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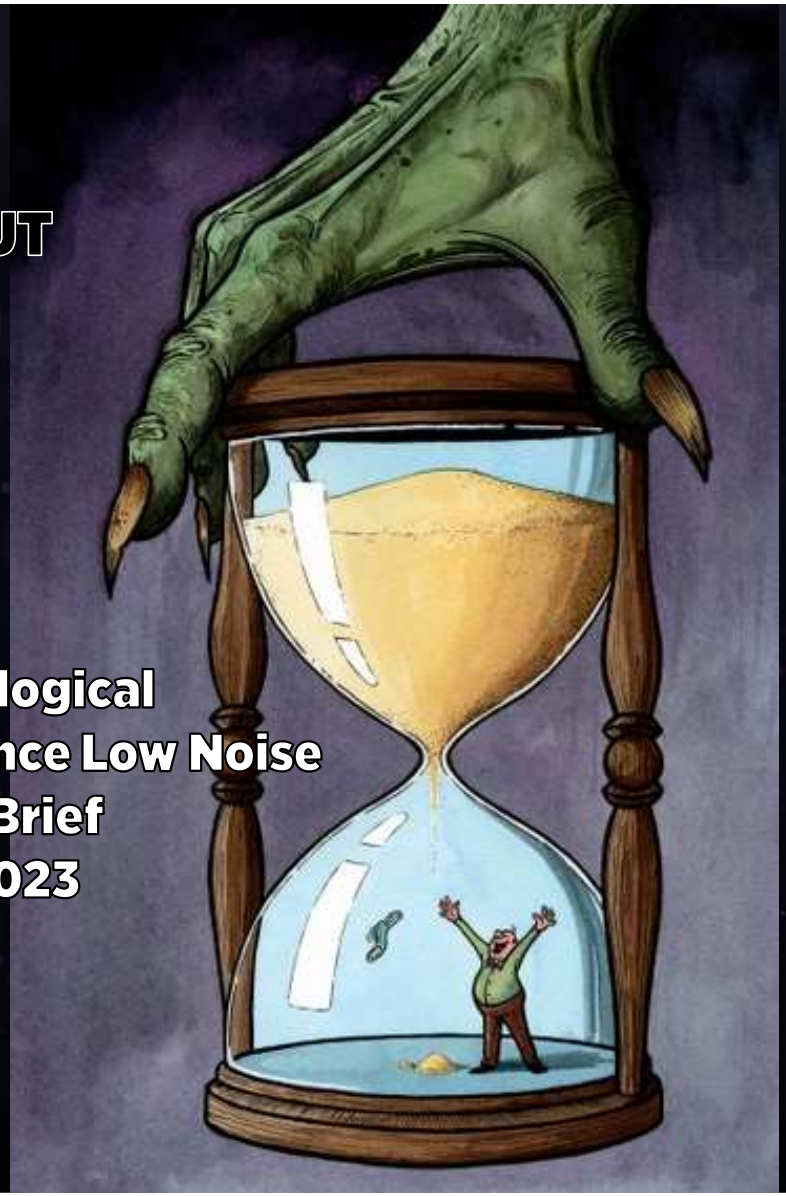
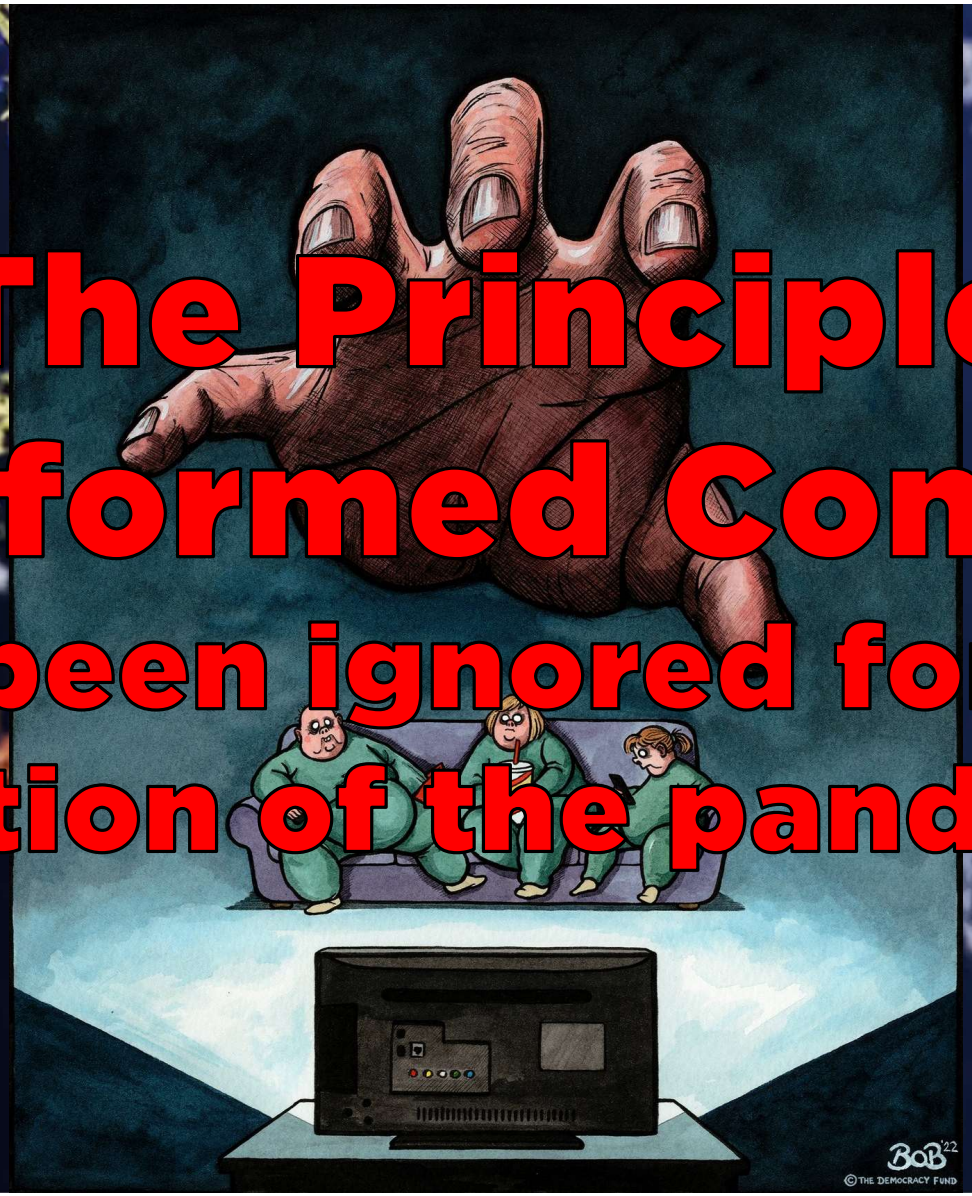


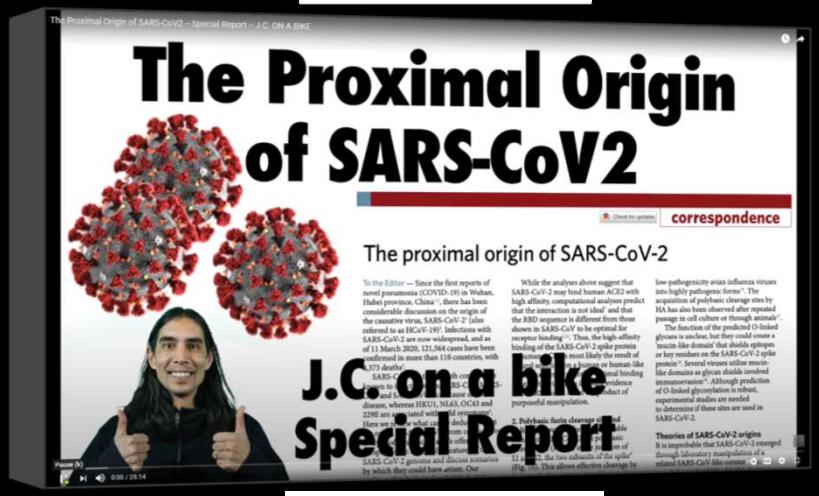
WHY ARE WE THEY DOING THIS?



