not eliminated viral transmission from infected to naïve hamsters. Intranasal vaccination of rhesus macaques with adenovirus vaccines also substantially reduced or even suppressed viral replication in the upper and lower respiratory tract. Human data on mucosal SARS-CoV-2 vaccines are so far limited to safety and immunogenicity studies. Aerosolized adenovirus vaccines given either as a booster or as primary immunization were safe and induced similar or superior immune response than injected vaccines while an aerosolized influenza vectored vaccine induced only a weak humoral and cellular immune response. Overall 100 mucosal SARS-CoV-2 vaccines are in development and 20 are in clinical trials. First human trials demonstrate that this will not be an easy task.

THE IMPACT OF CURRENT VACCINES ON THE PANDEMIC

Do we need new vaccines against COVID-19? In view of highly efficient, approved, widely distributed and safe vaccines, the question might seem rather rhetorical. It is key to emphasize that the availability of efficient vaccines against COVID-19 is a triumph of fundamental research, industrial development and public health efforts. Eight vaccine types have been approved for global use by the World Health Organization (WHO). They represent four different vaccine platforms: inactivated viruses (developed in China and India), mRNA vaccines (developed in US and Germany),

adenovirus-vectored vaccines (developed in UK and US) and adjuvanted protein vaccines (developed in US). Four vaccines have been approved in the US: the mRNA vaccines BNT162b2 (Pfizer / BioNTech) and mRNA-1273 (Moderna), the adenovirus-vectored Ad26. COV2.S (Johnson and Johnson) and the adjuvanted protein vaccine NVX-CoV2373 (Novavax). In clinical trials conducted in the US before the emergence of variant viruses, vaccine efficacy (VE) against symptomatic disease was 94% after two-shots of the mRNA or adenovirus-vectored vaccines. In addition, the vaccines approved by Western health authorities have a good safety record. The Centers for Disease Control and Prevention (CDC) recorded in the US 54 cases of vaccine-induced immune thrombotic thrombocytopenia (VITT) with the adenovirus-vectored vaccine, resulting in 9 deaths. CDC noted 10 deaths due to myocarditis associated with mRNA vaccine application (Barouch, 2022). The impact of the vaccines on adverting death has been tremendous. A recent mathematical modelling study analysed the global impact of vaccination during its first year of introduction (Watson et al., 2022). When the British researchers based their estimates on officially reported COVID-19 death data, they calculated for 2021 about 14 million deaths prevented by vaccination. When they based their calculation on excess deaths data from a model developed by the British weekly *The Economist*, they estimated nearly 20 million deaths averted by vaccinations. Overall, vaccination has reduced the death toll from COVID-19 in 2021 by 63%.

ROOM FOR IMPROVEMENT

Despite these achievements of the approved vaccines, there is still room for improvement. Vaccine efficacy (VE) against hospitalization was “only” 70% with both mRNA or adenovirus vectored vaccines in trials conducted in South Africa when the Omicron variant was the dominant viral isolate (Barouch, 2022) suggesting some need for amelioration with respect to protection against emerging and future viral variants. In addition, neutralizing antibody titres wane within several months after vaccination. Therefore current vaccine efforts concentrate on developing a booster vaccination scheme to cope with the waning of vaccine-induced humoral immunity (how many doses? At what time schedule? Homologous or heterologous booster?; Costa Clemens et al., 2022; Ledford, 2022). There are also intensive efforts to develop adapted mRNA vaccines that present two viral spike sequences (bivalent vaccines; Callaway, 2022) to cope with the immune evasion characteristics of novel variant viruses. Data from trials are now accumulating to answer these questions, but it is still to early to express definitive recommendations.

Other shortcomings of the current vaccines were also discussed. Fears towards new biotechnological methods used in the development of mRNA or recombinant viral vaccines in addition to politically and emotionally motivated general opposition towards vaccines resulted in vaccination coverage rates that just reached about 70% in high income countries. Due to other problems, vaccine coverage rates are 50%, 30% and 4% in upper-middle, lower-middle and low income countries, respectively. South-East Asia, the Eastern Mediterranean and particularly Africa showed low coverage raising political and economic problems of global vaccine equity that prevented a further reduction of the global COVID-19 death toll by vaccines (Watson et al., 2022). A combination of causes ranging from cost, freezing requirements, distribution logistic problems, business priorities for vaccine production and “vaccine nationalism” resulted in stark global health inequities (Barouch, 2022; Wagner et al., 2021). Therefore, the ease of vaccine application (with or without needles), vaccine effectiveness when used in routine programmes, need and frequency of boosters, cost, the need of cold-chain logistics, manufacturing scalability, acceptability by communities and scope for local or regional production are additional important factors for future vaccines (Nohynek & Wilder-Smith, 2022).

THE NEED FOR STERILIZING IMMUNITY

Another important challenge is the achievement of sterilizing immunity by vaccination. Vaccination has two effects: direct effects protecting the vaccinated person from disease and indirect effects for the contacts of the vaccinated person protecting them from disease. This can only be achieved if the vaccinated subject is not only protected from developing severe disease, but also shielded from infection or at least if the load of the virus excreted by the vaccinated person is substantially reduced in breakthrough infections. The indirect protection effect would prevent or at least reduce onward transmission and thus curtail the spread of the pandemic leading ultimately to its suppression. A modelling study revealed that the waning of natural immunity contributes to the evolutionary potential of the virus. Sustained viral transmission in regions with low access to vaccines as a consequence of vaccine nationalism will result in an increased potential for viral antigenic evolution, which may result in the emergence of novel variants that could affect epidemiological characteristics and the pandemic trajectory globally and thus revive the pandemic (Wagner et al., 2021). The study revealed that sharing vaccines with countries that have low vaccine availability decreases overall infections. The authors admit that projecting the trajectory of the pandemic is complicated and additional complexities

are introduced if natural and vaccination immunity is weak and only short-term with respect to viral transmission. The model, published a year ago, postulated a good transmission-blocking immunity after two vaccine doses or natural infection. A year ago there was still an expectation that the global effort in vaccination would bring the Covid-19 pandemic under control and transform the pandemic into an endemic phase. However, the authors of this opinion paper admitted that the actual pandemic trajectory is difficult to predict since it depends on too many, at that time not well defined factors, such as waning immunity, antigenic evolution of the virus and zoonotic reintroduction of the infection (Telenti et al., 2021). These authors noted that highly effective vaccines can achieve the elimination of disease even if the infection is not eliminated. However, a safer approach to the control of the pandemic would be vaccines that interfere with transmission of the infection by vaccinated subjects. The present report explores the impact of current vaccines on virus transmission by vaccinated subjects and then asks whether mucosal vaccines have a better chance to achieve that goal.

REINFECTION OF VACCINATED SUBJECTS

Immunological research has revealed a prominent role for antibodies to prevent asymptomatic infection with SARS-CoV-2 while T cells play a dominant role for averting severe disease, hospitalization and death from Covid-19 (Barouch, 2022). Therefore, the rapid waning of the antibody response after vaccination and the fact that injected vaccines induce a better systemic than a mucosal antibody response could represent a problem for preventing re-infection of the vaccinees and subsequent transmission of the infection to bystanders, thereby interfering with the interruption of infection chains and ultimately the suppression of the pandemic. As long as sterilizing immunity is not achieved, SARS-CoV-2 will continue to circulate even in a highly vaccinated populations allowing further evolution of the virus potentially also leading to higher virulence. Researchers have therefore also investigated the effect of injected intramuscular vaccines on mucosal antiviral immune response, viral exhalation and transmission of the infection and disease.

