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Opinion

Upper respiratory tract mucosal immunity for SARS-CoV-2 vaccines

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SARS-CoV-2 vaccination significantly reduces morbidity and mortality, but has less impact on viral transmission rates, thus aiding viral evolution, and the longevity of vaccine-induced immunity rapidly declines. Immune responses in respiratory tract mucosal tissues are crucial for early control of infection, and can generate long-term antigen-specific protection with prompt recall responses. However, currently approved SARS-CoV-2 vaccines are not amenable to adequate respiratory mucosal delivery, particularly in the upper airways, which could account for the high vaccine breakthrough infection rates and limited duration of vaccine-mediated protection. In view of these drawbacks, we outline a strategy that has the potential to enhance both the efficacy and durability of existing SARS-CoV-2 vaccines, by inducing robust memory responses in the upper respiratory tract (URT) mucosa.

Highlights

Existing SARS-CoV-2 vaccination programmes have several drawbacks: (i) low impact on vaccine breakthrough infections and subsequent transmission in the community (viral replication in vaccinated populations fuels vaccine escape to promote the emergence of new vaccine-resistant SARS-CoV-2 variants); (ii) limited duration of vaccine-mediated protection; and (iii) inefficient vaccine responses in vulnerable individuals at high risk of severe COVID-19, due to compromised host intrinsic antiviral mechanisms.

Early control of infection via robust antigen-specific mucosal responses in the upper airways could limit viral dissemination and reduce transmission rates. However, current licensed vaccines and their routes of administration induce mainly systemic, rather than mucosal, immunity.

A boosting strategy to induce potent antigen-specific memory responses in the upper respiratory tract mucosa may enhance overall efficacy and durability of vaccine-mediated protection, even in individuals with compromised antiviral mechanisms.

Synopsis

Existing licensed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines include mRNA vaccines by Moderna (mRNA-1273 and the more recent bivalent Wuhan–Omicron combinations, e.g., BA.1 mRNA-1273.214 and BA.4/5 mRNA-1273.222) and Pfizer/BioNTech (monovalent BNT162b2 and bivalent Omicron BA.4/5-adapted); a chimpanzee adenovirus-vectored vaccine by AstraZeneca/University of Oxford (ChAdOx1); recombinant, replication-incompetent human adenovirus type 26-vectored vaccine by Janssen (Ad26.COV2.S); and a protein-subunit vaccine encased around a nanoparticle core by Novavax (NVX-CoV2373). All these encode the full-length SARS-CoV-2 spike (S) glycoprotein. While we mainly refer to relevant evidence pertaining to these first-generation vaccines (and the Omicron-adapted bivalent mRNA vaccines), there are many other SARS-CoV-2 vaccines that are in clinical use globally, including inactivated whole virus vaccines, as well as other adenovirus-vectored vaccines and protein-subunit vaccines encoding the SARS-CoV-2 S glycoprotein or receptor-binding domain.

Early control of SARS-CoV-2 infection and prevention of transmission are heavily dependent on robust mucosal immune responses in the URT [1–4]. However, current licenced SARS-CoV-2 vaccines induce predominantly systemic responses, rather than potent, durable responses in the URT mucosa [5–16]. Thus, despite their effectiveness in reducing disease severity, hospitalisation rates, and mortality, these vaccines do not generate sterilising immunity, thereby having a limited role in preventing infection and blocking subsequent viral transmission [17–20]. Suboptimal vaccine-induced immunity that does not adequately inhibit transmission in the community, hence permitting ongoing cycles of viral replication, can promote the establishment of new, highly virulent, vaccine-resistant SARS-CoV-2 variants [21,22]. Although current licenced vaccines may be effective against severe disease and death, even if to variable degrees, against some SARS-CoV-2 variants of concern (VOCs) [23–26], the VOCs demonstrate an aptitude for immune escape with significantly reduced sensitivity to antibody neutralisation [17,27–40]. While Omicron-adapted bivalent vaccines can increase the breadth of neutralisation of different

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SARS-CoV-2 strains systemically [13,14,41], suggesting this approach may mitigate the impact of Omicron BA.4/5 as well as new emergent VOCs, transmission cannot be prevented without the induction of URT mucosal responses. Moreover, vaccine-mediated protection against high viral burden and symptomatic infection rapidly wanes within 3–6 months [42–50]. We therefore discuss the rationale for investigating the additional administration of a clinically approved protein formulation, alongside existing SARS-CoV-2 vaccination strategies (without the necessity to reconstruct each vaccine), which may be capable of inducing robust URT mucosal immunity to (i) reduce transmission rates through early control of SARS-CoV-2 infection in the upper airways; (ii) slow down viral evolution by allowing less efficient viral replication; and (iii) enhance the longevity of vaccine-induced protection.