The Principle of Informed Consent has been ignored for the duration of the pandemic

WHY ARE WE THEY DOING THIS?

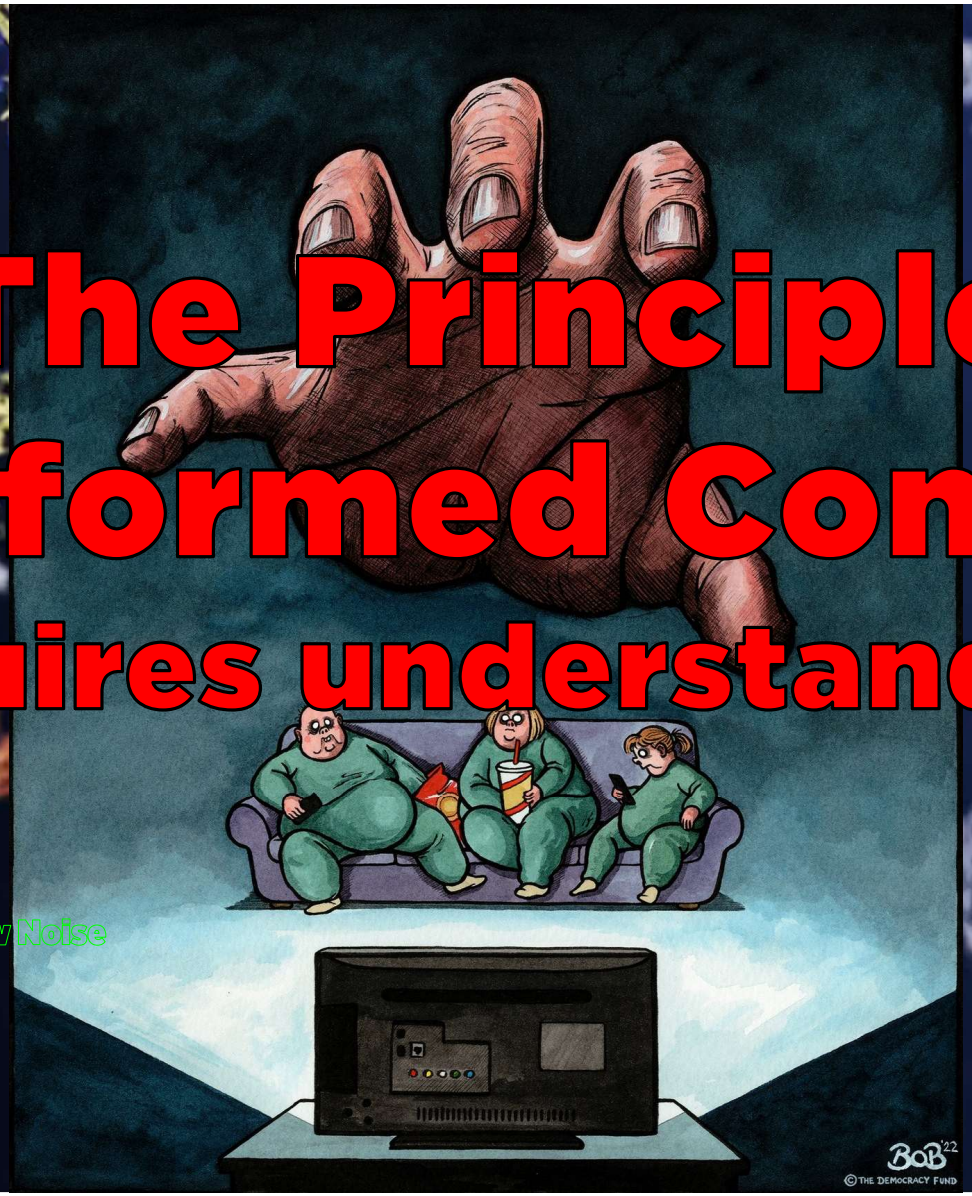
21 FEB 2020



20 MAR 2020

WHY ARE WE THEY DOING THIS?

The Principle of Informed Consent requires understanding



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Information Brief
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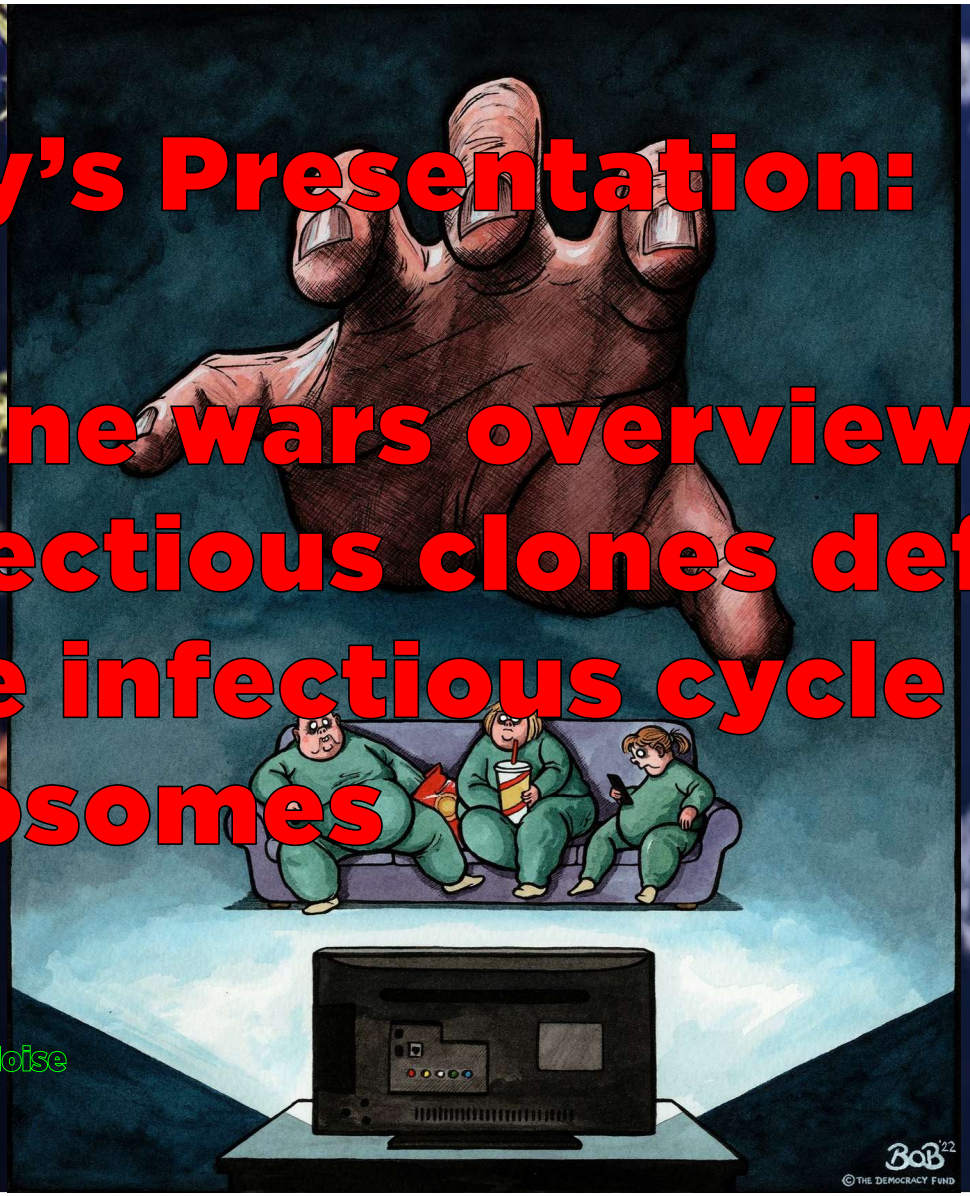
3. The infectious cycle
4. Exosomes