Local immunity

US scientists investigated salivary secretory IgA immunoglobulins (sIgA) as a marker for a mucosal antibody response in a small group of volunteers who received either the Moderna or the BioNTech mRNA vaccine. In seronegative subjects (i.e. persons without a prior natural SARS-CoV-2 infection) the injected mRNA vaccine induced only a negligeable sIgA response. Higher antiviral sIgA titres were induced by the mRNA vaccine in seropositive subjects (i.e. persons who had experienced a prior natural infection). However, salivary sIgA response was variable over time even in the same subject and did not correlate with serum anti-viral IgG titres (Sano et al., 2022).

Virus excretion: US studies

A number of research groups investigated the effect of vaccination on virus excretion in vaccinated as compared with unvaccinated infected subjects. The outcome differed by geographical area and epidemiological setting. US studies did not observe an effect of vaccination on virus excretion in breakthrough infections. Several studies used the Ct cycle threshold level of the diagnostic PCR assay as a proxy measure for the viral load (with lower Ct values indicating a higher viral load in the diagnostic sample). The analysis of a large outbreak in Massachusetts in July 2021 (more than 1000 infections at a mass gathering) revealed that 127 vaccinated persons with a mostly symptomatic breakthrough infection had a Ct value of 23 which was only marginally higher than the Ct value of 22 in unvaccinated infected persons (Brown et al., 2021). In addition, the analysis of genomic and epidemiological data from this outbreak supported the conclusion of multiple transmissions of the delta variant virus from and between fully vaccinated individuals (Siddle et al., 2022). Vaccinated and unvaccinated patients with delta infections who were hospitalized in Wisconsin and Texas also showed comparable and low Ct values, indicating that vaccination did not affect the delta viral load (Subbaraman, 2021). Vaccinated but uninfected control individuals from this outbreak showed a good antiviral serum antibody response, but no antiviral nasal antibodies. Vaccinated individuals with an infection showed a vigorous anamnestic serum antibody response (30-fold increase), but only a modest 4-fold nasal antiviral IgA increase (Collier et al., 2022). US researchers also directly investigated the physical exhalation of virus particles in the breath of 93 infected subjects displaying mild symptoms. They found that subjects infected with the alpha, delta and omicron variants excreted higher viral loads than those infected with the initial SARS-CoV-2 strain, that alpha variant load was higher in the fine than in the coarse air particles and that both vaccinated and even boosted subjects exhaled high viral loads (medRxiv preprint https://doi.org/10.1101/2022.07.27.22278121).

Virus excretion: UK studies

In contrast, studies from UK showed some, albeit variable effects of vaccination on viral load excretion.

In the REACT-1 study conducted in the summer 2021 in England, the researchers assessed reverse transcription polymerase chain reaction (RT-PCR) swab positivity. During summer 2021 when the alpha variant was replaced by the delta variant, 100,000 self-administered nose and throat swabs were analysed from a random sample of the population. Vaccinated subjects with a positive SARS-CoV-2 test ("breakthrough infections") showed a higher median Ct value of 28 compared with 23 in unvaccinated subjects (hence a lower viral load). The authors suggested that this could indicate a lower infectiousness of vaccinated but infected subjects compared with unvaccinated infected subjects. However, the Ct difference between both groups disappeared when a stricter Ct value was used for defining a positive sample (Ct threshold shifting from 37 to 33), questioning the biological relevance of their reported difference (Elliott et al., 2021). In a follow-up REACT-1 study report conducted when the delta variant was dominant, the authors reported a lower nasal viral load in infected subjects who had received a booster vaccination compared with those who had only received two vaccine doses, but this difference was lost when the omicron variant dominated the infection wave. Vaccinated and unvaccinated infected children showed similar Ct values during both the delta and the omicron wave. In contrast, asymptomatic subjects showed lower viral loads than symptomatic subjects both during the delta and the omicron wave (Elliott et al., 2022).

In another infection survey conducted in England during early 2021 the researchers assessed the effectiveness of the BNT162b2 and ChAdOx1 vaccines against any SARS-CoV-2 PCR-positive test result. The percentage of positive PCR tests and their Ct value distribution differed in vaccinated compared with unvaccinated subjects without a prior infection. Vaccine efficacy against infection was 64% after the first and 80% after the second vaccine dose. Breakthrough infections in vaccinated subjects showed a lower viral load than primary infections in unvaccinated subjects. Unvaccinated subjects experiencing a re-infection also showed a low viral load (Pritchard et al., 2021).

Virus excretion: Singapore and Israel

A study from Singapore compared 84 vaccinated with 130 unvaccinated subjects, both hospitalized with delta variant-associated COVID-19. Severe disease cases defined by oxygen support differed significantly between vaccinated and unvaccinated patients (3% vs. 53%), but viral load was high and comparable in both patient groups (Ct values of 19). However, viral load decreased more rapidly in vaccinated compared with unvaccinated subjects, parallel to a more rapid increase in serum neutralizing antibodies (Chia et al., 2022).

Before February 2021, researchers identified in Israel 5000 breakthrough infections in mRNA-vaccinated people. When plotting the Ct value against the days after first vaccination, they observed a fourfold decrease in viral load starting 12 days after vaccination, suggesting a potential reduction of infectiousness (Levine-Tiefenbrun, Yelin, Katz, et al., 2021). When repeating the analysis in mid-2021, the researchers found again a fourfold Ct decrease in viral load in 16,000 breakthrough infections with the delta variant, but the effect declined and finally vanished after 2 and 6 months, respectively, but could be restored by a booster vaccination (Levine-Tiefenbrun, Yelin, Alapi, et al., 2021). However, the impact of the booster on viral load decreased to a two-fold difference in delta virus excretion compared with infections in unvaccinated subjects and disappeared after 3 months (Levine-Tiefenbrun et al., 2022).

VACCINATION EFFECTS ON TRANSMISSION OF INFECTION

Viral load determinations are only a proxy measure for the infectiousness of subjects. It is thus important to explore actual transmission data for viral infections.

UK

Household data from health care workers (HCW) in Scotland, who were in 2020 among the first worldwide to be vaccinated with AstraZeneca (AZ) adenovirus or Pfizer mRNA vaccines, showed that family members from vaccinated compared with unvaccinated HCW were about 30% and 20% less likely to be infected or to be hospitalized, respectively (Shah et al., 2021). Household transmission data from early 2021 in England suggested that even partial vaccination with AZ or Pfizer vaccines halved the rate of secondary transmission (Harris et al., 2021).

Other researchers came to different results when they investigated the contagiousness of 470 UK index cases to 600 contacts between Sept 2020 and Sept 2021. Secondary attack rates (SAR) among household contacts exposed to fully vaccinated index cases was similar to household contacts exposed to unvaccinated index cases (25% vs. 23%): 39% of infections in fully vaccinated contacts arose from fully vaccinated, epidemiologically linked index cases. Peak viral load did not differ by vaccination status or variant type (pre-alpha, alpha, delta), but fully vaccinated cases with delta infection showed a more rapid decline of viral load than those infected with pre-alpha or alpha variant (Singanayagam et al., 2022).