Role of URT mucosal immunity in vaccine efficacy and durability

The airway, mouth, and ocular mucosa represent the main ports of SARS-CoV-2 viral entry, providing the first line of defence against infection [51,52]. Early control via respiratory mucosal immunity, particularly in the URT, is therefore imperative for limiting viral trajectory into the lower respiratory tract, thus preventing disease progression, and URT mucosal immunity can also generate sterilising immunity, in turn, blocking viral transmission [1,3,53,54]. Vaccines that prevent transmission can block viral evolution towards variants with increased virulence, while high transmission rates in a vaccinated population may promote the establishment of new vaccine-resistant SARS-CoV-2 VOC [21,22], associated with increased transmissibility and immune evasion [27,29,35,39,55–59]. It is generally accepted that natural selection removes highly virulent viral strains that cause severe disease and death, since this also eliminates the virus. Therefore, vaccines that allow hosts to survive but do not prevent transmission, allow more virulent strains to emerge, whereas if a vaccine is able to prevent transmission, viral evolution towards increased virulence is blocked [22]. Indeed, previous studies in animals, as well as mathematical modelling of SARS-CoV-2 transmission in vaccinated individuals, have indicated that the highest risk of new vaccine-resistant strain establishment and pathogen escape from host immunity, occur when a large proportion of the population has been vaccinated but viral transmission is not controlled [21,22,60]. Facilitation of such evolutionary developments can put individuals that do not produce efficacious vaccine-mediated protective responses (e.g., immunocompromised individuals, those with chronic inflammatory diseases, obese subjects, or the older population) [61–68] (or unvaccinated hosts) at greater risk of severe disease or death [22]. This underlines the urgent necessity to contain viral transmission, even among populations in which vaccination rates are high, to avoid the uncontrolled spread of SARS-CoV-2 that can lead to further accumulation of critical mutations.

An additional challenge of new emerging VOC is the necessity for constant reassessment of correlates of protection (CoP). Remarkably, a consensus on CoP for SARS-CoV-2 vaccine efficacy has not been universally agreed, although there has been support for SAR-CoV-2 S-specific IgG concentrations and anti-SARS-CoV-2 neutralising antibody titres as CoP against symptomatic coronavirus disease 2019 (COVID-19) [69]. However, due to the respiratory mucosal nature of SARS-CoV-2 infection, CoP for SARS-CoV-2 vaccines should include URT mucosal immunity, particularly when evaluating protection against infection, as opposed to symptomatic disease alone. SARS-CoV-2 S-specific URT mucosal IgA responses, for example, can provide protection against acquisition of SARS-CoV-2 infection [70], and durable SARS-CoV-2-specific T cell responses in the URT mucosa may also contribute to generating sterilising immunity [71].

Mucosal immunity also confers long-term protection [53,72,73], and despite the induction of robust respiratory tract mucosal memory responses in patients with a history of a milder COVID-19 disease course [74], there is evidence of steady and rapid decline in the longevity of

vaccine-mediated antiviral immune responses [42–50]. While several factors (including older age, comorbidities or immunosuppressed status of vaccinees, or change in dominance of SARS-CoV-2 variants) can contribute to the limited duration of vaccine-generated protective immunity, this declining immunogenicity may be significantly attributed to suboptimal vaccine-mediated induction of respiratory tract mucosal responses [10,12,54,75]. Tissue-resident memory T cells (TRMs), in particular, provide immune surveillance at mucosal barrier tissues, and can induce rapid antigen-specific recall responses [53,72,73]. Indeed, several studies have demonstrated that respiratory tract TRMs confer robust protection from respiratory pathogens, even in the absence of neutralising antibody responses [4,53,76,77], and reduced COVID-19 severity has been associated with increased numbers of airway $CD4^+$ and $CD8^+$ TRMs [78,79]. However, lung TRMs can only confer transient protection, as they undergo attrition and significantly decay in number over time, and are not self-sustaining [53,80]. Conversely, URT (nasal) TRMs can persist, with minimal decay, as nasopharyngeal tissue does not encounter the same erosion, and can thus confer durable protection against influenza virus infection, preventing the development of severe pulmonary disease [76]. Moreover, since SARS-CoV-2 infection initially affects the nasopharynx and oral mucosa, it is URT rather than pulmonary TRMs that can control the infection before it reaches the lower respiratory tract and terminal airways of the lungs to cause pulmonary pathology. As such, the design of vaccines optimised for potent URT TRM responses (in addition to circulating SARS-CoV-2-specific memory responses) may offer a valuable protection strategy that ensures optimal long-term SARS-CoV-2 vaccine efficacy, with durable respiratory mucosal immunogenicity.