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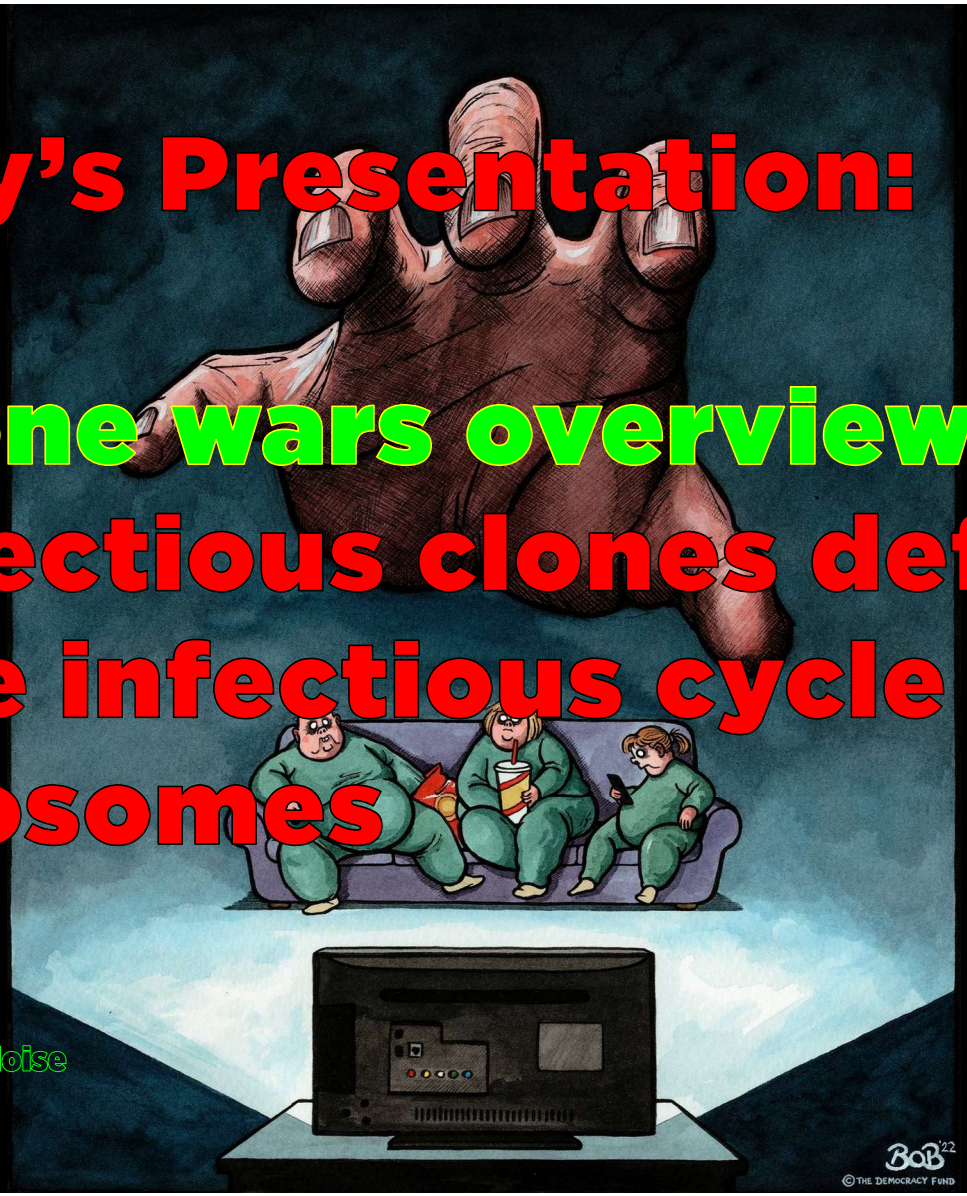