Researchers from Oxford used contact-testing data from England during the first half of 2021 for 150,000

tested contacts of 110,000 index patients to explore the effect of vaccination on infection transmission. Overall, 46% of contacts from 76,000 unvaccinated index patients experienced an infection. Only 35% of contacts from index patients with the first vaccination had an infection. The infection rate was 28% and 21% of contacts to index patients who were twice vaccinated with the AZ or the mRNA vaccine, respectively. Protection from transmission of the alpha variant was greater than that for the delta variant and decreased with time after vaccination to reach the level of transmission from unvaccinated patients 3 months after AZ vaccination. Partially vaccinated index patients showed comparable viral loads than unvaccinated index patients, while fully vaccinated index patients showed a lower viral load for the alpha, but not for the delta variant compared with unvaccinated patients. Asymptomatic patients showed lower viral loads than symptomatic patients in both vaccinated and unvaccinated patients. Vaccination decreased the viral load in asymptomatic patients for infections with the alpha variant, but much less for infections with the delta variant. Variation in the viral load explained only 23% of the variation in the reduction of the transmission by vaccination indicating further factors influencing transmission efficiency (Eyre et al., 2022).

US

Researchers investigated transmission of omicron variant infections in 110,000 inmates of Californian prisons in the first half of 2022. The risk of transmission to a close contact (cellmate) was 29%, but differed significantly according to the vaccination status of the index patient. Transmission rate was 36% for an unvaccinated index patient without prior infection; 27% for a vaccinated index patient and 22% for an index patient with a prior infection. The transmission rate decreased with additional doses of vaccination, but increased with time from last vaccination (medRxiv preprint doi: https://doi.org/10.1101/2022.08.08.22278547).

Israel

A household study from Israel with HCW conducted before May 2021 revealed that the secondary infection rate among contacts of unvaccinated index cases was 42% while that of vaccinated index cases was only 19% (Layan et al., 2022).

In Israel vaccination campaigns with the Pfizer mRNA vaccine started before the vaccine was approved in children and the detailed documentation of vaccination and infection events in the healthcare system allowed studies evaluating the indirect effect of vaccination in parents on the infection of their unvaccinated children. Hayek et al. (2022) investigated infections in 400,000 unvaccinated children and adolescents during the early alpha variant infection wave and found a 26% and 72% reduced infection rate in children from their household if one or both parents were vaccinated, respectively, as compared with households without vaccinated parents. During the later Delta infection wave they compared infection rates in unvaccinated children from households where parents had received a booster versus households where parents had only received two vaccine doses. If one parent was boosted, children experienced an 21% decreased infection rate compared with an 58% reduction if both parents were boosted. As a test for non-specific effects, the researchers investigated the rate of bacterial diarrhoea in the children and did not observe an effect of parental vaccination with SARS-CoV-2.

Another group of researchers analysed data from 2.5 million subjects in Israel for household transmission of infection. During the alpha wave they observed a 23% reduced infection transmission risk by vaccination of the index case which eroded to a 7% reduced transmission 3 months after the second vaccination of the index case. When restricting the susceptible population to unvaccinated children they observed during the alpha wave 41% vaccine efficacy against transmission while during the delta wave no significant effect of vaccination of index cases on transmission was observed (Prunas et al., 2022). The difference between the two study outcomes might be that the first household study from Israel investigated effects occurring early after vaccination and concentrated on children (Dean & Halloran, 2022).

The effect of vaccination on infection transmission was also investigated in Israel at the population level. Researchers relied again on health insurance data from early 2021 where 50% of the population was vaccinated with the Pfizer vaccine. They separated the dataset into 177 communities with different kinetics of vaccination coverage and used the unvaccinated paediatric population as bystander and indicator population. The researchers observed that for each 20 percentage points of individuals who are vaccinated in a given population, the positive test fraction for the unvaccinated population decreased approximately twofold, suggesting a substantial herd immunity effect (Milman et al., 2021).

Scandinavia

A Danish household study conducted through the delta and omicron waves found increased rates of secondary infections if the index case was unvaccinated compared with a fully vaccinated index case. The researchers described decreased transmission rates if the index case was boosted compared with index cases who had received two vaccine doses (medRxiv

preprint doi: https://doi.org/10.1101/2021.12.27.21268278). Ct values among unvaccinated compared with vaccinated secondary cases infected with delta were comparable (Lyngse et al., 2022).

Adult household members of single-vaccinated HCW from Finland assessed before May 2021 experienced a 23% risk reduction for infection compared with adults living with unvaccinated HCW. After the second vaccine dose, adult household members demonstrated even a 39% reduction in infection risk. However, the vaccination status of HCW had no significant impact on the infection risk of children and adolescents living with them (Salo et al., 2022).

Taken together, the transmission studies indicate a significant and substantial effect of vaccination on the infectiousness of breakthrough infections. However, the degree of the effect varies with the type of contact group, the virus variant and some data indicate a substantial erosion of this effect with time after vaccination. In view of the weak local antibody response, an Israeli research group thinks that it is highly unlikely that population-level transmission of SARS-CoV-2 can be eliminated through vaccination with current vaccines alone (Prunas et al., 2022) raising the issue of complementation with nasally applied vaccines.

ANIMAL EXPERIMENTS WITH NASAL VACCINES

Theoretically, nasal vaccines represent clear advantages over injected vaccines since such vaccines could stop the transmission of coronavirus to other persons by suppressing the viral infection already in the nose of the vaccinated person and thus interrupt infection chains. Acceptance rate of a nasally applied vaccine would also be greater than for a muscular vaccine applied with a syringe. Nasal drops are also safer to apply than an injected vaccine, which needs clean syringes and might represent a problem in developing countries. Therefore, researchers started with animal studies on nasal SARS-CoV-2 vaccines already in the early phase of the pandemic.

Mice

US researchers developed a chimpanzee adenovirus vector that encodes a pre-fusion stabilized spike (S) protein. Upon intramuscular injection in mice this vaccine induced spike-specific IgG, but no IgA serum antibodies. No spike protein-specific T cells were detected in the lungs nor were IgA-secreting plasma cells observed in the spleen. By intranasal immunization in mice with the same vaccine, both spike protein-specific IgG and IgA were seen in the serum, vaccine-induced resident memory T cells were detected in the lungs, and plasma cells producing both IgA or IgG were seen in spleens. Anti-viral IgA-producing plasma cells were 5-times more frequent than those producing IgG. Notably, intranasal vaccine vector application suppressed viral replication also in the nose of virus-challenged mice where the virus detected in the nose only represented the residual challenge virus. Intranasal immunization conferred sterilizing immunity since no antibody response to the viral nucleoprotein (not contained in the viral vector, but potentially expressed by the challenge virus) was seen in intranasally in contrast to intramuscularly vaccinated mice (Hassan et al., 2020). Since the protective effects of the nasal vaccine was seen in mice after a single vaccine application, a nasal application might offer another advantage over intramuscular application of adenovirus vectored vaccines that need two injections for full protection.