Finally, mucosal immunogenicity can also induce trained immunity, which refers to the epigenetic memory (with transcriptional and functional reprogramming) of innate immune cells to inflammatory pathogenic encounters, and confers enhanced innate immune responsiveness to subsequent viral triggers [54,81,82]. Trained immunity has the added benefit of being an immediate response (compared to the longer adaptive immune response time), can boost host defence responses by overcoming virus-imposed innate immunosuppression, while also facilitating downstream adaptive mechanisms [54]. Vaccines that induce trained immunity can stimulate both nonspecific and specific immune mechanisms via long-term enhancement of innate responses that mobilise adaptive immunity, and represent a valuable attribute to provide broader protection beyond conventional vaccines [81,83]. Harnessing this function in the development of SARS-CoV-2 vaccines could therefore elicit stronger responses to SARS-CoV-2 encounter following vaccination [54,84].

The route of vaccine administration and induction of respiratory tract mucosal immunity

Mucosal immune responses are dependent on the site of inflammatory induction due to their anatomical compartmentalisation. The route of vaccine administration can therefore strongly impact the type of local immunity induced. The amenability of mRNA vaccines to induce respiratory mucosal immunity has been questioned [54], and while some viral vectors demonstrate natural tropism for the respiratory mucosa [85], parenteral vaccines against respiratory pathogens often fail to induce strong respiratory mucosal memory responses [86–90]. Conversely, respiratory mucosal vaccination can effectively generate robust airway and lung TRMs, T follicular helper (T_{fh}) cells that are critical in mediating antibody responses, as well as mucosal IgA antibodies [51,91–95]. Indeed, murine studies have demonstrated complete protection from lethal SARS-CoV infection after intranasal, but not subcutaneous, vaccination [1]. Likewise, several preclinical animal studies investigating mucosal SARS-CoV-2 vaccines have reported that intranasal immunisation can provide durable protection in the upper and lower respiratory tracts, against both historical and emerging SARS-CoV-2 variants [91,93,96–98]. However, many intranasal vaccine platforms face significant challenges in terms of their efficacy, and are often deemed unsafe

for human use, compared to parenteral vaccines [54,99–101]. One of the biggest challenges in developing intranasal vaccines is nasal mucociliary clearing, whereby the vaccine is diluted in mucosal secretions, captured in mucus gels, subjected to enzymatic breakdown, and eliminated by epithelial barriers, thus affecting drug absorption and bioavailability. Considerably higher vaccine doses are therefore necessary to deliver sufficient levels of vaccine antigen to antigen-presenting cells (APCs) within the URT mucosa, that is required to elicit the desired protective responses [99,101], which may exceed safety limits for human use.

The BNT621b2, mRNA-1273, ChAdOx1, Ad26.COV2.S, and NVX-CoV2373 vaccination programmes all have parenteral administration (intramuscular injection) routes [51] (all SARS-CoV-2 vaccines in clinical use are parenterally administered) [100], and induce strong systemic immune responses associated with high serum IgG antibody titres (via the recruitment of circulating B cells, alongside circulating T_h1-biased $CD4^+$ T cell and $CD8^+$ cytotoxic T lymphocyte responses) in healthy adults [5–9]. These vaccines also elicit SARS-CoV-2 S-specific serum IgA responses, and while serum IgA is clonally related to mucosal IgA and may reach mucosal surfaces via different routes, SARS-CoV-2 S-specific serum IgA levels decay significantly faster than S-specific IgG [75,102–104]. Conversely, robust mucosal IgA responses in the URT, where cells are initially targeted by SARS-CoV-2, can prevent viral dissemination [54], in turn, preventing disease progression and inhibiting community transmission. Several studies have demonstrated that the approved first-generation SARS-CoV-2 mRNA vaccines are unable to elicit effective mucosal responses in the respiratory tract, thus offering limited protection against breakthrough infection [10,11], and challenge studies of ChAdOx1-vaccinated primates did not reduce upper respiratory viral loads [12]. The inability of the URT mucosa of vaccinated subjects to respond to viral challenge suggests a lack of, or suboptimal vaccine-induced URT TRM establishment, since airway TRMs (principally those in the URT) provide first line of defence against respiratory pathogens such as SARS-CoV-2 [1,105]. Upon viral encounter, $CD4^+$ TRMs can generate rapid antigen-specific recall responses via proliferation, and inflammatory cytokine/chemokine release to generate a T_h1-biased local tissue environment, which subsequently promotes the recruitment of other immune cells to establish an antiviral state, and $CD8^+$ TRMs can elicit immediate antiviral effector functions through degranulation and cytotoxicity, in addition to cytokine production [106,107].