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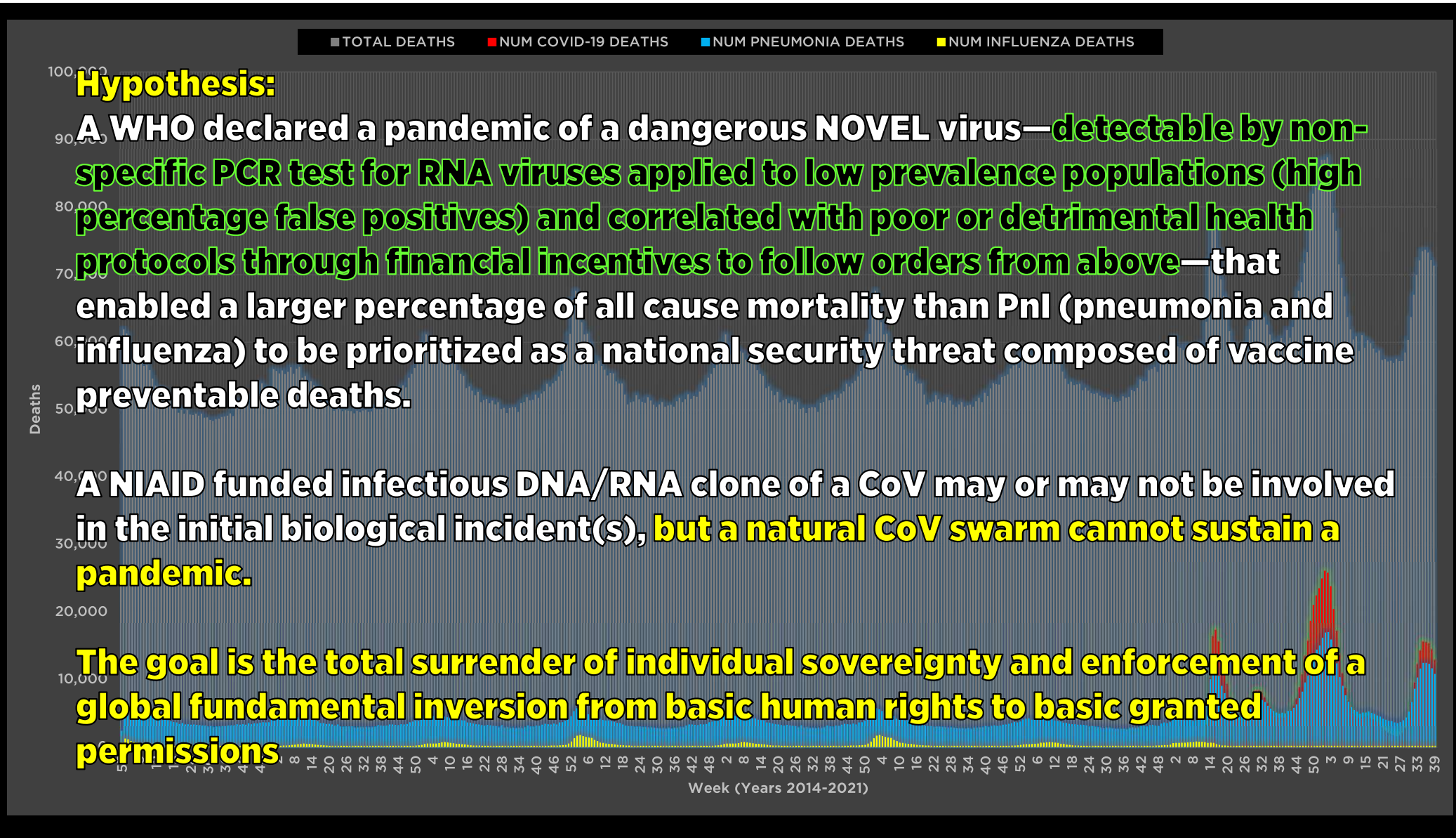
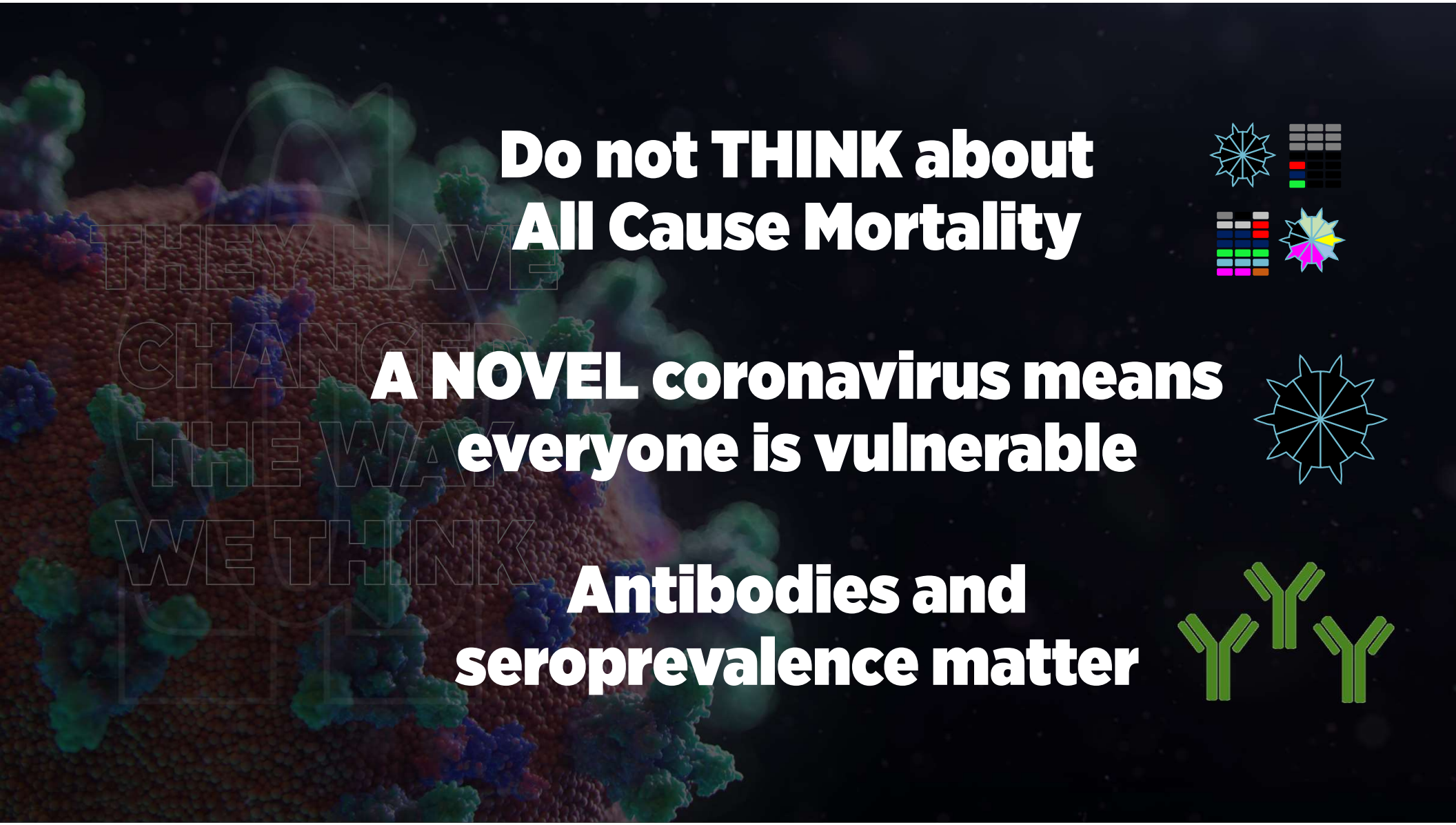

TOTAL DEATHS
NUM COVID-19 DEATHS
NUM PNEUMONIA DEATHS
NUM INFLUENZA DEATHS
Deaths
Week (Years 2014-2021)
Hypothesis:
A WHO declared a pandemic of a dangerous NOVEL virus—detectable by non-specific PCR test for RNA viruses applied to low prevalence populations (high percentage false positives) and correlated with poor or detrimental health protocols through financial incentives to follow orders from above—that enabled a larger percentage of all cause mortality than PnI (pneumonia and influenza) to be prioritized as a national security threat composed of vaccine preventable deaths.
A NIAID funded infectious DNA/RNA clone of a CoV may or may not be involved in the initial biological incident(s), but a natural CoV swarm cannot sustain a pandemic.
The goal is the total surrender of individual sovereignty and enforcement of a global fundamental inversion from basic human rights to basic granted permissions