Canadian scientists developed trivalent adenovirus vectors based on either a human serotype 5 or a chimpanzee adenovirus. These vectors expressed the S1 domain of the spike protein, the full length nucleocapsid (N) protein and a segment of the nonstructural RNA-dependent RNA polymerase (RdRp) protein in order to broaden the T cell immune response to additional SARS-CoV-2 antigens. After documenting safety of the vaccines in mice, they demonstrated that a single dose of intranasal immunization in mice not only led to a superior humoral anti-spike antibody response, but also a superior airway T cell response compared with intramuscular injection of the same trivalent vaccine. Notably, multifunctional $CD8^+$ T cells with cytotoxic potential were induced in the respiratory tract. The chimpanzee adenovirus vector turned out to be better than the human adenovirus vector. The intranasal, but not the intramuscular vaccines induced mucosal tissue-resident memory T cells (TRM) which play a pivotal role in host defence against reinfection. Intranasal in contrast to intramuscular immunization also induced trained airway macrophages critical for trained innate immunity (TII). Experiments using mice strains that displayed mutations in different arms of the immune system revealed that both humoral and T cell immunity are required for protection by intranasal immunization, while TII improves clinical outcomes but did not control viral replication. The researchers also tested vectors expressing all three, two or one viral antigens and observed that inclusion of the N / RdRp antigens offered additional protection via T cell immunity and TII. The intranasal trivalent vaccine provided sterilizing lung immunity also to two virus variants of concern (Afkhami et al., 2022).

US researchers developed another interesting vaccine vector for intranasal immunization. They used the *Escherichia coli* bacteriophage T4 as a nanovaccine. By CRISPR engineering they constructed a phage that expressed the SARS-CoV-2 spike and E proteins on the surface of the phage capsid and the SARS-CoV-2 N

protein within the phage capsid. They induced in mice spike-specific helper and effector T cells, killer T cells and broad neutralization of Beta, Delta, and Omicron variant viruses. Two doses of the intranasal phage-vectored vaccine protected mice from weight loss or death (according to mouse Covid-19 disease model), prevented lung pathology and established sterilizing viral immunity in the lung. T4 phage is noninfectious in humans and has demonstrated safety in clinical trials of phage therapy approaches (Sarker et al., 2012, 2016, 2017). The T4 vectored vaccine is stable for at least 10 weeks at ambient temperature and together with the low cost of phage production offers attractive prospects as a mucosal nanovaccine particularly for developing countries (Zhu, Jain, et al., 2022).

Hamster

US virologists immunized hamsters with an approved adenovirus vaccine intranasally (IN). The animals mounted a higher serum neutralizing antibody titre than animals after intramuscular (IM) immunization. After challenge, infectious virus was detected in the oropharyngeal swabs of all immunized animals, but IN vaccinated hamsters showed a 10-fold lower titre than IM vaccinated hamsters. Neither IN nor IM vaccinated hamsters showed viral RNA, infectious virus or pathology in the lungs while unvaccinated controls showed clear signs of interstitial pneumonia. Similar observations were made when the challenge virus was applied by intranasal inoculation or by transmission from infected cage mates (van Doremalen et al., 2021).

US researchers also used a different virus vector, parainfluenza virus type 3, expressing the native (S) or prefusion-stabilized (S-2P) SARS-CoV-2 S spike protein. In hamsters a single intranasal immunization with these vectors induced significantly increased serum IgG titres against the spike protein. However, only hamsters immunized with the prefusion-stabilized spike protein showed significant neutralizing antibody titres against various SARS-CoV-2 isolates. Both vaccines protected against weight loss induced by viral challenge and prevented detection of infectious virus in the lung. Hamsters intranasally immunized with the prefusion-stabilized spike protein showed upon challenge no infectious virus in nasal turbinates, while the S protein vaccine only decreased and shortened nasal virus excretion (Liu et al., 2021).

Still another group of US scientists compared oral and intranasal immunization of hamsters with an adenovirus type 5 vector expressing the full length spike protein. Serum anti-spike IgG and IgA titres were higher after both oral and nasal than after intramuscular vaccine injection. Both mucosal vaccination routes suppressed detection of infectious virus in the lung upon viral challenge. Only nasal immunization reduced albeit not prevented infectious virus detection in the nasal swab of challenged hamsters. All vaccine routes decreased lung pathology in challenged animals. 63%, 44% and 100%, respectively, of naïve hamsters exposed to orally, nasally or intramuscularly immunized and subsequently infected hamsters experienced a transmission of the infection. Mucosal vaccination did not completely prevented transmission, but was more efficient than intramuscular immunization and likely reduced the effective dose reaching the exposed hamsters as indicated by a less affected weight development (Langel et al., 2022).

Other approaches than conventional vaccines have also be explored for their capacity to reduce virus transmission. A concept borrowed from classical virological research is defective interfering (DI) particles, viruses that contain shortened, internally deleted viral genomes that successfully compete with the replication of viruses containing the complete genome and thereby reduce infectivity. US researcher developed 2-kb non-coding mRNA from SARS-CoV-2 as therapeutic interfering particles that suppressed viral burst size and reduced cell-to-cell virus transmission (Chaturvedi et al., 2021). A single, intranasal, postexposure dose of these particles lowers SARS-CoV-2 nasal shedding and reduced transmission of SARS-CoV-2 including Delta variant from infected to uninfected hamsters (Chaturvedi et al., 2022). However, DI mRNA as well as nasal application of engineered IgM that displays broad range potent neutralization of SARS-CoV-2 and its variants and protects mice prophylactically – and at 10-times higher doses also therapeutically – against lung disease (Ku et al., 2021) belong more to nasal antivirals than to the subject of nasal vaccines.

Rhesus macaques

Chinese virologists engineered a replication-incompetent recombinant serotype 5 adenovirus carrying a codon-optimized gene encoding Spike protein (S) and compared its immunogenicity and protection in rhesus macaques after intra-muscular (IM) and intranasal (IN) application. IM vaccination induced a good serum ELISA and neutralizing antibody response, but no mucosal antibodies while IN vaccination induced both serum and mucosal antibody titres. Serum antibody titres increased over time in IM injected, but not in IN immunized animals resulting in 10–100 fold higher titres in IM immunized animals. IN vaccination induced weaker cell-mediated immune (CMI) responses to the spike protein than IM vaccination. In challenge experiments control monkeys showed high viral loads in pharyngeal swabs while this was not observed in either IM or IN immunized monkeys, demonstrating that IN vaccination can confer effective protection of monkeys against SARS-CoV-2 infection. IN induced less

anti-vector antibodies than IM immunization which is a possible advantage for repeat immunizations with the same adenovirus vector vaccine (Feng et al., 2020). US researchers showed that intranasal vaccination with a S protein expressing vector induced serum IgG, IgA and neutralizing antibody titres and T cell responses. Upon intranasal and intrabronchial challenge with SARS-CoV-2 animals that had received the nasal vaccine showed a lower clinical score, lower viral presence in the lungs and lower nasal virus titres than animals receiving the empty control vector (Hassan et al., 2021). Another US group confirmed the observation when reporting significantly decreased subgenomic RNA (indicative of replicating virus) or infectious virus in nasal turbinates of rhesus macaques intranasally immunized with adenovirus vector vaccine when they underwent viral challenge compared to systemically immunized animals (van Doremalen et al., 2021).

NIH researchers who had developed a parainfluenza vector for SARS-CoV-2 in hamsters reported in a recent preprint combined intranasal/ intratracheal immunization experiments with this vector in rhesus macaques. The vaccine induced mucosal antibodies in both the upper and lower respiratory tract in addition to neutralizing serum antibodies displaying activity also against variant viruses. Immunization induced $CD4^+$ T-cells and cytotoxic $CD8^+$ T-cells in the airways which showed transition to tissue-resident memory phenotypes and protected rhesus macaques against replication by a challenge virus in both the upper and lower airways (https://doi.org/10.1101/2022.05.21.492923).