It is generally accepted that local antigen encounter is needed in the airways (especially in the lungs) to establish TRMs [108], although this would be not the case after systemic (intramuscular) vaccination. However, there are reports of induction of lower respiratory tract mucosal responses following intramuscular SARS-CoV-2 vaccination [12,109,110]. To reach the lungs, it is possible that the SARS-CoV-2 vaccine antigen diffuses from the injection site to regional draining lymph nodes, and is subsequently taken up by local APCs, that then migrate to the pulmonary mucosa-associated lymphoid tissue, as well as nasopharynx-associated lymphoid tissue [111–113]. Indeed, there are descriptions of nasal immune responses following vaccination with BNT162b2 and mRNA-1273 [104,107,114], and nasal TRM establishment can be induced independently of local antigen encounter [76,115]. Nonetheless, the high rates of breakthrough infections and community transmission following vaccination indicate that any responses in the URT are insufficient or transient [116]. Another study detected nasal SARS-CoV-2-specific $CD4^+$ and $CD8^+$ TRMs almost exclusively in vaccinees with SARS-CoV-2 breakthrough infections, but not in vaccinated subjects that did not encounter circulating SARS-CoV-2 [117]. Notably, that there is no reduction in peak viral loads in vaccinated individuals with breakthrough infections, compared to infections in unvaccinated cases, in settings with elevated viral transmission [17,18], emphasises the necessity for supplementary measures to control infection and transmission rates.

Type 1 interferon signalling in the URT: early infection control and generation of sterilising immunity

Effective, long-lasting vaccine-induced protection from severe COVID-19 is dependent upon the proficiency of host innate and adaptive responses in promoting the generation of SARS-CoV-2-specific memory T cell subsets, germinal centre (GC)-derived memory B cells, and long-lived plasma cells. Host innate immunity also plays a pivotal role in the development of vaccine-mediated immunological memory, by providing the necessary signals for T cell activation, thus mobilising antigen-specific adaptive memory responses [118,119]. Human type 1 interferons (IFN-1s; which include 13 partially homologous IFN-α subtypes, a single IFN-β subtype, and the lesser known κ, ε, δ, τ, and ω subtypes) are typically produced by APCs at mucosal surfaces in response to viral antigenic presence, which is detected by intrinsic pattern recognition receptors: a cascade of IFN-1-mediated inflammatory events leads to the induction of IFN-stimulated gene (ISG) transcription programmes that interfere with viral replication and promote the deployment of innate and antigen-specific, adaptive antiviral effects [120–125]. A rapid canonical IFN-1 response with T_h1 activation, followed by cytotoxic lymphocyte effector functions, is fundamental for bestowing prompt and effectual antiviral protection against SARS-CoV-2 [120,126–128]. IFN-1s also promote T_{fh} cell differentiation, and, in turn, GC reactions, which are necessary for durable antibody responses with potent neutralising capacity [123,129], as well as directly stimulating B cell responses [130–132]. In addition to these antigen-specific adaptive antiviral effects, IFN-1 signalling can further modify innate immune responses upon pathogen encounter, to induce trained immunity through epigenetic mechanisms [133,134].

Following initial SARS-CoV-2 infection, exponential viral replication strongly induces ISG expression in the nasopharynx within the first week, and IFN/ISG-mediated defences in the URT can promptly curtail SARS-CoV-2 replication, and would therefore also be predicted to reduce peak viral load, viral transmission, and host susceptibility [3]. In patients with mild–moderate COVID-19 who achieve recovery, there is an early, transient wave of circulatory IFN-α (and an absence of circulating IFN-β), while severe or critical cases demonstrate low or no IFN-α responses, accompanied by clinical deterioration and transfer to intensive care units, indicating that IFN-α is crucial for early control of SARS-CoV-2 infection [127]. Indeed, severe COVID-19 is characterised by a hallmark of blunted canonical IFN-α expression, that is likely due to a combination of impaired host antiviral mechanisms [127,135–137], and a degree of viral antagonism [138]. IFN-α signalling in the respiratory tract mucosa may also be important for TRM differentiation: a recent report demonstrated that IFN-α is required for the adequate expansion and function of airway $CD8^+$ TRMs during respiratory syncytial virus re-infection [139].

