

SARS-CoV2 Live Virus Skin Vaccine

Draft March 25, 2020, last updated May 14 - [Pablo Mayrgundter](#), Austin TX
<https://tinyurl.com/y8ujrcze>

The work is an open resource to medical doctors and researchers.

Summary and Asks

Skin cells express the same ACE2 and TMPRSS2 membrane proteins¹ used by CoV2 to enter the respiratory epithelial cells, leading to primary Covid pathology. There are many clinical reports of viral exanthems in the skin associated with early Covid progression, but none of serious CoV2 infection².

Live viruses have been the principal vaccines used for Smallpox, Polio, Measles and Adenovirus³. These vaccines use attenuated (polio and measles) or related (smallpox) strains when the inoculation target would otherwise be susceptible to primary pathology. Live-unattenuated virus (adenovirus) has been used when the inoculation site yields only benign infection (gut, to avoid respiratory infection).

These facts suggest the possibility of a **Live Virus Skin Vaccine (LVSF)** for Covid, or possibly a GI vaccine, though the risks there still seem high. This document investigates the vaccinology, clinical testing and manufacturing logistics required for widespread vaccine use.

Questions & status for virologists, immunologists or infectious disease specialists:

1. Can skin be infected with SARS-CoV2? **Mouse: likely, Human: likely**
2. Is initiating CoV2 infection via skin inoculation safe? **Unknown**
3. Should CoV2 skin infection confer immunity to Covid? **Mouse: likely**
4. Should an attenuated strain be used? **TBD, logistically difficult**
5. What dose, where and how? **Unknown**

Reviewers

¹ [Appendix: Candidate Tissues for Inoculation](#)

² [Appendix: SARS-CoV2 Skin Infection Literature Review](#)

³ [Appendix: Live Virus Vaccines](#)

This proposal is being led by an independent software developer with experience in epidemic disaster response epidemiology (Haiti cholera outbreak, 2010) in association with the [C-TIG working group](#) and has been privately reviewed by 3 doctors: a dermatologist, an infectious disease specialist, a family practice doctor; and a biochemist, a biostatistician, and a humanitarian director at Doctors Without Borders. References are available upon request.

Key Considerations

Can skin be infected with SARS-CoV2?

Likely yes:

- A typical clinical report is that there are many markers of (benign?) viral infection: *“viral exanthems (ie, morbilliform rash, petechial rash co-existing with thrombocytopenia, erythematous-to-purpuric coalescing macules, widespread urticaria, and varicella-like vesicles).”* However, there is not yet direct evidence of local viral replication. [See [Appendix: ARS-CoV2 Skin Infection Literature Review](#)]
- Skin has the highest expression of ACE2 and TMPRSS2 membrane proteins of any tissue not implicated in primary Covid pathology. [See [Appendix: Candidate Tissues for Inoculation](#)]
- A study of the CoV2 spike protein found a new furin-type cleavage site that was not present in CoV, possibly leading to a *“gain of fusion activity that might result in increased viral entry in tissues with low density of ACE2 expression”*⁴.
- **Comparison infections**
 - **Respiratory infection:** the CoV2 virus infects respiratory epithelial cells via binding to the ACE2 membrane protein and fusion via the TMPRSS2 membrane proteins; these membrane proteins are currently thought to be necessary and sufficient for CoV2 infection.
 - TODO: incorporate this model [Replication of SARS-CoV-2 in human respiratory epithelium](#)
 - **Kidney infection:** ACE2-based infection in kidney pathology: *“In line with this distribution of ACE2, we observed virus particles in tubular epithelium and podocytes, sites of known ACE2 expression. Collectively, the tubular and glomerular visceral epithelial cells of the kidney are the main targets of SARS-CoV-2. Based on our observations, the endothelium thus is not*

⁴ [Clinical presentation and virological assessment of hospitalized cases of coronavirus disease 2019 in a travel-associated transmission cluster](#) Woelfel et al. 05 Mar 2020

expected to be directly infected with SARS-CoV-2.”⁵

Is skin infection safe?