Mucosal vaccination as booster

German virologists investigated mucosal vaccinations with adenoviral serotype 5 and 19a vectors in mice with or without prior systemic priming (Lapuente et al., 2021). Neutralizing antibodies against virus was detected in sera and lung washes from mice primed with a plasmid expressing SARS-CoV-2 spike S and nucleocapsid N proteins, followed by a boost with a nasal adenovirus vaccine vector. This was however not observed after nasal vaccination with the adenovirus vector alone. In contrast to two intramuscular applications of an mRNA vaccine, intranasal boosts with adenoviral vectors induced high levels of mucosal IgA and lung-resident memory T cells. Since intranasal boost strategies led to complete protection against SARS-CoV-2 infection in the mouse model, mucosal booster immunizations after mRNA priming might be a promising approach to increase mucosal immunity. Data on the duration of immune response after mixed IM/IN vaccination are still lacking.

US researchers found that single dose of unadjuvanted recombinant spike protein by intranasal injection was not sufficiently immunogenic in mice. Using this vaccine as a boost after an intramuscular mRNA vaccine elicited local B cell responses in the lung and stimulated resident memory T cells in the airways which was not achieved by mRNA intramuscular vaccination alone. The IM/ IN vaccination schedule induced not only mucosal immunity, but also protected mice from lung disease and mortality upon challenge not only with the homologous SARS-CoV-2 virus but also with some variant viruses (bioRxiv preprint doi: https://doi.org/10.1101/2022.01.24.477597). So far, mRNA vaccines for nasal application are not explored.

HUMAN TRIALS WITH MUCOSAL VACCINES

So far only few clinical trials have been published reporting data with mucosal vaccination against Covid-19, but this situation is likely to change soon since a number of trials are reported as ongoing (Waltz, 2022a).

The strategic advisory board of WHO has recommended a third injection for persons older than 60 years who received two intramuscular injections of the inactivated Chinese CoronaVac vaccine. To comply with this request, Chinese health authorities conducted a safety and immunogenicity trial in 420 healthy adults. A third of them received as booster a dose of 10^{10} viral particles of an adenovirus-based vaccine developed in China by oral aerosol application. Another third received twice as much oral vaccine while the last third received the initial inactivated CoronaVac vaccine intramuscularly. Adverse reactions were less frequent with the aerosolized boost than with the injected boost and consisted of dry mouth and pharyngeal swelling in the oral vaccine groups. Immunogenicity was studied in subgroups of 50 vaccinees per group. All three boosts increased the serum neutralizing or spike-specific antibody titres, but the increases were about 10-fold higher in the oral than in the intramuscular boost recipients, with no difference between high and low oral dose. The same was true for the stimulation of the virus-specific T cell response measured by interferon and interleukin ELISpot responses. The orally boosted vaccinees showed Th1-skewed cellular immune responses (Li et al., 2022). The vaccinees were followed for further 6 months for antibody titre development. Both oral booster groups showed neutralizing antibody titres decreases by 80%, but remained over the entire time period about 30-fold higher than titres in the group receiving a homologous intramuscular CoronaVac boost. Orally boosted, but not intramuscularly boosted vaccinees also showed titres against the omicron variant albeit at lower levels (medRxiv preprint doi: https://doi.org/10.1101/2022.07.26.22278072).

Chinese researchers conducted a phase 1 safety and immunogenicity trial with Ad5-nCoV, a Chinese replication-defective adenovirus type-5 vectored

vaccine that encodes the SARS-CoV-2 spike protein which was also used in the above-mentioned booster study. They compared five groups each comprising 26 participants. The participants received either two doses of low or high aerosolized vaccine by nebulization inhalation, a mixed intramuscular injection followed by an aerosol application, or one or two doses of intramuscular vaccines. Adverse events occurred more frequently after intramuscular than after aerosol vaccination, mostly manifested as a higher percentage of fever reaction after intramuscular injection. Serum neutralizing antibody titres were comparable after aerosol and intramuscular vaccine application. Neutralizing and IgG and IgA serum antibody titres after mixed vaccine application tended to be higher than in the other groups. All vaccination groups showed comparable T cell stimulation as measured by viral spike-specific IFN-γ ELISpot responses (Wu et al., 2021).

Another group of Chinese scientists conducted a combine phase 1/ phase 2 safety and immunogenicity trial with a nasal vaccine in more than 1000 healthy adults lacking antibodies to SARS-CoV-2 at baseline. The intranasal vaccine was a cold-adapted influenza strain lacking the non-structural influenza virus protein 1 (NS1) which expressed the RBD domain of SARS-CoV-2 spike protein. The vaccine diluent was used as placebo. The products were applied with a sprayer that atomized the liquid into a fine mist of droplets. Overall, 19% of the vaccine recipients reported mostly mild adverse events. Seroconversion for RBD IgG antibodies were seen in 20% of the vaccinees. Nasopharyngeal sIgA was only detected in 13% of the vaccinees, while 40% of vaccinees showed a weak T cell response against the viral spike protein. A similar rate of T cell response was seen in placebo recipients which was explained by the fact that both products were nebulized in the same small room leading to cross-contamination (Zhu, Zhuang, et al., 2022). If this interpretation is correct, even very small amounts of nebulized vaccine can induce a T cell response.

British scientists conducted an open-label phase 1 clinical trial in 30 vaccine-naïve healthy adults with the ChAdOx1 nCoV-19 adenovirus vaccine approved for intramuscular injection which they applied intranasally by a commercial nebulization device. Adverse effects were mild (sore throat, nasal discharge, headache). Nasal spike-specific IgA antibody titres were barely increased over preimmune titres after one and two intranasal vaccinations. Also serum spike-specific IgG antibodies were not significantly increased after intranasal vaccination while cellular immune response measured with the interferon-γ ELISpot test showed increases after intranasal vaccination, but remained below values measured in convalescent subjects. Intranasal booster given after two intramuscularly mRNA immunization in 6 subjects did not increase nasal anti-spike IgA titres. Infection of 7 from the 42 intranasally-immunized subjects within 16 weeks of follow-up was considered as a discouraging outcome by the study authors even if vaccine efficacy was not planned in this study (Madhavan et al., 2022). Based on this disappointing results Astra Zeneca abandoned its nasal vaccine project.

ONGOING ACTIVITIES

There are more than 100 mucosal vaccines against Covid-19 in development of which 20 are in clinical trials (Waltz, 2022a). Most advanced are mucosal vaccines from Bharat Biotech (Hyderabad, India), a non-replicating adenoviral vector applied as intranasal drops which completed two -so far unpublished- phase III trials and has received approval in India as a two-dose primary inoculation. Bharat compared its intranasal vaccine to Covaxin, an injected vaccine from India, for serum antibody production (Waltz, 2022b). Likewise, CanSino Biologics (Tianjin, China), using an aerosolized version of an approved intramuscular adenovirus vector vaccine for inhalation through nose and mouth, has finished unpublished phase III trials and has likewise received approval by Chinese authorities as a booster dose. Beijing Wantai Biological Pharmacy currently tests a live attenuated vaccine applied as an intranasal spray in an ongoing phase III trial with 40,000 subjects. A similar approach is followed by Codagenix (Farmingdale, New York) and Serum Institute of India (Pune) in a combined phase II/III efficacy study in 20,000 people from Africa. This clinical test is part of the WHO's Solidarity Trial Vaccines, which compares also other vaccines against a shared placebo group. Such trials meet difficulties since it is increasing difficult to recruit unvaccinated and uninfected subjects into trials and because it is ethically difficult to justify placebo groups when efficient injected vaccines are available.