The vaccines described here induce IFN-1 secretion and intracellular SARS-CoV-2 S production by APCs, upon uptake of vaccine-derived nucleic acids/antigens at the injection site, and the APCs subsequently deliver inflammatory and antigenic signals to T cells in lymph nodes draining the injection site, to mediate predominantly systemic SARS-CoV-2-S-specific adaptive immunity, but with limited delivery to the respiratory tract mucosa, and particularly to the URT [10–12]. It is therefore unlikely that there is significant IFN-1/ISG induction or development of antigen-specific immune memory in URT mucosa following intramuscular immunisation with the current licensed SARS-CoV-2 vaccines, which may, at least partly, explain why these vaccines do not prevent initial URT SARS-CoV-2 infection and subsequent transmission.

Induction of URT mucosal immunity with intranasal IFN-α coadministration could enhance SARS-CoV-2 vaccine efficacy and durability

The high rates of viral transmission even in vaccinated populations, and the limited duration of vaccine-mediated protection, may be attributed to the insufficient vaccine-mediated induction

of URT mucosal responses with suboptimal URT TRM establishment [10–12,116,140]. We therefore propose targeting URT mucosal immunity via the addition of an intranasal IFN-1 adjuvant alongside existing SARS-CoV-2 vaccines (all parenterally administered), which could potentially enhance both vaccine efficacy and durability, by initiating an inflammatory signal in the nasopharyngeal mucosa to evoke URT TRM development, as well as promoting URT mucosal IgA responses (Figure 1, Key figure; and Box 1).

Early canonical IFN-1 signalling within the URT mucosa can act on local vascular beds and alveolar capillaries to induce the endothelial expression of leukocyte trafficking molecules, and stimulate the production of chemotactic cues for recruitment of antigen-specific lymphocytes and innate antiviral effector cells [141–144]. As described previously, although intranasal

Key figure

Proposed protection conferred by vaccine alone versus vaccine plus intranasal interferon (IFN)-α