Do not THINK about
All Cause Mortality
A NOVEL coronavirus means
everyone is vulnerable
Antibodies and
seroprevalence matter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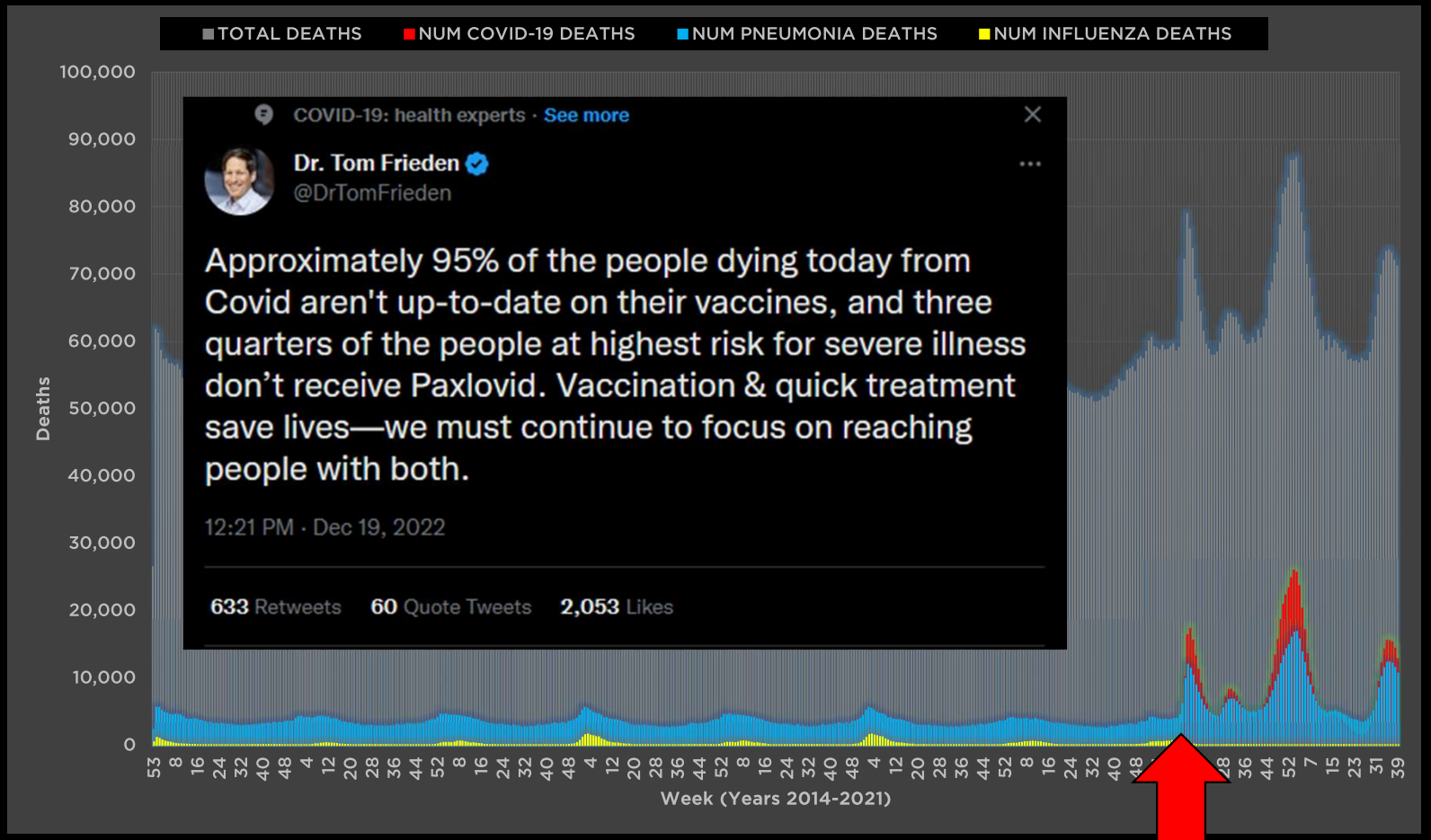
Our Common Narrative

Is the biological (ideological) bifurcation of America that starts with a "conspiracy theory"

Was it Natural or was it a Lab Leak?



Something changed here, and it wasn't a novel cause of death

Our Common Narrative

Is the biological (ideological) bifurcation of America that starts with a "conspiracy theory"

Was it Natural or was it a Lab Leak?

Antivaxxers are a national security issue

We have already tested it on millions of people

Coronaviruses from caves can pandemic and GOF is real

We can vaccinate against respiratory coronavirus

Transfection is a state of art new methodology

Antibodies ARE immunity

There are GOF viruses that can endanger billions (lab escape)

Transfection of the masses might have helped save old lives?

Previous vaccines obviously work great

Immunity is NK/T cell based and oriented inside-out

Transfection is not equivalent to immunization

We have never successfully immunized against a coronavirus

Attempts often result in enhanced disease after immunization

GOF is exaggerated on all levels to their advantage

cDNA clones are REAL

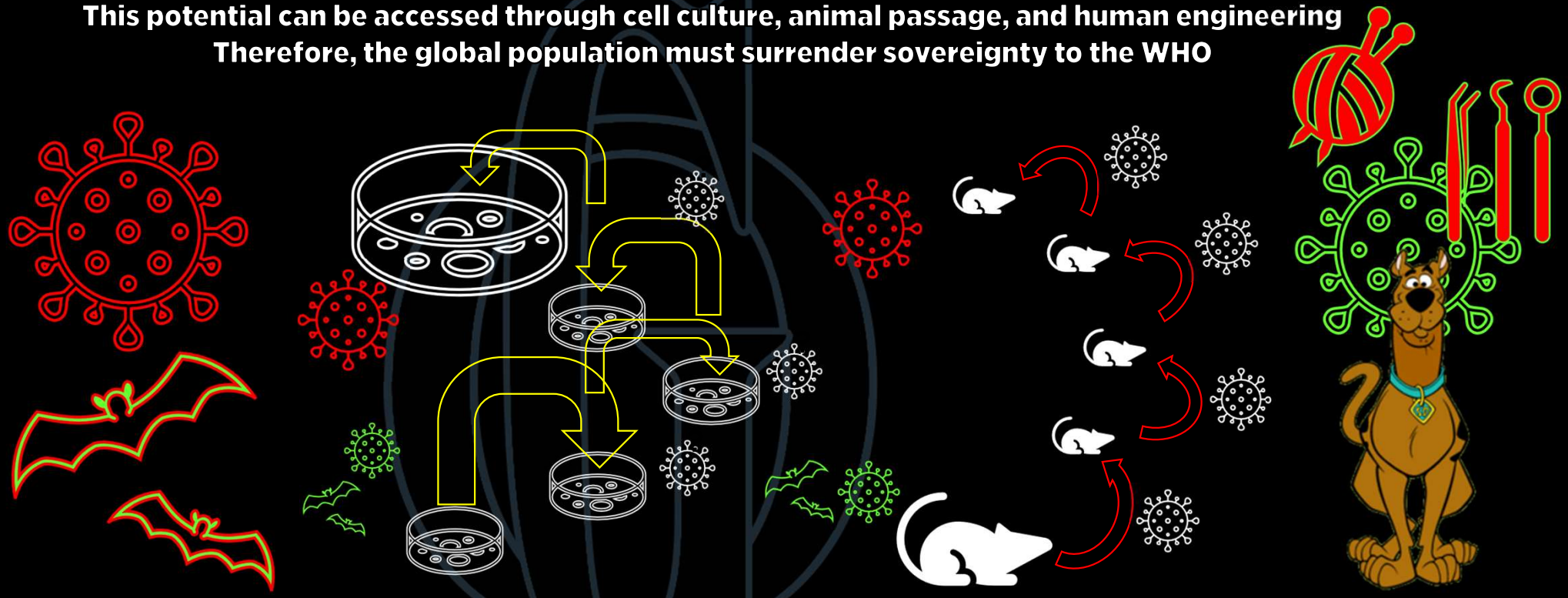
The entire vaccination schedule in the US should be reevaluated

