UJEMI PERSPECTIVES

Slow vaccination and immunity profile homogeny as a cause of SARS-CoV-2 antigenic drift

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SUMMARY Delays in vaccine production for severe respiratory syndrome coronavirus 2 (SARS-CoV-2), have resulted in countries dependent on foreign biomanufacturing capabilities to underperform in terms of their population immunization rates. The implications of slow vaccination and a comprehensive strategy to combat it have yet to be devised. Of particular concern is the exposure of SARS-CoV-2 to pressures created by vaccinated individuals which could promote antigenic drift negating vaccination efforts. Uncertainty also remains around the usage of the novel mRNA vaccines due to limited characterization of vaccinated host immunity profiles. If unable to induce lymphocytes of high diversity and longevity, mRNA vaccination could further accelerate the development of vaccine escape. This paper outlines the potential consequences of a slow vaccination rate with mRNA-based vaccines and recommends counteracting measures. It discusses **(i)** the mutable rate of key SARS-CoV-2 spike (S) protein sites that when altered, could lead to a loss of immunity; **(ii)** the potential of slow vaccination and mRNA vaccines causing antigenic drift; and **(iii)** methods of improving immunization rates through production-based means. Comparisons to previous pathogen cases suggest high risk of SARS-CoV-2 antigenic drift in response to slow vaccination and novel vaccines. These mutations are predicted to occur at several S protein epitopes. The results call for genomic surveillance initiatives to track development of immune evasion and longitudinal research of vaccinated host immunity profiles to measure durability and diversity of the induced humoral response. Recommendations for improving vaccination rate include the construction of production facilities and improvement of already existing distribution networks, while maintaining communication channels and transparency with the public.

INTRODUCTION

SARS-CoV-2, the causative agent of the ongoing pandemic, is a highly pathogenic virus originating from horseshoe bats (1). The positive sense RNA virus enters a human host via their respiratory pathways and interacts with cell angiotensinogen receptor 2 (ACE2) with its trimer S protein, promoting endosome mediated entry or direct membrane fusion (2). These infections can cause respiratory irritation and have led to severe illness and death in some patients (2). Recent research suggests SARS-CoV-2 can also exploit other entry receptors including neuropilin-1 which is expressed by neuronal and epithelial cells, suggesting a broader cell tropism (3).

In response to SARS-CoV-2's global spread, many countries have implemented quarantine measures and siphoned large amounts of money into prophylactic development and distribution (4–6). Still, vaccine production has continued to be hit with large scale setbacks resulting in a strain on supplies and vaccination delays. This has particularly impacted countries who rely on foreign production such as Canada (7). Despite Canada's robust response to the pandemic, it has fallen to 54th place globally in terms of its percentage of population vaccinated, underperforming in comparison to other western countries (8). This has risked pushing the government of Canada's deadline of September 2021 for a fully vaccinated populace (9).

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A slow global vaccination rate has important implications on the continued evolution of the virus. As of yet, SARS-CoV-2 mutant strains have generally only displayed increased transmissibility compared to predecessors (10,11). However, as vaccine administration progresses, a situation may arise in which large proportions of vaccinated and susceptible individuals live within close proximity for long periods. This would provide SARS-CoV-2 hosts in which it can replicate under the selective pressure of an immunized group which could cause a shift in evolutionary focus from transmissibility to host immune evasion (10). Ramifications could include the hampering of vaccination efforts and a "cat-and-mouse game" of continuous prophylactic development against rapidly evolving endemic SARS-CoV-2 strains, a situation similar to that of avian-origin influenza subtype H6N2. A decade of vaccine usage against this influenza strain has created a strong selective pressure for antigenic drift leading to continuous loss of vaccine efficacy and need for constant prophylactic redevelopment (12).

Recent advances in nucleic acid stability and delivery into cells allowed for the early development and approval of the Pfizer and Moderna mRNA vaccines against SARS-CoV-2 (13,14). Creation of said vaccines requires altering the sequence of RNA molecules making them relatively easy to produce (15). This contrast vaccines that introduce macromolecules to hosts as vaccines based on these methods require unique alterations to their production procedure based on their physio-chemical properties (15). Due to their novelty, many questions about mRNA vaccines remain such as their capability to provide durable humoral immunity. Of particular concern is their two dose requirement and potential to hamper immunity profile diversity by only exposing hosts to the S protein (14,16,17). Introducing single proteins limits the number of epitopes that can be targeted by immunized hosts reducing the number of evasive-conferring mutations needed by the virus to escape humoral responses.