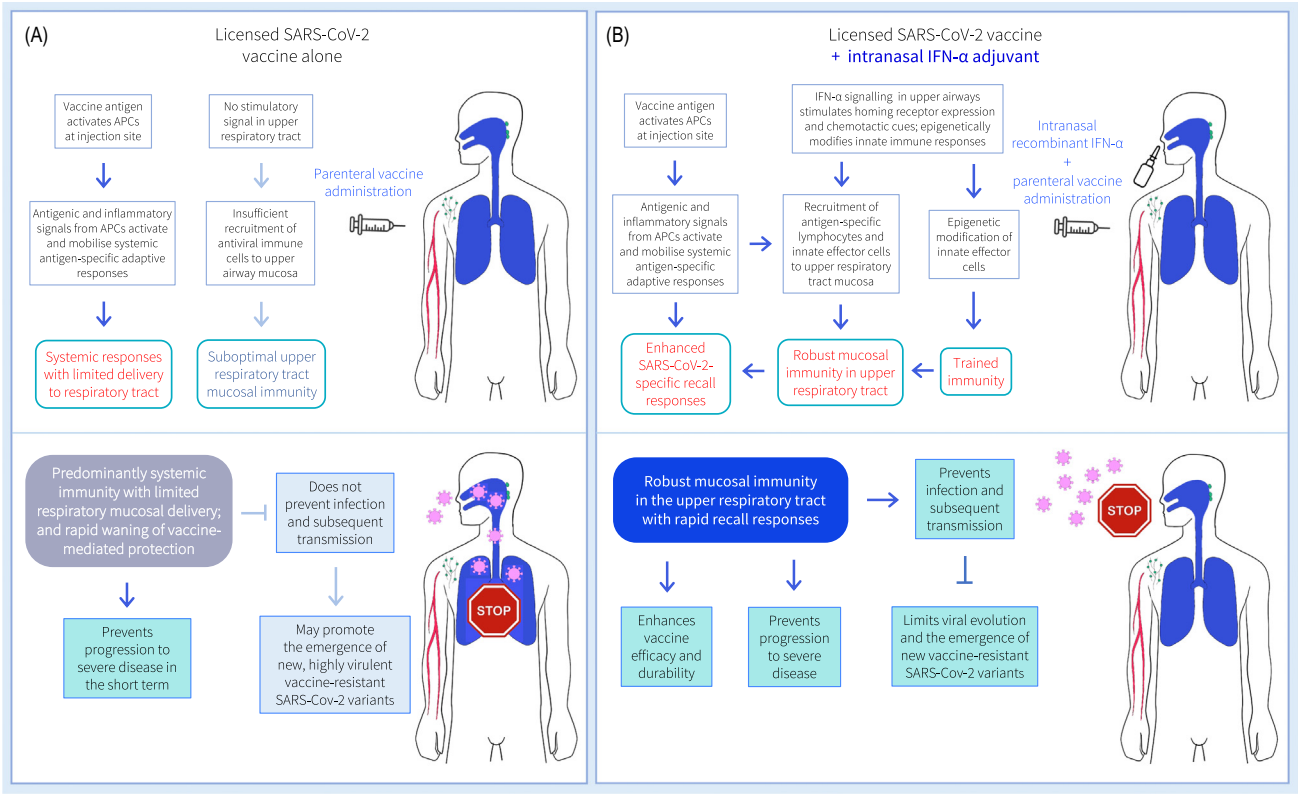

Figure 1. (A) Currently licensed SARS-CoV-2 vaccines and their routes of administration activate antigen-presenting cells (APCs) at the injection site, which produce antigenic and inflammatory signals to activate predominantly systemic responses, with limited delivery to the respiratory tract mucosae. Robust systemic responses can prevent progression to severe disease in the short term, but in the absence of upper respiratory tract mucosal immunity, does not prevent infection and subsequent community transmission. (B) Intranasal coadministration of a clinically approved IFN-α formulation, as a vaccine adjuvant alongside licensed parenteral SARS-CoV-2 vaccination programmes, may increase the efficacy and durability of existing SARS-CoV-2 vaccines via the induction of upper respiratory tract mucosal memory responses, trained immunity, and enhanced overall immunogenicity. Early control of SARS-CoV-2 infection in the upper respiratory tract could help to reduce transmission rates, enhance the durability of vaccine-mediated protection, increase vaccine efficacy even in individuals with compromised intrinsic antiviral mechanisms, and slow down viral evolution.

Box 1. Advantages of using an intranasal IFN-α as an adjuvant for current licensed SARS-CoV-2 vaccines

- Existing SARS-CoV-2 vaccines predominantly induce systemic SARS-CoV-2-specific responses, rather than potent and durable responses in the URT mucosa; these vaccines therefore do not generate sterilising immunity, since infection and subsequent transmission cannot be prevented without the induction of URT mucosal responses.
- The additional administration of a clinically approved IFN-α formulation, alongside existing SARS-CoV-2 vaccination strategies, may be capable of inducing robust URT mucosal immunity.
- IFN-α is produced in the URT mucosa during SARS-CoV-2 infection, and intranasal IFN-α administration thereby functionally mimics the natural host immune response.
- IFN-α signalling in the URT may induce an environmental cue with a specific ISG-mediated inflammatory milieu that promotes a chemotactic gradient, with local lymphocyte homing receptor expression for the induction of URT TRM cell establishment.
- URT TRMs provide the first line of defence against respiratory pathogens like SARS-CoV-2 (even in the absence of neutralising antibodies), can persist with minimal decay, and can block viral spread before it reaches the lungs to cause pulmonary pathology.
- IFN-α signalling has been implicated in the generation of $CD8^+$ TRMs, and is a potent inducer of the TRM cell surface activation marker, CD69.
- IFN-α signalling induces a T_h1-polarised local tissue environment, with a TNF-α-, IL-2-, IL-12-, and IL-15-rich cytokine profile, and these cytokines can evoke TRM differentiation.
- IL-12 and IL-15 expression may also be able to promote nonspecific $CD4^+$ TRM activation, which can subsequently differentiate into polyfunctional T cells with potent antiviral effector functions.
- IFN-α signalling induces CXCL10 expression as part of the ISG response, promoting the recruitment of $CXCR3^+$ lymphocytes to the T_h1-polarised tissue (CXCR3 is the cognate receptor for CXCL10).
- SARS-CoV-2 can antagonise the host IFN-1 response by various mechanisms, which is why SARS-CoV-2 infection is a weak inducer of the IFN-1 response compared to other respiratory RNA viruses, and may be the reason why URT TRM responses are transient following SARS-CoV-2 infection (in both vaccinated and unvaccinated subjects). The addition of an exogenous intranasal IFN-α formulation would overcome this problem, and may promote more durable TRM responses.
- IFN-α promotes T_{fh} cell differentiation, and, in turn, GC reactions (GCs are transient microanatomical structures within secondary lymphoid organs, where B cells acquire memory): exogenous IFN-α can generate sufficient adjuvant activity to induce GC formation, which are required for durable antibody responses with potent neutralising capacity.
- IFN-α signalling in URT can potentiate mucosal IgA responses within the nasopharyngeal mucosa, which may provide early control of infection.
- The adjuvant activity of exogenous intranasal IFN-α, when administered concomitantly with parenteral SARS-CoV-2 vaccines, could also augment IFN-activated APC trafficking to the injection site, thereby enhancing systemic responses to vaccination. IFN-α may be able to promote cross-priming of circulating $CD8^+$ cytotoxic T lymphocytes, whereby responses can be generated against antigens that are not expressed directly within IFN-1-activated APCs, therefore also enhancing the systemic responses to vaccination.
- There are several clinically approved IFN-α formulations, with established dosing and safety profiles, that are available for human use. We also note the development of viral human challenge models, which could be an additional arena for testing adjuvant IFN-α dosing.
- The addition of a clinically approved IFN-α adjuvant to existing vaccination strategies would allow the use of the first-generation SARS-CoV-2 vaccines without the necessity to reconstruct each vaccine.