Unclear:

- If skin infection could lead to systemic infection and typical Covid progression, that would invalidate this approach.
- However, CoV2 skin infection has not been noted as a significant transmission mechanism in the Covid epidemic, so if it is achievable, the local skin infection is likely to be mild and not become a source of viremia.
- “There is no significant viremia in Covid” - [TWiV 608: Daniel Griffin's COVID-19 clinical report | This Week in Virology](#) @43m
- Skin is a highly immunocompetent tissue and the frontline of our immune response.
- Perhaps the risk of systemic infection via this pathway should be less dangerous than the comparable risks in typical respiratory covid progression, given the greater vasculature in the respiratory system, particularly the lungs; similar for the GI tract infections.
- Respiratory Covid appears to proceed to high viral load without causing symptoms, which suggests that the immune response is being suppressed. Interferon suppression has been hypothesized⁶. It is unclear how quickly the skin would detect the infection and handle it locally. The mouse study looked at IgG antibody response (measured via ELISA), which arrived at week 2.
- TODO: immune cell infection
- TODO: [Microneedle and mucosal delivery of influenza vaccines](#), Kang et al., *Expert Rev Vaccines*. 2012 May
- TODO: Incorporate new interferon information from Michaelck and [The Airway Epithelium: Soldier in the Fight against Respiratory Viruses](#)
- Work In Progress: [Comparing Viral Kinetics of CoV2 Infection in Respiratory. Skin and GI](#)

Should CoV2 skin infection confer immunity to Covid?

Likely yes:

- In the U. Pitt study⁷ of the intradermal SARS-CoV-2 subunit vaccine:

⁵ [Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China](#), Su et al, *Kidney International*, 1 Apr 2020

⁶ todo

⁷ [COVID-19 Vaccine Candidate Shows Promise, Microneedle array delivered recombinant coronavirus vaccines: Immunogenicity and rapid translational development](#), E. Kim et al., *Lancet*, March 18 2020, [Dr. Falo](#) corresponding

- *“The skin is an ideal target for immunization. It contains a rich population of antigen presenting and immune accessory cells capable of inducing a proinflammatory microenvironment favoring the induction of potent and durable adaptive immunity.”*
- *“[Intradermal microneedle array (MNA)] delivery of SARS-CoV-2 S1 subunit vaccines elicited potent antigen-specific antibody responses that were evident beginning 2 weeks after immunization... MNA delivery of coronaviruses-S1 subunit vaccines is a promising immunization strategy against coronavirus infection.” (ibid)*
- Notably, MNA delivery of these vaccines generated significantly stronger immune responses than those administered by traditional subcutaneous needle injection, indicating the improved immunogenicity by skin-targeted delivery.

Skin Immune Response

TODO: adapt respiratory immune response to skin

TODO: What inflammatory mediators would come from the skin?

TODO: macrophage recruitment

TODO: B&T Cell interaction in target tissue

TODO: Incorporate these

- [The Dynamics of the Skin's Immune System](#), by Alan V. Nguyen, Athena M. Soulika, Int. J. Mol. Sci. 2019, 20(8), 1811; Department of Dermatology, School of Medicine, University of California Davis, Sacramento, USA
- [Skin Immunity](#), by Agata Matejuk, Arch. Immunol. Ther. Exp.(2018). Wrocław Medical University, Wrocław, Poland
- [Immunology and Skin in Health and Disease](#), by Jillian M. Richmond and John E. Harris, 2014, Cold Spring Harbor Perspectives in Medicine

Comparison: Primary Immune Response in Respiratory Covid

Macrophage emits cytokines IL-1, IL-6, TNF- α , leads to constriction of surrounding smooth muscle dilation (vasodilation), endothelial cell contraction and so increases its permeability, plasma leaks into interstitial space, (leading to collapse of alveoli in ARDS). Neutrophils are pulled in by cytokines to destroy the virus, release proteases to oxidize/kill the virus, but also damages surrounding tissue. Cytokines also travel in the bloodstream and signal hypothalamus to release prostaglandins (PGE2?); changes body temp set point and fever. CoV2 respiratory infection is distinct for early high viral load with delayed onset of primary immune response symptoms like fever.