Despite the potential costs of slow vaccination combined with the novel mRNA vaccine, methods of mediating the current situation have yet to be implemented. Limited understanding of SARS-CoV-2 viral immune evasion in response to vaccination calls for longitudinal monitoring and research studies. These projects will however depend on the largely unexplored areas of vaccine-associated selective pressure and prediction of mutations that promote vaccine escape. Additional research that studies the molecular factors and driving forces of viral evolution as well as identifies barriers in global vaccination is vital for combating SARS-CoV-2.

PROPOSED RESEARCH QUESTIONS

The slow global vaccination rate with mRNA vaccines against SARS-CoV-2 risks evolution of new variants capable of vaccine escape. Methods of monitoring and combating the arising such strains remain to be explored. Fast improvements in vaccination rate through production and logistical means would bring the virus under control and lessen the probability of evasive strains emerging. Monitoring could prevent a worst-case scenario caused by antigenic drift by quickly allowing for identification of novel evasive strains and immediate development of appropriate prophylactics. This review explores three questions that are important for implementing mutant monitoring and for improving vaccination rates against SARS-CoV-2:

1. What is the identity and mutability of SARS-CoV-2 S protein regions that when altered, allow for vaccine escape?
2. Could usage of novel mRNA vaccines coupled with slow vaccination rates act as a promoter of SARS-CoV-2 antigenic drift?
3. How can we improve Canadian and global immunization rates to avoid development of evasive SARS-CoV-2 strains?

PROPOSED PROJECT NARRATIVE

High mutability and humoral evasion capabilities of SARS-CoV-2's S protein. Viruses with RNA genomes are typically associated with high mutation rates allowing for diverse populations of strains and quick adaptation to selective pressures (18). A delicate balance in

mutability must be maintained by the virus to maximize evolution without significantly high levels of error catastrophe (18). This phenomenon results from mutations that retard functionality of genes required for successful completion of the viral life cycle. Thought to be a byproduct of a long complex genome, coronaviruses have uniquely evolved proofreading ability through their non-structural protein (nsp) 14 exoribonuclease domain (18). This causes SARS-CoV-2 to have slower genomic change compared to other RNA viruses, with only 10^{-6} polymorphisms per site per cycle versus influenza's 10^{-5} per site per cycle (19,20).

Despite SARS-CoV-2's low predicted mutability, it has displayed robust evolutionary processes. The high transmissibility of the virus has allowed for rapid infection of hosts and the creation of large numbers of virions, increasing the probability of variants arising. Recent reports have also found 68% of infection cases are characterized by high intra-host diversity of SARS-CoV-2, a common occurrence with highly mutable viral species such as hepatitis C virus (HCV) (21,22). The presence of quasispecies in HCV subjects necessitates treatment with a multi-drug regimen to prevent formation of drug resistant strains, suggesting novel therapeutic interventions for SARS-CoV-2 may require the same (22). These combination therapies employ multiple drugs acting upon distinct pathways, requiring the virus to undergo the unlikely simultaneous formation of several resistance-conferring mutations to escape treatment (23–26).

This also has broad implications for vaccination efforts. Commonly, experimental vaccines for viral quasispecies fail due to their rapid evolution and diversification (27). As said by Dr. Holland at the University of California, the treatment of quasispecies can be viewed as a fight against a mutant continuum of viruses rather than a single species (28). Many functionally viable and selectively advantageous strains of SARS-CoV-2 are likely arising in patients only to be lost due to stochasticity (10). As vaccination continues at a slow pace, there is a risk of the growing selective pressure promoting their stochastic escape, leading to a shift in SARS-CoV-2 master sequence to a novel evasive strain (28).