SARS-CoV-2 vaccination can elicit strong memory responses in the URT mucosa in experimental animals [91,92,94,145,146], unlike parenteral vaccination [10–12,86,87], few are effective in humans compared to the parenteral vaccination route and have largely failed to progress beyond Phase 1 clinical trials. URT IFN-1 stimulation, on the other hand, can generate robust responses in the respiratory tract mucosa [132,147].

URT mucosal (oromucosal) administration of recombinant IFN-1s, in combination with parenteral influenza vaccination, can significantly enhance vaccine efficacy by mimicking natural mucosal IFN-1 protection, promoting dendritic cell maturation, antigen presentation and trafficking, and stimulating T_h1-mediated cellular immunity (while parenteral IFN-1 administration is associated with systemic toxicity) [147–150], and exogenous IFN-1s can generate sufficient adjuvant activity to induce GC formation upon antigen stimulation [132]. Using a similar approach, a recent study demonstrated that coadministration of mucosal CCL27 enhances SARS-CoV-2 DNA vaccine-induced responses [151], and another study found that stimulating the appropriate local inflammatory milieu (via intranasal zymosan administration) can bypass the requirement for local antigen

encounter in respiratory tract TRM development, but may cause excessive pulmonary inflammation, thus making this choice of adjuvant unsafe in clinical practice [152]. However, there are several clinically approved IFN-1 formulations with established dosing and safety profiles available for human use [153–156].

The adjuvant activity of exogenous IFN-1s, administered via an intranasal route, concomitantly with parenteral administration of SARS-CoV-2 vaccines, could enhance systemic responses to vaccination via augmented trafficking of IFN-1-activated APCs to the injection site and cross-priming of $CD8^+$ cytotoxic T lymphocytes [147,157–159], and may also be capable of promoting local lymphocyte homing receptor expression and environmental cues in the URT [3,76,160–162] (Box 1). It is possible that the induction of this additional exogenous IFN-1-mediated inflammatory response in the URT could provide a chemotactic gradient with a specific ISG-mediated inflammatory milieu, to mobilise SARS-CoV-2-specific lymphocytes from the lungs up to the nasopharyngeal mucosa, and promote their recruitment from circulating T cell pools [3,160,163], in order to establish URT TRMs. For example, the ISG response in the URT includes chemokine CXC ligand (CXCL)10 expression, which may promote the recruitment of $CD4^+$ T_h1 cells, $CD8^+$ cytotoxic T lymphocytes, and natural killer cells, due to their expression of its cognate receptor, CXCR3 [3,160]. CXCR3 mediates chemotaxis towards T_h1-polarised inflamed tissue, and its upregulated expression is a characteristic of airway TRMs [80,144,164,165]. IFN-1/ISG signalling in the URT may generate additional T_h1-biased inflammatory signals, including the expression of cytokines such as tumour necrosis factor (TNF)-α, IL-2, IL-12, and IL-15, which can also promote TRM differentiation [76,161,162]. Further, IFN-α has been demonstrated as a potent inducer of the TRM cell surface activation marker, CD69 [163], and IFN-α signalling has been recently implicated in the generation and regulation of $CD8^+$ TRM responses to RSV infection [139]. Of note, $CD4^+$ TRMs can also be nonspecifically activated by several proinflammatory cytokines (including IL-12 and IL-15), and these TRMs subsequently differentiate into polyfunctional T cells with potent antipathogenic capacity [166]. IFN-α signalling has also been implicated in potentiating mucosal IgA production at the URT mucosa [167], production of which can limit viral spread to prevent the development of severe pulmonary disease, as well as inhibit viral transmission [54].