Should an attenuated virus be used?

Empirically we know that immune response to natural (live, unattenuated) infection tends to confer the strongest immunity. If the risk for viremia and systemic infection originating from the skin inoculation is found to be low/highly unlikely, a wild-type CoV2 should be preferred in order to present the most promising immunogenic agent.

- TODO: quantify benefit of immunity from natural infection in other diseases.
- TODO: quantify risk of systemic infection via viremia.

Attenuation/Killed Virus

If the risk of serious or systemic infection is too high, attenuation or killing of the virus should be considered.

- TODO: incorporate idea from <https://news.ycombinator.com/item?id=22830320>. Roughly, it would be beneficial to look for infected communities with a less pathogenic strain, e.g. by correlation of the data at nextstrain.org with epi reports. Quite a bit of work. Would skin need an attenuated virus at all if risk of productive infection is low to non-existent?
- “We therefore hypothesise that the major deletion revealed in this study may lead to an attenuated phenotype of SARS-CoV-2.” [Discovery of a 382-nt deletion during the early evolution of SARS-CoV-2](#), Su et al, Mar 11 2020

What dose, where and how?

According to the Master Question List for COVID-19 maintained by the Department of Homeland Security:

“The human infectious dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unknown by all exposure routes. SARS-CoV-2 is the cause of coronavirus disease 19 (COVID-19).”⁸

The same document goes on to list a number of results from animal models indicating a range of infectious doses to the respiratory tract of ~100K-5M PFUs.

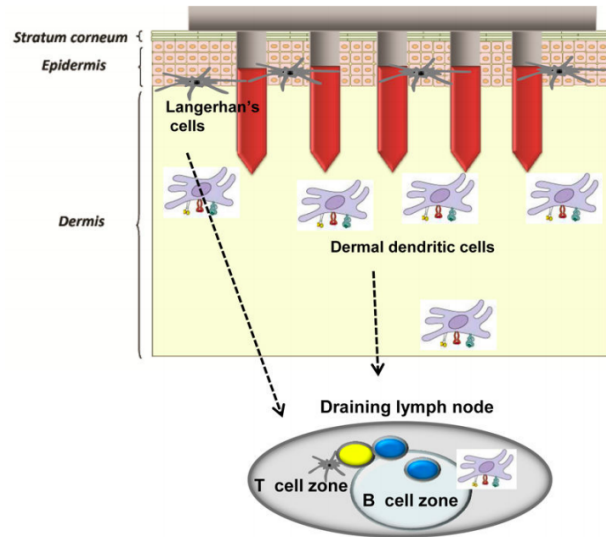
Assuming the dose for skin infection can be determined (i.e. in vitro and in Phase I trials), we proceed to the mechanism of injection. Known options include: intradermal injection of dilute virus tincture with syringe, [bifurcated needle](#) or [Microneedle array](#) into

⁸ [Master Question List for COVID-19](#), US Department of Homeland Security, 5 Apr 2020

skin of shoulder or any better candidate tissue.

20 µg of SARS-CoV-2-S1 recombinant vaccine was used for mouse skin vaccination, administered via Miro-Needle Array, but MNA looks very complicated. Intradermal injection a la botulotoxin seems more widely practical.

TODO: Dereference sources from quote in article “Up to one hundred vaccinations can be given from one vial of the reconstituted vaccine. The established technique is to dip the needle in the vaccine, and then puncture a person's upper arm fifteen times in a small circular area. When done correctly a trace of blood appears at the vaccination site.”



Preclinical Models

In Silico

See [CoV2 Skin Infection Simulation with PhysiCell](#).