There are no pathogenic viruses

Western medicine is all fake

It is lies all the way down

The TV and algorithms have told us that coronaviruses have pandemic potential
This potential can be accessed through cell culture, animal passage, and human engineering
Therefore, the global population must surrender sovereignty to the WHO



To coerce a surrender of individual sovereignty and a global fundamental inversion of human rights from freedom to fascism

They changed how we THINK

They changed how we THINK about the
Human Coronavirus Swarm

They changed how we THINK about
All Cause Mortality

They changed how we THINK about
our immune response to a respiratory virus

They changed how we THINK about
Immunization, vaccination, and immunity

THEY HAVE
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WE THINK

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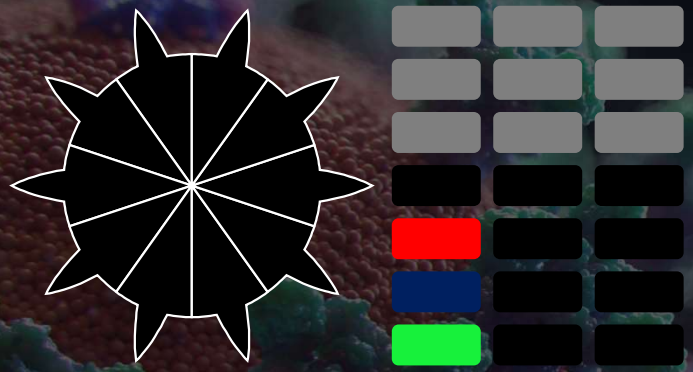
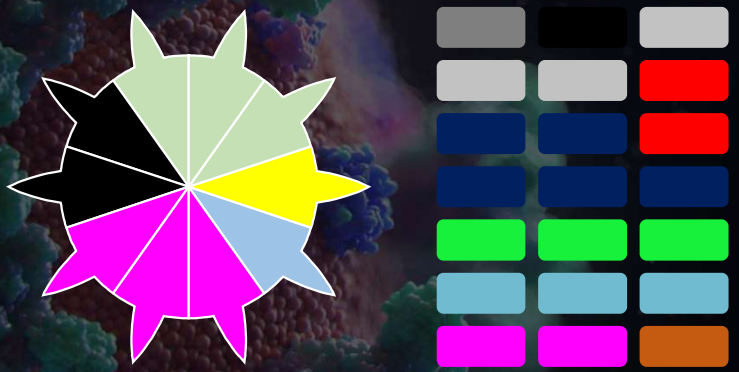
They changed how we THINK about the Human Coronavirus Swarm





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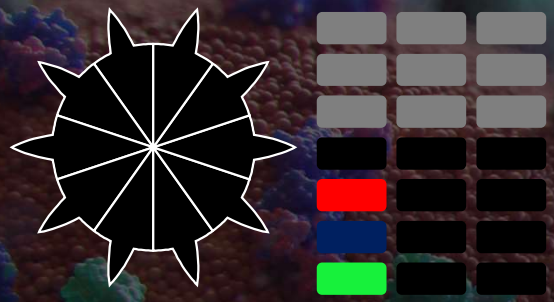

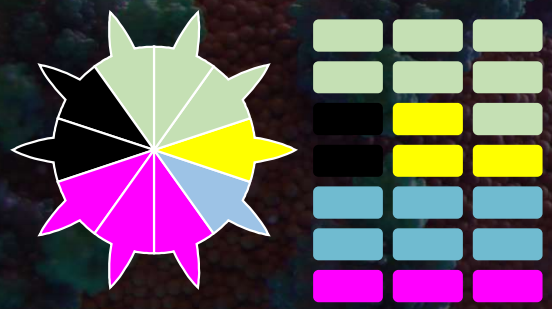

There used to be several hundred possible causes of respiratory disease that lead to pneumonia and were included in the general category of P&I or *Pneumonia and Influenza*

From 2020, we reformulated the counting of all DEATH based on hundreds of EUA products purported to be specific for a single NOVEL and DEADLY virus

The tests and purported sequences are the only evidence of its existence as a NOVEL and DEADLY virus

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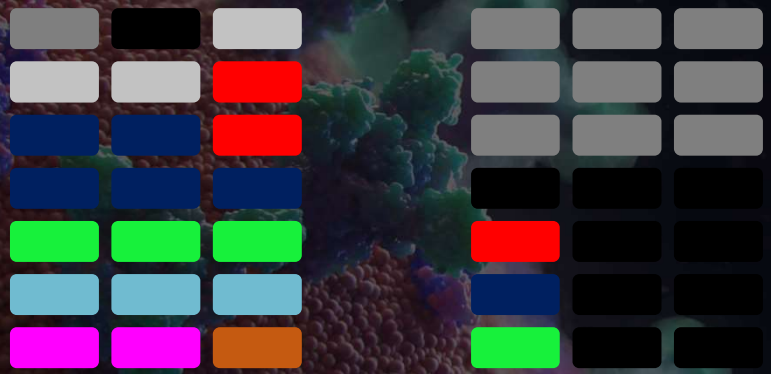
They changed how we THINK about the All-Cause Mortality Rate



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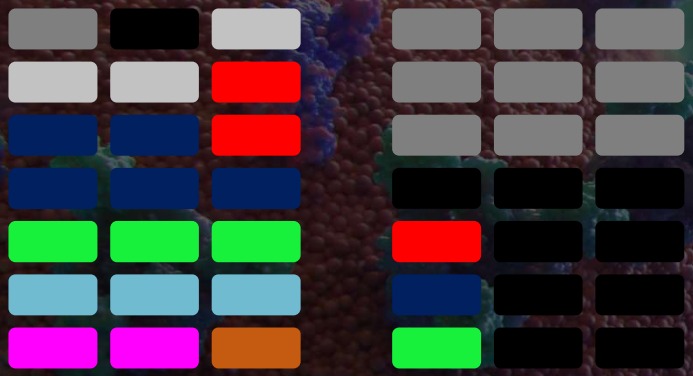

They have led us to believe there is a NEW CAUSE OF DEATH that killed no one before 2020

They convinced the public and governments around the world that the PCR tests are evidence of a NOVEL PATHOGEN

They have purposefully omitted ALL CAUSE MORTALITY from the discussion because there is no evidence for a NEW CAUSE OF DEATH nor for new effective treatments for it