Razi Vaccine (Karaj, Iran) developed a protein subunit vaccine applied as nasal spray and has received emergency authorization in Iran. Russia has approved an intranasal-spray version of Sputnik V. No published data are available for these two vaccines. Several other viral vector and a protein subunit vaccine from Cuba are in phase I or II clinical trials.

OUTLOOK

The constitution of a placebo group is not the only problem for these trials. Another issue is the definition of clinical endpoint. Many of the aforementioned clinical trials will measure serum neutralizing antibody titres and compare it with those achieved by intramuscular vaccination and take these data as indicators for protection from disease. Other trials will also measure secretory IgA and tissue-resident memory T cells as indicators

for (sterilizing) mucosal immunity. However, mucosal correlates for protection against infection are much less clear than serum correlates against disease. None of these trials test for prevention of infection or prevention of transmission of infection. Such an outcome is in fact an ambitious goal for mucosal vaccines. Few mucosal vaccines are approved, most are oral vaccines. Some of them are very successful (oral polio, oral rotavirus vaccines), others are less impressive (cholera vaccine) while the experience with nasal vaccines against respiratory infections is mixed. The Swiss vaccine producer Berna retracted in 2001 its intranasal inactivated influenza vaccine from the market because of a significant increase in Bell's palsy (facial paralysis) after vaccination (Mutsch et al., 2004). The FluMist nasal influenza vaccine developed by MedImmune was approved in 2003 in the US. There is a clinical trial in nearly 8000 children which showed superior efficacy of a live attenuated influenza virus vaccine administered as an intranasal spray compared with an inactivated influenza virus vaccine injected intramuscularly. There were 55% fewer cases of cultured-confirmed influenza cases in the group that received the nasal as compared with the injected vaccine recipients. The study nevertheless called for some caution since children younger than 1 year showed in a post-hoc analysis a significant higher hospitalization rate with respiratory diagnosis than those receiving the injected vaccine (Belshe et al., 2007). In addition, during the 2015–2016 season, vaccine efficacy against influenza A(H1N1) in US children was significant for inactivated injected vaccine but not for live attenuated nasal vaccine recipients (Poehling et al., 2018). These concerns regarding lack of effectiveness led to the CDC Advisory Committee on Immunization Practices (ACIP) recommendation to administer inactivated influenza vaccines and not the nasal vaccine, during the 2016–17 and 2017–18 seasons (Grohskopf et al., 2017).

Success with a nasal vaccine against a viral respiratory infection is thus an ambitious goal and far from being guaranteed. Clinical trials have to document several outcomes. First, clinical trials of nasal SARS-CoV-2 vaccines should demonstrate that they are safe. Second, trials should prove that their protection efficacy against severe disease is as good as that achieved with injected Covid-19 vaccines. If that is the case, intranasal or oral SARS-CoV-2 vaccines could be an interesting alternative to injected vaccines for psychological (needle fear) and logistic reasons (no need for sterile syringes, vaccine application could be done by non-medical personnel). Third, epidemiological research should prove that nasal vaccination reduces the transmission of breakthrough infections in vaccinated subjects to bystanders, at least at a rate greater than achieved by intramuscular vaccinations. This would be the most valuable asset for nasal vaccines, but probably also the most ambitious outcome that is so far not part of the test protocols in the ongoing clinical trials with nasal vaccines. This would then be a contribution to end the circulation of SARS-CoV-2. With the exception of China which opted for a no-covid policy, most governments have now settled to limit the hospital burden of Covid-19 for their societies which can be achieved by injected vaccines. However, this strategy carries an inherent risk: if no barrier to viral replication is erected in the upper respiratory tract by vaccination, the virus will continue to circulate and to mutate, raising the spectre of the emergence of a viral variant that undermines the current vaccine protection and if combined with a substantial virulence gain could reignite a renewed acute pandemic phase. As this is more than a theoretical risk and as our knowledge level for mucosal vaccines against respiratory infections is limited, it is certainly justified to continue with fundamental and clinical research on mucosal vaccines against SARS-CoV-2. Hurdles are manyfold ranging from lack of standardized tests of mucosal immunity, lack of correlates for mucosal protection to lack of financial investment into nasal vaccine making it a commercially high risk, and only potentially high reward industrial activity (Akst, 2022). To address this issue, some prominent immunologists have requested a new Lightning Speed Operation as for the first injected Covid-19 vaccines, now for mucosal vaccines against Covid-19 (Topol & Iwasaki, 2022).[1]

AUTHOR CONTRIBUTIONS

Harald Brussow: Writing – original draft (equal).

ACKNOWLEDGEMENTS

I thank Dr. Sophie Zuber for critical reading of the manuscript.

ORCID

Harald Brüssow https://orcid.org/0000-0003-4783-8583

ENDNOTE

[1]Since submission of the review four important reports were published, two were mentioned as preprints in this review. Mao et al (Science. 2022 Oct 27:eabo2523) immunized hamsters after an intramuscular mRNA shot with an unadjuvanted intranasal spike protein boost. Compared to hamsters receiving two mRNA injections, these animals showed reduced viral shedding when exposed to infected cage mates. Le Nouën et al (Cell 12691) generated a live-attenuated parainfluenza virus-vectored vaccine candidate expressing the spike protein. A single intranasal/intratracheal dose induced in macaques strong spike-specific airway mucosal IgA and IgG responses. Following challenge, SARS-CoV-2 replication was undetectable in airways and lung tissues of immunized macaques. Ashhurst et al (Nature Communications 13:6972) developed a subunit vaccine consisting of spike protein with a Toll-like-receptor 2 (TLR2) adjuvant, an activating innate immune receptor expressed in respiratory epithelia. Mucosal vaccination in mice provided complete protection against disease and sterilizing lung immunity against SARS-CoV-2. Liu et al (Nature Communications 13:6792) engineered by deletion and mutation an attenuated SARS-CoV-2 vaccine candidate. Intranasal immunization induced a robust antibody response and protected hamsters not only against disease, but also reduced infectious viral titers in nasal wash samples 1000-fold.

REFERENCES

Afkhami, S., D'Agostino, M.R., Zhang, A., Stacey, H.D., Marzok, A., Kang, A. et al. (2022) Respiratory mucosal delivery of next-generation COVID-19 vaccine provides robust protection against both ancestral and variant strains of SARS-CoV-2. *Cell*, 185(5), 896–915.

Akst, J. (2022) Nasal vaccines are commercially high risk, perhaps high reward. *The Scientist*, 13 June 2022; nasal vaccines are commercially high risk, perhaps high reward | TS digest | the scientist. Available from: the-scientist.com

Barouch, D.H. (2022) Covid-19 vaccines - immunity, variants, boosters. *The New England Journal of Medicine*, 387(11), 1011–1020.

Belshe, R.B., Edwards, K.M., Vesikari, T., Black, S.V., Walker, R.E., Hultquist, M. et al. (2007) Live attenuated versus inactivated influenza vaccine in infants and young children. *The New England Journal of Medicine*, 356(7), 685–696.