In Vitro

ASK: find a lab that will test in vitro skin infection: 1) is the infection productive, 2) is there evidence of immunogenic activity.

Candidate partners:

- [This McMaster lab](#) is studying infection of different cell types (from [Isolation, sequence, infectivity and replication kinetics of SARS-CoV-2](#), Banerjee et al, April 11, 2020). Pablo has an ask out to Banerjee in reply [to his ask for IT help as of April 14](#). Not interested, tho didn't reply explicitly about this doc.
- [Generating organotypic 3D skin cultures as an in-vitro model of the human epidermis](#)

Animal

- The U. Pitt vaccine was tested on mice.⁹

⁹ [Microneedle array delivered recombinant coronavirus vaccines: Immunogenicity and rapid translational](#)

Clinical Trials

... The following is very much in draft ...

TODO: incorporate advice from [Optimizing the Trade-off Between Learning and Doing in the COVID-19 Pandemic](#), and [WHO: Standards and operational guidance for ethics review of health-related research with human participants](#)

TODO: find someone who has run a phased trial before (Ron?)

Therapeutic target

A positive vaccine therapy will yield an adaptive immune response which will be identifiable by antibody test in host blood.

The trial will sample blood at regular intervals for 4 weeks, comparing levels against convalescent or recovered hosts.

Failure Criteria

1. Infection beyond the inoculation site, e.g. respiratory or systemic infection.
2. Fails to elicit immune reaction; no antibodies detected.

Success Criteria

1. Antibodies are detected in blood on par with recovered hosts.
2. Partial success if lower antibody rates are sufficient to achieve a prophylactic effect.

Clinical Trials

Phase I - Safety

- Number of volunteers?
 - Demographic and health qualifications
- Care protocol
 - Safety Monitoring
 - Maybe daily blood PCR test to detect systemic spread and immediately administer antiviral.

- Wound care - The patient should be given materials and guidance for self-care of the inoculation site. The wound should be protected with sterile gauze, to allow the infection to proceed while preventing co-infection and to inhibit aerosol spreading.
- Isolation - The patient should self-isolate until the infection has cleared and the case exit criteria determined.
- Antiviral therapy for acute pathological reaction
- Phase exit criteria

Maybe a follow-on safety test for elderly?

Phase II - Efficacy

- Optimize dose, delivery method for IgG response.

Phase III - Reliability

Manufacture

There are significant logistical problems with bringing novel vaccines to large-scale production. LVSV works around much of this problem by using the local infected population as the source of the principal raw vaccine ingredient, namely the virus itself. The problem is shifted to purification at or near clinical usage endpoints.

See [CoV2 Collection and Dose Preparation](#).

Appendixes

Live Virus Vaccines

Adenovirus¹⁰

Virus: Adenovirus type 4 & 7

Transmission: aerosol, oral

Primary infection: respiratory leading to ARDS & pneumonia

Progression: respiratory -> GI

Vaccines:

1. Inactivated type 4 & 7, Milleman and Werner et al at WRAIR 1950

¹⁰ [Military Infectious Diseases Research Program: Adenovirus Vaccine](#)

2. Live oral type 4, Chanock et al at NIH
3. Live oral type 7, WRAIR 1980 (nb: no adjuvants noted:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7151885/>)

Poliovirus¹¹

Virus: Enterovirus C

Transmission: fecal-oral, oral-oral

Primary infection: pharynx and intestinal mucosa

Progression: GI -> tonsils & intestinal lymphoid -> viremia, occasional CNS infection

Vaccines:

- 1) Intravenous (IV) attenuated vaccine, Kolmer 1935
 - Outrage at experimental deaths of children stopped work despite positive results
- 2) Inactivated vaccine, Brodie 1935
 - Chemically killed with formaldehyde
 - Also cancelled because of Kolmer's work