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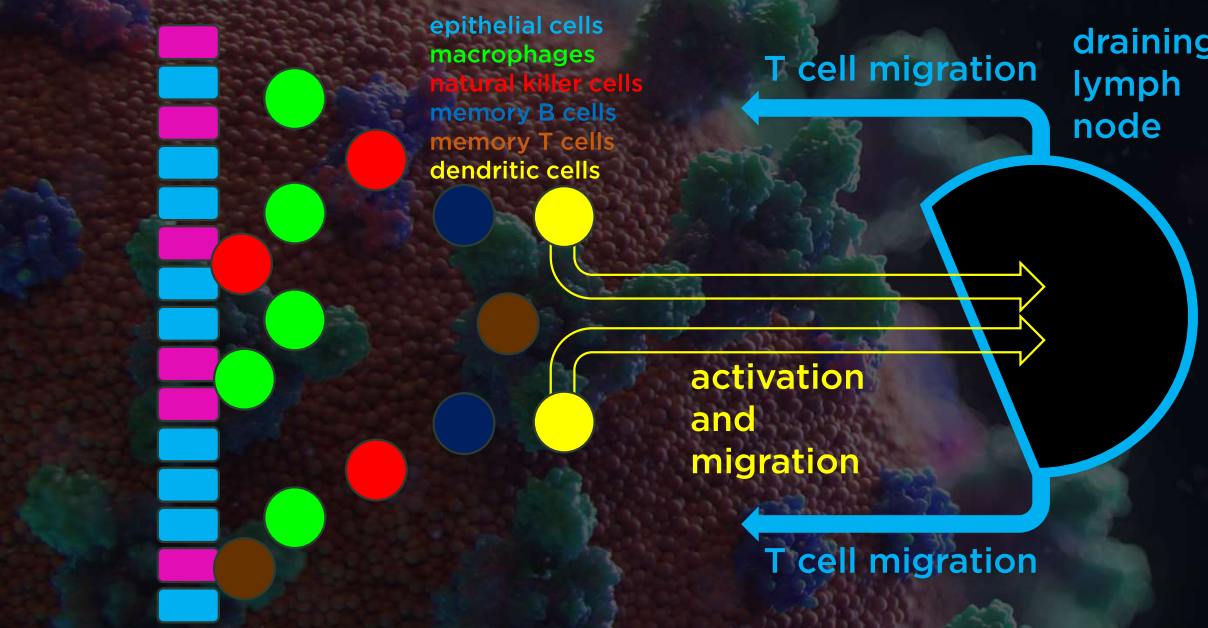
They changed how we THINK about the Human Immune System



THEY HAVE CHANGED THE WAY WE THINK

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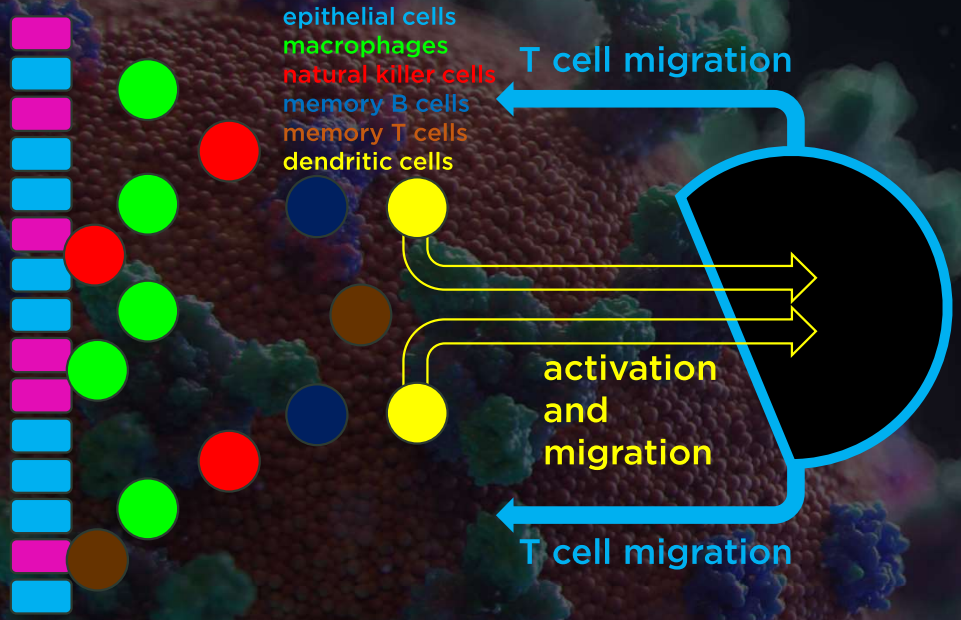

They have misled us about the value of seroprevalence for national security reasons and to claim Product effectiveness

They have accomplished this by disingenuously emphasizing antibodies to structural proteins as unproven correlates of immunity

They have oversimplified the public's understanding of the immune response to avoid any loss of countermeasure uptake from informed consent non-participation

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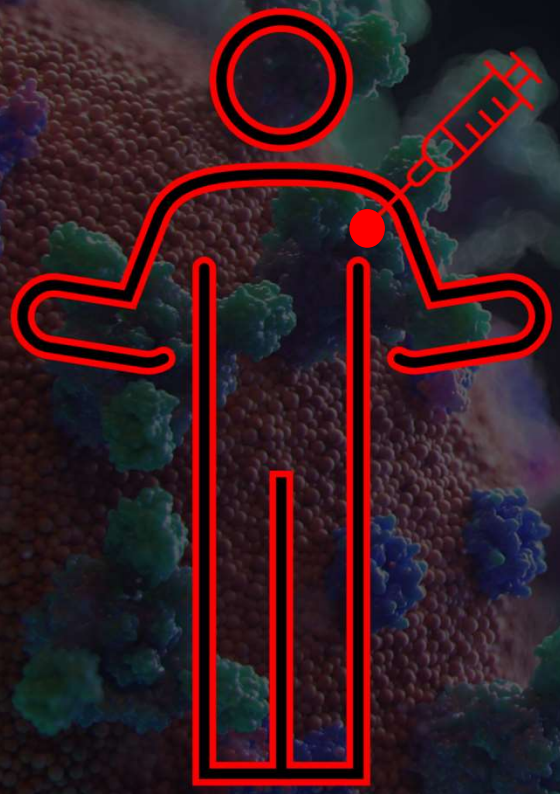
They changed how we THINK about the concept of vaccination



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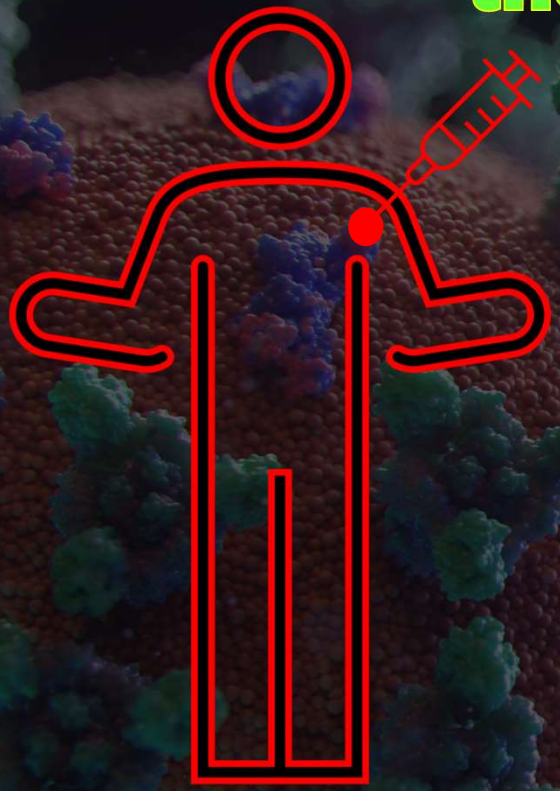

They have misled the public about the biological definition of a vaccine for national security reasons

They have accomplished this by disingenuously emphasizing antibodies as “correlates” of immunity

They are currently focused on convincing the public to accept transfection as proven safe and effective due to a serendipitous consequence of the pandemic

ALMOST NOTHING ABOUT THESE PRODUCTS IS KNOWN (content, purity, variability…fidelity of product, tissue specificity, individual variability, long term effects, etc.)