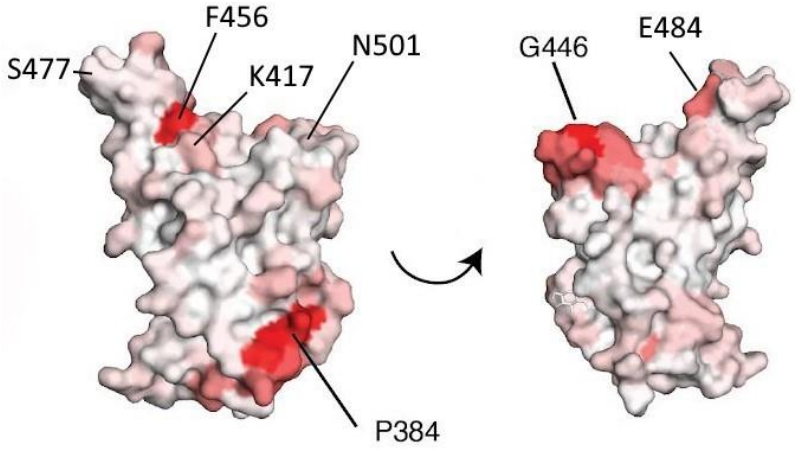

FIG. 1 Regions of the S protein RBD recognized by previously generated host antibodies. Mutations in white regions do not impact antibody recognition whereas mutations within dark red areas drastically reduce RBD affinity to previously generated antibodies. Figure adapted from Fig. 3 in Ref. (35).

The high observed mutability of SARS-CoV-2 suggests the need for longitudinal monitoring studies as vaccination progresses. To monitor the virus's antigenic drift, it is important that sites are identified that when mutated could impact host antibody recognition. Of significance are mutations that affect the binding of neutralizing antibodies as their levels have been negatively correlated to disease progression (29,30). These antibodies bind to viral S proteins in a manner that prevents entry (31,32). Limited work by Greaney et al. was done to predict sites that when altered, could impact binding of neutralizing antibodies that obstruct ACE2-S RBD protein interactions (29). A yeast library containing all functional variants of the S protein monomer was tested with collected human serum samples from immunized hosts to generate Figure 1. Determined sites include the N501 and E484 which have been shown to be mutated in the growing U.K. (501Y.V1) and South African (501Y.V2) strains (Table 1) (30,33,34). These strains as predicted, have shown to have lower affinity to previously generated host antibodies (30,33). Greaney et al.'s study was limited by the usage

of the single S protein monomer which fails to take in account how shifting of the trimer components relative to one another may affect antibody recognition (32). It also does not consider the S protein interaction with newly identified entry receptors such as neuropilin-1. Further research is necessary to determine SARS-CoV-2's receptor range and any additional sites of concern.

Slow vaccination with mRNA vaccines risks promoting antigenic drift. It is important to examine the likelihood of vaccination impacting antigenic drift of SARS-CoV-2 and its possible implications. General knowledge suggests the acquirement of vaccine resistance is less robust when compared to drug resistance development (16). This can be attributed to two factors: 1) the redundancy and host-specificity of virus epitopes targeted, and 2) the suppression of virus growth in immunized hosts (16,39). Host-specific recognition of multiple epitopes implies several mutations must arise in pathogens to evade immune responses, and that these mutations may not confer protection from all vaccinated hosts. Sterilizing immunity works cooperatively by reducing the number of virions present, and thus the probability of resistance mutations emerging. These factors are both of concern for SARS-CoV-2 due to slow vaccination rates and the usage of novel mRNA vaccines.

Table 1 Mutations present in SARS-CoV-2 lineages of concern predicted to promote viral escape (29,30,34,36–38)**.**

Viral Strain	Mutations	Description
U.K. - 501Y.V1 (Lineage B.1.1.7)	N501Y	- Slight to no decrease in vaccine efficacy - Increased transmissibility - May have higher disease severity
South African - 501Y.V2 (Lineage B.1.351)	E484K, N501Y, K417Y	- Moderate to high decrease in vaccine efficacy - Reduced therapeutic antibody efficacy
Brazil – 501Y.V3 (Lineage P.1 or B.1.1.28.1)	E484K, N501Y, K417T	- Under investigation - Preliminary reports of reduced vaccine efficacy - Reduced therapeutic antibody efficacy