Enders cultivates polio in human tissues 1948

- 3) Oral attenuated vaccine, Hilary Koprowski 1950
 - Attenuated by repeated passage through albino mice brain and rat
 - NIH administered animal trials ensured low neuropathy in monkeys
 - Used in Belgian Congo and Poland 1958
- 4) IV inactivated Polio Vaccine (IPV), Jonas Salk 1955
 - Chemically killed with formalin
- 5) Oral attenuated Vaccine (Oral Polio Vaccine OPV), Albert Sabin 1957
 - Attenuated by repeated cold-growth cycles in non-human cell lines
 - Approved 1962
- 6) NB: no adjuvants used for OPV or IPV (as implied by
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3529007/>)

Measles¹²

Virus: Measles morbillivirus

Transmission: aerosol or secretion

Primary infection: epithelial cells of mucosa

Progression: viremia -> lungs and skin -> exhaled or secretions

Immunosuppressive: yes

Isolation: Thomas Peebles from throat swab of 11 year old David Edmonston of

¹¹ https://en.wikipedia.org/wiki/Polio_vaccine

¹² https://en.wikipedia.org/wiki/Measles_vaccine

Southborough, MA. (Date?)

Vaccines:

1. Injected subdermal or intramuscular, attenuated Edmonston B version, Enders
 - Successful trial use by David Morley for outbreak in Nigeria, 1960
 - Successful trial use of Edmonston B by WHO for outbreak in NYC, 1962
 - Licensed 1963
2. Further attenuated 1968

Smallpox¹³

Virus: smallpox

Transmission: aerosol

Primary infection: respiratory

Vaccines:

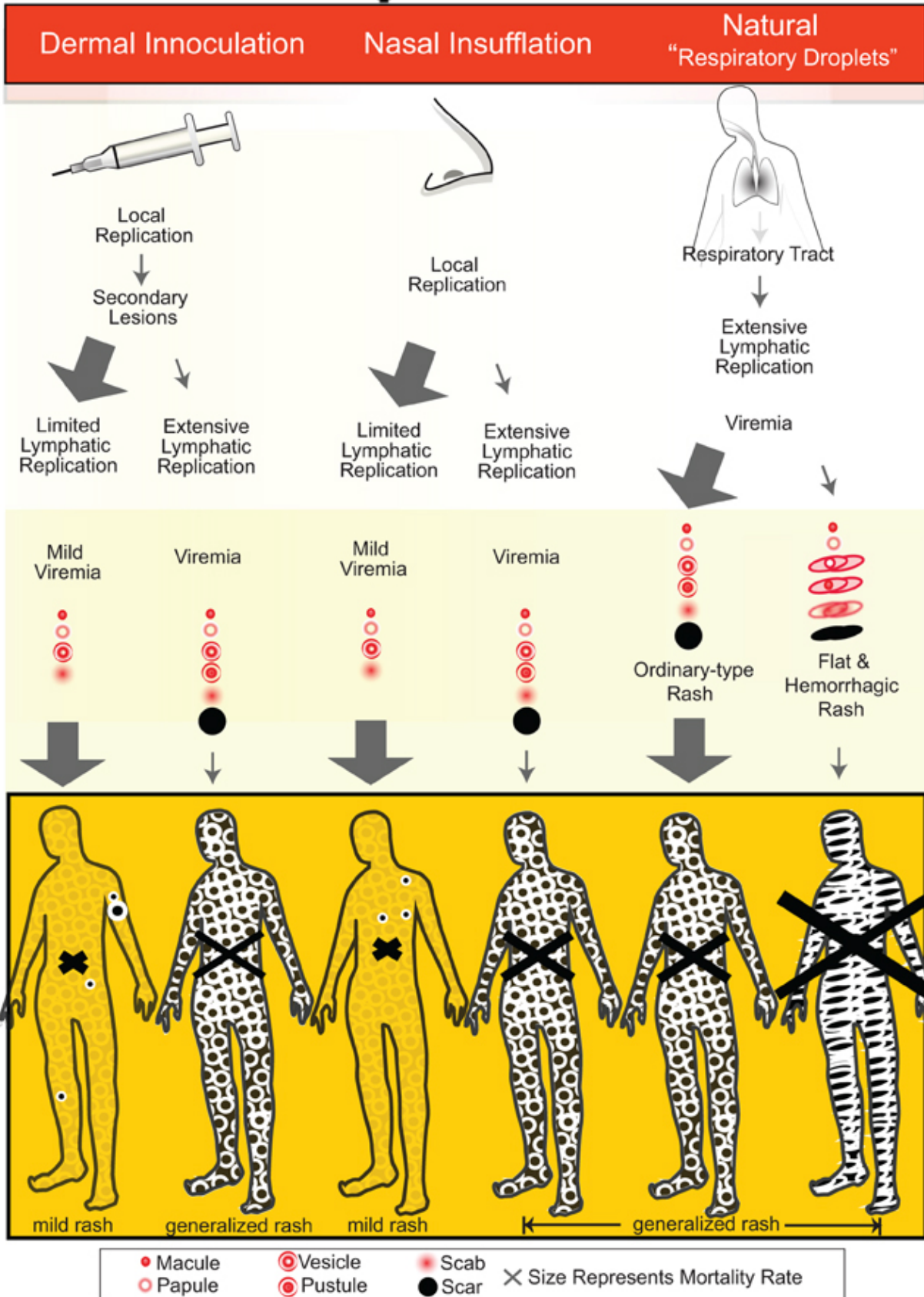
1. Live virus cowpox, Jenner, 1796

“The figure below shows graphically how these different routes of exposure may have produced altered patterns of viral replication within the host and resulted in different risks of extensive viremia and severe disease.”¹⁴

¹³ https://en.wikipedia.org/wiki/Smallpox_vaccine

¹⁴ [What was the primary mode of smallpox transmission? Implications for biodefense](#)

Smallpox Viremia



Candidate Tissues for Inoculation

SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2¹⁵. The skin has the highest expression of both ACE2 and TMPRSS2 (by simple sum) of tissues unrelated to typical Covid pathology. ***The small intestine might also be considered if there are no sequela and viremia seems unlikely.***

Gene expression by cell type (Transcripts Per Million)

Cell type	ACE2 (TPM)	TMPRSS2 (TPM)
Lung	1.01	43.23
Esophagus - Mucosa	2.399	28.07
Stomach	0.3821	113.6
Small Intestine	25.75	42.10
<i>Adipose - subcutaneous</i>	<i>2.317</i>	<i>0.032</i>
<i>Skin</i>	<i>0.4235</i>	<i>6.954</i>
<i>Skeletal Muscle</i>	<i>0.2139</i>	<i>0.01980</i>

The high rate of expression in the tissues associated with Covid (in the top of the table) supports the current understanding that both are required in cells for infection to occur. However, lung ACE2 expression doesn't seem that high. However, as noted above, the furin cleavage site in CoV2 may be responsible for the infection of cells expressing lower ACE2 levels. TMPRSS2 expression is also higher in skin than the other 2 considered tissues.

Do skin cells have the rest of the viral replication machinery seen in the lung pneumocytes? I.e. RNA-dependent RNA, ribosomes and polymerase and proteinases? It may actually prove useful to not support a productive infection, so long as the immune response is triggered and antigens make it back to the lymphatic system for adaptive response.

¹⁵ [SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor](#), Hoffmann, M. et al., Cell (2020)

SARS-CoV2 Skin Infection Literature Review

[The University of Nottingham Center for Evidence Based Dermatology](#) are maintaining a list of all relevant COVID-19 research, including a category for [covid skin disease research papers](#). Many of the papers are restricted access, but I did find a few that are full-text and this one explicitly claims evidence of viral skin infection:

"clinical features that indicate viral exanthems (ie, morbilliform rash, petechial rash co-existing with thrombocytopenia, erythematous-to-purpuric coalescing macules, widespread urticaria, and varicella-like vesicles)" - [Cutaneous manifestations in COVID-19: Lessons learned from current evidence](#), Suchonwanit et al, Journal of the American Academy of Dermatology, 21 Apr 2020