Brown, C.M., Vostok, J., Johnson, H., Burns, M., Gharpure, R., Sami, S. et al. (2021) Outbreak of SARS-CoV-2 infections, including COVID-19 vaccine breakthrough infections, associated with large public gatherings - Barnstable County, Massachusetts, July 2021. *MMWR. Morbidity and Mortality Weekly Report*, 70(31), 1059–1062.

Callaway, E. (2022) New omicron-specific vaccines offer similar protection to existing boosters. *Nature*, 609(7926), 232–233.

Chaturvedi, S., Beutler, N., Vasen, G., Pablo, M., Chen, X., Calia, G. et al. (2022) A single-administration therapeutic interfering particle reduces SARS-CoV-2 viral shedding and pathogenesis in hamsters. *Proceedings of the National Academy of Sciences of the United States of America*, 119(39), e2204624119.

Chaturvedi, S., Vasen, G., Pablo, M., Chen, X., Beutler, N., Kumar, A. et al. (2021) Identification of a therapeutic interfering particle-a single-dose SARS-CoV-2 antiviral intervention with a high barrier to resistance. *Cell*, 184(25), 6022–6036.

Chia, P.Y., Ong, S.W.X., Chiew, C.J., Ang, L.W., Chavatte, J.M., Mak, T.M. et al. (2022) Virological and serological kinetics of SARS-CoV-2 Delta variant vaccine breakthrough infections: a multicentre cohort study. *Clinical Microbiology and Infection*, 28(4), 612.e1–612.e7.

Collier, A.Y., Brown, C.M., McMahan, K.A., Yu, J., Liu, J., Jacob-Dolan, C. et al. (2022) Characterization of immune responses in fully vaccinated individuals after breakthrough infection with the SARS-CoV-2 delta variant. *Science Translational Medicine*, 14(641), eabn6150.

Costa Clemens, S.A., Weckx, L., Clemens, R., Almeida Mendes, A.V., Ramos Souza, A., Silveira, M.B.V. et al. (2022) Heterologous versus homologous COVID-19 booster vaccination in previous recipients of two doses of CoronaVac COVID-19 vaccine in Brazil (RHH-001): a phase 4, non-inferiority, single blind, randomised study. *Lancet*, 399(10324), 521–529.

Dean, N.E. & Halloran, M.E. (2022) Protecting the herd with vaccination. *Science*, 375(6585), 1088–1089.

Elliott, P., Eales, O., Bodinier, B., Tang, D., Wang, H., Jonnerby, J. et al. (2022) Dynamics of a national omicron SARS-CoV-2 epidemic during January 2022 in England. *Nature Communications*, 13(1), 4500.

Elliott, P., Haw, D., Wang, H., Eales, O., Walters, C.E., Ainslie, K.E.C. et al. (2021) Exponential growth, high prevalence of SARS-CoV-2, and vaccine effectiveness associated with the Delta variant. *Science*, 374(6574), eabl9551.

Eyre, D.W., Taylor, D., Purver, M., Chapman, D., Fowler, T., Pouwels, K.B. et al. (2022) Effect of Covid-19 vaccination on transmission of alpha and Delta variants. *The New England Journal of Medicine*, 386(8), 744–756.

Feng, L., Wang, Q., Shan, C., Yang, C., Feng, Y., Wu, J. et al. (2020) An adenovirus-vectored COVID-19 vaccine confers protection from SARS-COV-2 challenge in rhesus macaques. *Nature Communications*, 11(1), 4207.

Grohskopf, L.A., Sokolow, L.Z., Broder, K.R., Walter, E.B., Bresee, J.S., Fry, A.M. et al. (2017) Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices - United States, 2017-18 influenza season. *MMWR - Recommendations and Reports*, 66(2), 1–20.

Harris, R.J., Hall, J.A., Zaidi, A., Andrews, N.J., Dunbar, J.K. & Dabrera, G. (2021) Effect of vaccination on household transmission of SARS-CoV-2 in England. *The New England Journal of Medicine*, 385(8), 759–760.

Hassan, A.O., Feldmann, F., Zhao, H., Curiel, D.T., Okumura, A., Tang-Huau, T.L. et al. (2021) A single intranasal dose of chimpanzee adenovirus vectored vaccine protects against SARS-CoV-2 infection in rhesus macaques. *Cell Reports Medicine*, 2(4), 100230.

Hassan, A.O., Kafai, N.M., Dmitriev, I.P., Fox, J.M., Smith, B.K., Harvey, I.B. et al. (2020) A single-dose intranasal Chad vaccine protects upper and lower respiratory tracts against SARS-CoV-2. *Cell*, 183(1), 169–184.

Hayek, S., Shaham, G., Ben-Shlomo, Y., Kepten, E., Dagan, N., Nevo, D. et al. (2022) Indirect protection of children from SARS-CoV-2 infection through parental vaccination. *Science*, 375(6585), 1155–1159.

Ku, Z., Xie, X., Hinton, P.R., Liu, X., Ye, X., Muruato, A.E. et al. (2021) Nasal delivery of an IgM offers broad protection from SARS-CoV-2 variants. *Nature*, 595(7869), 718–723.

Langel, S.N., Johnson, S., Martinez, C.I., Tedjakusuma, S.N., Peinovich, N., Dora, E.G. et al. (2022) Adenovirus type 5 SARS-CoV-2 vaccines delivered orally or intranasally reduced disease severity and transmission in a hamster model. *Science Translational Medicine*, 14(658), eabn6868.

Lapuente, D., Fuchs, J., Willar, J., Vieira Antão, A., Eberlein, V., Uhlig, N., et al. (2021) Protective mucosal immunity against SARS-CoV-2 after heterologous systemic prime-mucosal boost immunization. *Nature Communication* 12(1), 6871.

Layan, M., Gilboa, M., Gonen, T., Goldenfeld, M., Meltzer, L., Andronico, A. et al. (2022) Impact of BNT162b2 vaccination and isolation on SARS-CoV-2 transmission in Israeli households: an observational study. *American Journal of Epidemiology*, 191(7), 1224–1234.

Ledford, H. (2022) Which COVID boosters to take and when: a guide for the perplexed. *Nature*, 609(7925), 18–19.

Levine-Tiefenbrun, M., Yelin, I., Alapi, H., Herzel, E., Kuint, J., Chodick, G. et al. (2022) Waning of SARS-CoV-2 booster viral-load reduction effectiveness. *Nature Communications*, 13(1), 1237.

Levine-Tiefenbrun, M., Yelin, I., Alapi, H., Katz, R., Herzel, E., Kuint, J. et al. (2021) Viral loads of Delta-variant SARS-CoV-2 breakthrough infections after vaccination and booster with BNT162b2. *Nature Medicine*, 27(12), 2108–2110.

Levine-Tiefenbrun, M., Yelin, I., Katz, R., Herzel, E., Golan, Z., Schreiber, L. et al. (2021) Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine. *Nature Medicine*, 27(5), 790–792.

Li, J.X., Wu, S.P., Guo, X.L., Tang, R., Huang, B.Y., Chen, X.Q. et al. (2022) Safety and immunogenicity of heterologous boost immunisation with an orally administered aerosolised Ad5-nCoV after two-dose priming with an inactivated SARS-CoV-2 vaccine in Chinese adults: a randomised, open-label, single-Centre trial. *The Lancet Respiratory Medicine*, 10(8), 739–748.