Cases of whooping cough caused by *Bordetella pertussis* have dramatically risen in recent years (40). This has been attributed to the usage of a newer acellular vaccine (ACV) at the turn of the century which introduces purified proteins in contrast to whole cell vaccines (WCV), reducing post injection fever and soreness. While found to be as efficacious as WCV, research performed a decade later has found important differences in the immunity profile generated by ACV (40). These differences cumulate in faster waning of *B. pertussis* resistance which have allowed for resurgence and development of mutations associated with vaccine escape (40). Current immunization efforts against SARS-CoV-2 have taken advantage of novel mRNA vaccines developed by Pfizer and Moderna. Similarly to ACVs, mRNA vaccines introduce pre-fusion conformation S protein rather than whole viral particles for

antibody development (41). Introduction of singular particles in next gen vaccines reduces both intra and inter-host diversity of epitopes targeted, which results in fewer mutations needed to evade host immune responses (16). Additionally, due to the novelty of these vaccines, little research exists on the longevity of the provided humoral immunity in humans. To avoid repeating mistakes made with *B. pertussis*, future research should look into differences in SARS-CoV-2 immune profiles between vaccinated and infected hosts, to study the diversity of epitope binding antibodies, and monitor vaccine escape through antigenic drift and waning of host immunity.

SARS-CoV-2 has already displayed remarkable adaptation in response to selective pressures from host immune responses. B cell epitopes in nsp16, neuraminidase (N), and S proteins show greater variability between viral samples than non-epitope positions indicating variation is being promoted by host immune interactions (42). Growing strains including the U.K. 501Y.V1 and South African 501Y.V2 contain mutations that provide reduced affinity for previously generated antibodies, suggesting their selection is partly due to growing immunity (30,43). Rapid SARS-CoV-2 evolution has also been observed in studies of immunosuppressed and compromised individuals who have experienced persistent infection (33,44,45). Weak patient responses have allowed for high viral loads and subsequent evasion of both host and therapeutic antibodies, with the majority of escape mutations centered in the S gene (33,44).

Slow vaccination rates could provide a community environment similar to immunosuppressed patients where a large population of virions supported by susceptible hosts are subject to a strong selective pressure created by vaccinated individuals. Exposure of the large numbers of virions to the selective pressure could promote the emergence of resistant strains. This issue is compounded with the mRNA vaccine's two-dose requirement (17). Since a single dose only provides partial protection to hosts, delays in 2nd dose application may provide additional immunosuppressed-like environments for rapid viral evolution (17). As vaccination progresses, it is important researchers monitor for acceleration of SARS-CoV-2 antigenic drift.

Addressing distribution and concerns of vaccination. Given the risk of vaccine mediated antigenic drift, it is imperative methods of increasing vaccination rates are identified. Major issues include biomanufacturing capacity, distribution, and vaccine hesitance. Due to falling profit margins and high liabilities associated with vaccine production, countries such as the U.S. and Canada have decreased their capacity significantly in the last 60 years (46). This has amounted to a weak response against the ongoing pandemic. Canada particularly has nearly zero biomanufacturing capabilities, relying completely on foreign production (47). Delays in shipments have amounted to the country falling off schedule, risking pushback of the governmental deadline for a fully vaccinated populace (47).

Canada could benefit by adopting funding policies similar to the U.K., that focus on large-scale repurposing of facilities for vaccine production (48,49). However, biomanufacturing remains a costly endeavor. Facilities require importing complex equipment, sources of raw materials, salaries for educated personnel, and compliance with Health Canada's current good manufacturing practices (cGMP), all while operating on slim margins (50). If domestic facilities are built, Canada will need to be prepared for returns that only accrue in the long term (50).

In response to delays in vaccine shipments, the Canadian government has started funding two facilities with expected operational dates of 2021, and one with an opening date of 2024 (51,52). Critics argue their founding is too late for Canada, but global perspectives and preparation for future pandemics should be considered. The Coalition for Epidemic Preparedness Innovations (CEPI) predicts enough vaccine doses for the world population will only be manufactured by 2023 to 2024 (53). Majority of early doses have been pre-ordered by high-income countries without regard to distribution equity, a decision that could lead to nearly double the number of expected coronavirus disease 2019 (COVID-19) -associated deaths (53). Initiatives such as the World Health Organization's (WHO) COVID-19 Vaccines Global Access (COVAX) have attempted to reduce this gap, with the goal of purchasing 2 billion vaccines for developing countries by the end of 2021. Hindrances are also expected in

terms of vaccine distribution in low- and middle-income countries (LMIC). Despite the approval of the rotavirus vaccine in 2006, only 60% of children in the world have received all three required doses, depicting the accessibility gap (53).