At this point, it is important to highlight that timing, dose, and type of IFN-1 administration are crucial for generating effective antiviral responses, without causing hyperinflammatory tissue damage [168–170]. Although there are several ongoing clinical trials assessing the therapeutic use of IFN-1s for COVID-19 [169,171], this approach should be treated with caution, as it may be too late to administer a safe and efficacious IFN-1 therapy once SARS-CoV-2 infection is established [169,170,172]. An effectual antiviral response in SARS-CoV-2 infection is reliant on a prompt and transient canonical IFN-α response [126,127], whereas IFN-β expression is absent from all patients in early SARS-CoV-2 infection [127], and severe COVID-19 is associated with absent early canonical IFN-α signatures but pronounced late noncanonical IFN-β signalling, which activates hyperinflammatory nuclear factor-κB-dependent programmes that restrain antiviral responses [173–175]. Thus, late IFN-1 administration (particularly IFN-β) could induce noncanonical hyperinflammatory response pathways (that do not inhibit viral replication) to exacerbate viral persistence, inflammation, and disease pathogenesis [168,176]. Conversely, when coadministered intranasally with parenteral SARS-CoV-2 vaccines, IFN-α may be considerably more effective in mediating robust URT mucosal memory by inducing inflammatory chemotactic cues that could further stimulate the development of nasal TRM cells; thereby promoting early control of infection in the upper airways and preventing progression to severe disease, blocking viral transmission in the community, and generating SARS-CoV-2-specific long-term protection with rapid recall responses (Figure 1). While several prime–pull strategies with mucosal administration of cytokines/chemokines following vaccination, to enhance mucosal delivery of antigen-

Clinician's corner

Current SARS-CoV-2 vaccines elicit inefficient URT mucosal responses, which are vital for preventing disease progression and generating sterilising immunity.

Intranasal IFN-α co-administration with current SARS-CoV-2 vaccines may offer a convenient solution to induce URT mucosal responses:

- IFN-α functionally mimics the natural host immune response
- several approved IFN-α formulations are available for human use
- easy to administer

specific lymphocytes, have been explored in the context of other infectious diseases [177,178], this strategy appears to be missing from the current SARS-CoV-2 literature. We propose the concurrent administration of intranasal IFN-α with the first-generation SARS-CoV-2 parenteral vaccines (rather than sequentially, following vaccine administration), since URT mucosal IFN-1 coadministration with parenteral vaccination has been reported as an effectual and well-tolerated means of enhancing vaccine efficacy [147,148].

We therefore postulate that existing, clinically approved, intranasally administered IFN-α formulations might represent a promising strategy as vaccine adjuvants with current licensed SARS-CoV-2 vaccination programmes, that is worth investigating in order to test their capacity to reduce SARS-CoV-2 infection and transmission rates while improving the durability of vaccine-mediated protection.

Concluding remarks

The COVID-19 pandemic, as well as several other respiratory pathogen outbreaks throughout history, has had devastating effects on human health, with significant economic burden. Current SARS-CoV-2 vaccination programmes do not adequately induce URT mucosal immune responses, which may be reflected in high vaccine breakthrough infection and transmission rates, and the limited duration of vaccine-mediated protection. However, the induction of SARS-CoV-2-specific URT mucosal immunity may reduce transmission rates, limit viral evolution, and improve the longevity of vaccine-induced immunity (see Outstanding questions), and could thus help to bring the pandemic to an end. We therefore consider that intranasal IFN-α, coadministered as an adjuvant to existing SARS-CoV-2 vaccines, could be a valuable strategy to enhance overall vaccine efficacy and durability, through its potential for the induction of robust URT TRM responses for conferring the first line of SARS-CoV-2-specific immunological defence.

Outstanding questions

Will the coadministration of intranasal IFN-α alongside current licensed SARS-CoV-2 be able to elicit levels of URT mucosal responses that are potentially sufficient to confer sterilising immunity?

How will the impact of the additional intranasal IFN-α administration on viral transmission be measured accurately?

Will different optimum IFN-α doses need to be stratified for different demographic groups (e.g., higher doses in vulnerable individuals at high risk of developing severe COVID-19, including those with an immunosuppressed status, comorbidities, or age-related susceptibility, who produce inefficient vaccine responses due to compromised intrinsic immune mechanisms)?

Is there likely to be both nonspecific and SARS-CoV-2-specific upper respiratory tract mucosal responses induced by the addition of an intranasal IFN-α, that can also provide protection from potential new SARS-CoV-2 variants?

What are the exact mechanisms of vaccine-mediated protection from SARS-CoV-2 infection that might be induced upon additional IFN-α administration?

Author contributions

R.F. conceptualised the scope and focus of the manuscript, wrote the article, and prepared the figure. R.F., A.O-R., A.M., and D.H.D. critically appraised, revised, and contributed to the final version.

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Declaration of interests

R.F., A.O-R., and A.M. declare no competing interests. D.H.D. reports participation in Data and Safety Monitoring Boards for COV HIC001, COV HIC002, and Oxford SARS-CoV-2 CHIM study in seropositive volunteers, and acts as Commissioner for Medicines and Healthcare products Regulatory Agency (MHRA), UK.

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