Here's a study with large N discussing rashes and early vesicular eruptions that seem more likely to come from CoV2 infection than be later immune symptoms or from clotting:

"Lesions may be classified as acral areas of erythema with vesicles or pustules (Pseudo-chilblain) (19%), other vesicular eruptions (9%), urticarial lesions (19%), maculopapular eruptions (47%) and livedo or necrosis (6%). Vesicular eruptions appear early in the course of the disease (15% before other symptoms). The pseudo-chilblain pattern frequently appears late in the evolution of the COVID-19 disease (59% after other symptoms), while the rest tend to appear with other symptoms of COVID-19. Severity of COVID-19 shows a gradient from less severe disease in acral lesions to most severe in the latter groups. Results are similar for confirmed and suspected cases, both in terms of clinical and epidemiological findings. Alternative diagnoses are discussed but seem unlikely for the most specific patterns (pseudo-chilblain and vesicular)." - [Classification of the cutaneous manifestations of COVID-19: a rapid prospective nationwide consensus study in Spain with 375 cases](#), Casas et al, British Journal of Dermatology, 29 Apr 2020

However, there is not yet direct evidence of local viral replication. It is unclear to the author if this would be a desirable finding or not for the purposes of a skin-based vaccine.

TODO: more to review:

- [Characterization of acute acro-ischemic lesions in non-hospitalized patients: a case series of 132 patients during the COVID-19 outbreak](#) - Fernandez-Nieto et al, Journal of the American Academy of Dermatology, 21 Apr 2020
- [Dermatologic findings in two patients with COVID-19](#) - Rivera-Oyola et al, JAAD Case

Registered Vaccine Trials

Of 220 registered clinical trials related to Covid-19, there are 5 for novel CoV2 vaccinations (see [Registered Vaccine Trials](#) below) with rolling completion dates through 2020. Alternative approaches to therapy are being considered. For example, passive antibody serum has been deployed despite it being a treatment from the 1890s and “a return to the past” [1,2]. There are 9 clinical trials involving Traditional Chinese Medicine (of interest for non-standard ethics and also the history of variolation in TCM). There are as yet no trials planned for live virus. (Reviewed March 30, 2020, by reading all abstract titles, keyword search for “vaccine”, “vaccination”, “traditional chinese medicine” and “variolation”).

Pathology

Viremia

Clinical testing for viremia is complicated because labs will sometimes just test for virus RNA, so-called “RNAemia”, which is not direct evidence of intact virions; as the virus destroys tissues the viral RNA in infected cells may (will probably?) carry into the bloodstream, so perhaps the RNAemia is an overestimate.

“Since we did not perform tests for detecting infectious virus in blood, we avoided the term viraemia and used RNAemia instead. RNAemia was defined as a positive result for real-time RT-PCR in the plasma sample. RNAemia: AllPatients 6 (15%), ICU 2 (15%), No ICU care 4 (14%)”¹⁶

Viremia in SARS

“At the peak of viraemia, viral RNA was detectable in 75 % of blood samples from patients who were clinically diagnosed with SARS 1 or 2 weeks before the test.”¹⁷

Notes

- Personal correspondence (April 5) from a doctor asking her colleagues: “One infectious disease doctor and her PhD colleague in her office wrote this

¹⁶ [The Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China](#); Huang et al; Lancet; Jan 24 2020

¹⁷ [Antibody response and viraemia during the course of severe acute respiratory syndrome \(SARS\)-associated coronavirus infection](#); Chen W et al; J Med Biology; May 2004

response: 'Clinically does not infect skin, since touching the infected droplets does not cause infection. Infection occurs when introduced through mucous membrane. However mucous membranes are epithelial cells, and so are skin. So theoretically it would work, but problems will be attenuating the virus and maintaining immunogenicity.'