Slow vaccination in the developing world is both an ethical issue and a risk for first world countries. Interconnectedness with globalization and public backlash against quarantine measures including travel restrictions, highlights the potential of countries being reinfected despite eradication within their borders. This has been seen in island countries such as New Zealand and Taiwan who have approached elimination repeatedly only to be reinfected (54). If unimpeded, SARS-CoV-2 could continue to replicate and evolve in response to selective pressures in LMIC populations conferring strains that can undergo vaccine escape and spread globally. As such, it is imperative Canada and other countries engage in concerted funding of biomanufacturing facilities and identify methods of improving distribution reach to increase world production capacity and vaccinate all populations against SARS-CoV-2.

Vaccine hesitance must also be addressed. Acceptance of vaccines ranges from as high as 90% in China to 55% in Russia (53). Misinformation and deception risk undermining efforts, slowing vaccination rates and increasing the burden of SARS-CoV-2. Fears of adverse effects may be addressed with transparency and positive social influences to build trust and consensus on the necessity of vaccination (53,55). Going forward, governmental research is needed on both the scientific objectives of improving vaccine biomanufacturing and global distribution and psychological objectives of anticipating and dealing with mistrust to improve vaccination rates.

CONCLUSIONS

Global vaccination efforts are well underway in an attempt to inhibit the spread of SARS-CoV-2, the agent behind the current viral pandemic. However, the slow rate of immunization and the usage of novel mRNA vaccines could lead to the appearance of evasive strains. This paper describes the current research in SARS-CoV-2 mutations that could promote evasion of host antibody recognition. It highlights the necessity of monitoring viral evolution and overcoming hurdles of vaccine production, distribution, and public mistrust.

SARS-CoV-2 has already displayed high mutability through the presence of quasispecies in patients, arising of novel strains, and cases of persistent infections. It is thus important the evolution of SARS-CoV-2 be fully explored. Work has been done to determine sites on the S protein RBD that when mutated, reduce affinity for antibodies that neutralize ACE2-S protein interactions (29). But further research, perhaps using a similar yeast library method as employed by Greaney et al., is necessary to identify and study other receptors like neuropilin-2, and to understand how changes in the trimeric S protein affect antibody binding. It is important these regions be quickly determined and monitored using global sequencing initiatives to study the evolution of the virus and allow for immediate action against emerging strains, such as the development of vaccine boosters. United approaches based on the U.S.'s defunct PREDICT epidemiological research program which intended to predict pandemics, could be established for worldwide research of and response to viral evolution (53).

Cross-sectional and longitudinal studies are also needed to characterize the immunity profiles of populations vaccinated with the novel mRNA vaccines. Serum from vaccinated individuals could be compared to that collected from hosts who have cleared the infection to study antibody diversity. To determine duration of humoral immunity, vaccine efficacy could be studied by collecting the number of vaccinated hosts infected at different time points, and comparing the results to that of other SARS-CoV-2 vaccines based on alternative platforms (56).

Methods of speeding up vaccination remain to be identified. Low numbers of biomanufacturing facilities have resulted in early setbacks for countries without production capabilities. Difficulties in purchasing and distribution are also expected to impact LMICs. It is imperative that global effort be put into combating these issues by funding distribution networks and domestic facilities. SARS-CoV-2 vaccine distributors in LMICs may take advantage of the existing global system that provides 80% worldwide coverage for childhood vaccination (53). To address the needs of SARS-CoV-2 vaccines, these networks will need

to be improved to reach all age groups and have their cold-storage chains modified to transport the various SARS-CoV-2 vaccines. Throughout these efforts' public health measures such as quarantine and masks must be maintained to minimize viral spread and evolution, lowering slow vaccination consequences.

It is important proper communication channels also be maintained with the public to address the vaccine confidence gap. Informative governmental programs will need to be funded to identify community vaccine perceptions and provide trustworthy information. The entire immunization community must engage with and put an emphasis on listening to public concerns rather than providing definite statements to the public on what they should believe to better communicate with vaccine skeptics while reaffirming the beliefs of acceptors (57,58).

Quickly researching and implementing measures that monitor viral evolution and increase immunization rates of the public could prevent the formation of evasive strains and allow for complete eradication of SARS-CoV-2. This would drastically reduce overall morbidity, prevent endemic SARS-CoV-2, and may help establish the much-needed programs and vaccination networks needed to combat the inevitable next pandemic.

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