Liu, X., Luongo, C., Matsuoka, Y., Park, H.S., Santos, C., Yang, L. et al. (2021) A single intranasal dose of a live-attenuated parainfluenza virus-vectored SARS-CoV-2 vaccine is protective in hamsters. *Proceedings of the National Academy of Sciences of the United States of America*, 118(50), e2109744118.

Lyngse, F.P., Mølbak, K., Denwood, M., Christiansen, L.E., Møller, C.H., Rasmussen, M. et al. (2022) Effect of vaccination on household transmission of SARS-CoV-2 Delta variant of concern. *Nature Communications*, 13(1), 3764.

Madhavan, M., Ritchie, A.J., Aboagye, J., Jenkin, D., Provstgaad-Morys, S., Tarbet, I. et al. (2022) Tolerability and immunogenicity of an intranasally-administered adenovirus-vectored COVID-19 vaccine: an open-label partially-randomised ascending dose phase I trial. *eBioMedicine*, 6, 104298.

Milman, O., Yelin, I., Aharony, N., Katz, R., Herzel, E., Ben-Tov, A. et al. (2021) Community-level evidence for SARS-CoV-2 vaccine protection of unvaccinated individuals. *Nature Medicine*, 27(8), 1367–1369.

Mutsch, M., Zhou, W., Rhodes, P., Bopp, M., Chen, R.T., Linder, T. et al. (2004) Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. *The New England Journal of Medicine*, 350(9), 896–903.

Nohynek, H. & Wilder-Smith, A. (2022) Does the world still need new Covid-19 vaccines? *The New England Journal of Medicine*, 386(22), 2140–2142.

Poehling, K.A., Caspard, H., Peters, T.R., Belongia, E.A., Congeni, B., Gaglani, M. et al. (2018) 2015-2016 vaccine effectiveness of live attenuated and inactivated influenza vaccines in children in the United States. *Clinical Infectious Diseases*, 66(5), 665–672.

Pritchard, E., Matthews, P.C., Stoesser, N., Eyre, D.W., Gethings, O., Vihta, K.D. et al. (2021) Impact of vaccination on new SARS-CoV-2 infections in the United Kingdom. *Nature Medicine*, 27(8), 1370–1378.

Prunas, O., Warren, J.L., Crawford, F.W., Gazit, S., Patalon, T., Weinberger, D.M. et al. (2022) Vaccination with BNT162b2 reduces transmission of SARS-CoV-2 to household contacts in Israel. *Science*, 375(6585), 1151–1154.

Salo, J., Hägg, M., Kortelainen, M., Leino, T., Saxell, T., Siikanen, M. et al. (2022) The indirect effect of mRNA-based COVID-19 vaccination on healthcare workers' unvaccinated household members. *Nature Communications*, 13(1), 1162.

Sano, K., Bhavsar, D., Singh, G., Floda, D., Srivastava, K., Gleason, C. et al. (2022) SARS-CoV-2 vaccination induces mucosal antibody responses in previously infected individuals. *Nature Communications*, 13(1), 5135.

Sarker, S.A., Berger, B., Deng, Y., Kieser, S., Foata, F., Moine, D. et al. (2017) Oral application of Escherichia coli bacteriophage: safety tests in healthy and diarrheal children from Bangladesh. *Environmental Microbiology*, 19(1), 237–250.

Sarker, S.A., McCallin, S., Barretto, C., Berger, B., Pittet, A.C., Sultana, S. et al. (2012) Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. *Virology*, 434(2), 222–232.

Sarker, S.A., Sultana, S., Reuteler, G., Moine, D., Descombes, P., Charton, F. et al. (2016) Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. *eBioMedicine*, 4, 124–137.

Shah, A.S.V., Gribben, C., Bishop, J., Hanlon, P., Caldwell, D., Wood, R. et al. (2021) Effect of vaccination on transmission of SARS-CoV-2. *The New England Journal of Medicine*, 385(18), 1718–1720.

Siddle, K.J., Krasilnikova, L.A., Moreno, G.K., Schaffner, S.F., Vostok, J., Fitzgerald, N.A. et al. (2022) Transmission from vaccinated individuals in a large SARS-CoV-2 Delta variant outbreak. *Cell*, 185(3), 485–492.

Singanayagam, A., Hakki, S., Dunning, J., Madon, K.J., Crone, M.A., Koycheva, A. et al. (2022) Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. *The Lancet Infectious Diseases*, 22(2), 183–195.

Subbaraman, N. (2021) How do vaccinated people spread Delta? What the science says. *Nature*, 596(7872), 327–328.

Telenti, A., Arvin, A., Corey, L., Corti, D., Diamond, M.S., García-Sastre, A. et al. (2021) After the pandemic: perspectives on the future trajectory of COVID-19. *Nature*, 596(7873), 495–504.

Topol, E.J. & Iwasaki, A. (2022) Operation nasal vaccine-lightning speed to counter COVID-19. *Science Immunology*, 7(74), eadd9947.

van Doremalen, N., Purushotham, J.N., Schulz, J.E., Holbrook, M.G., Bushmaker, T., Carmody, A. et al. (2021) Intranasal ChAdOx1 nCoV-19/AZD1222 vaccination reduces viral shedding after SARS-CoV-2 D614G challenge in preclinical models. *Science Translational Medicine*, 13(607), eabh0755.

Wagner, C.E., Saad-Roy, C.M., Morris, S.E., Baker, R.E., Mina, M.J., Farrar, J. et al. (2021) Vaccine nationalism and the dynamics and control of SARS-CoV-2. *Science*, 373(6562), eabj7364.

Waltz, E. (2022a) How nasal-spray vaccines could change the pandemic. *Nature*, 609(7926), 240–242.

Waltz, E. (2022b) China and India approve nasal COVID vaccines - are they a game changer? *Nature*, 609(7927), 450.

Watson, O.J., Barnsley, G., Toor, J., Hogan, A.B., Winskill, P. & Ghani, A.C. (2022) Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. *The Lancet Infectious Diseases*, 22(9), 1293–1302.

Wu, S., Huang, J., Zhang, Z., Wu, J., Zhang, J., Hu, H. et al. (2021) Safety, tolerability, and immunogenicity of an aerosolised adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in adults: preliminary report of an open-label and randomised phase 1 clinical trial. *The Lancet Infectious Diseases*, 21(12), 1654–1664.

Zhu, F., Zhuang, C., Chu, K., Zhang, L., Zhao, H., Huang, S. et al. (2022) Safety and immunogenicity of a live-attenuated influenza virus vector-based intranasal SARS-CoV-2 vaccine in adults: randomised, double-blind, placebo-controlled, phase 1 and 2 trials. *The Lancet Respiratory Medicine*, 10(8), 749–760.

Zhu, J., Jain, S., Sha, J., Batra, H., Ananthaswamy, N., Kilgore, P.B. et al. (2022) A bacteriophage-based, highly efficacious, needle- and adjuvant-free, mucosal COVID-19 vaccine. *MBio*, 13(4), e0182222.

How to cite this article: Brüssow, H. (2023) Do we need nasal vaccines against COVID 19 to suppress the transmission of infections? *Microbial Biotechnology*, 16, 3–14. Available from: https://doi.org/10.1111/1751-7915.14181