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8 February 2023

They changed how we THINK about the concept of vaccination

Taking the Product will protect your Grandmother

We all have a duty to society that includes masking, social distancing, testing, and regular transfection

They are currently moving forward under a continuing emergency to justify further transfection Products.

Transfecting pregnant women to protect the unborn child is currently a viable medical position that can only be explained as part of the effort to normalize transfection as proven safe and effective

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8 February 2023

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our immune response to a respiratory virus

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Immunization, vaccination, and immunity

They ventilated to prevent spread and killed many

+

Remdesivir kills people

+

Untreated secondary bacterial pneumonia kills people

+

Shutting down schools hurt kids

+

Masking hurt kids

+

Social distancing hurt families and the communities in which they exist

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IMMUNOLOGY 101-2020
They changed how we THINK
IMMUNOLOGY 101-2020

IMMUNOMYTHOLOGY 101-2020

The Proximal Origin

The Fauci Emails
Fusion Inhibition
Endosomal Entry
3CL inhibitors

The Molecular Signatures

GP120 HIV
DC-SIGN
Furin Cleavage site

The Singular Narrative

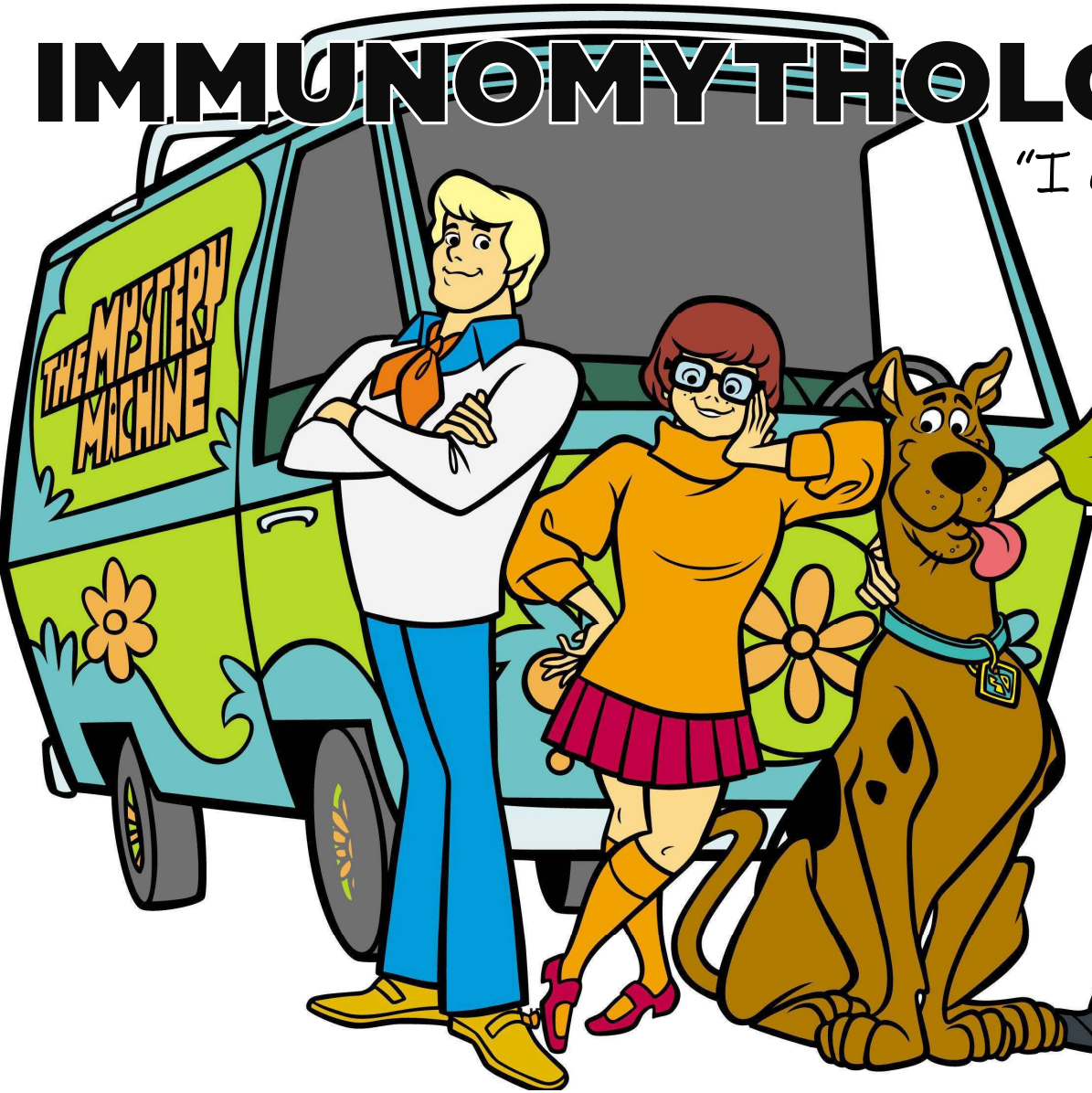
Lockstep lockdowns
Novel virus/No previous immunity
Slogans
Vaccine redefined
Trillions of dollars globally

They changed how we THINK

IMMUNOMYTHOLOGY 101-2020

IMMUNOMYTHOLOGY 101-2020

"I can't believe we figured it out!?"



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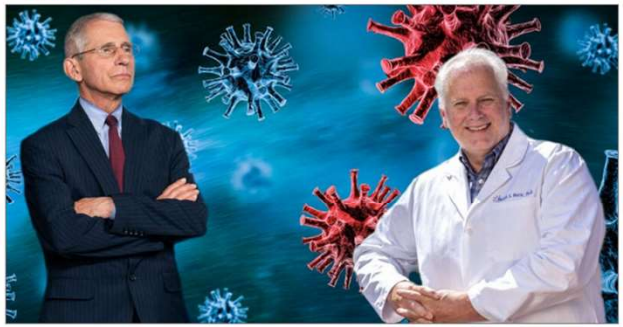
New Analysis of COVID Virus Suggests Fauci and Baric's Fingerprints on Pandemic Bug

Critics have long questioned why the National Institutes of Health would fund experiments by University of North Carolina at Chapel Hill professor Ralph Baric to develop a technique for hiding evidence of human tampering in laboratory-created super viruses.

By Robert F. Kennedy, Jr.
J. Jay Couey, Ph.D.
Charles Rixey

157



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Critics have long questioned why the National Institutes of Health (NIH) would fund experiments by University of North Carolina at Chapel Hill (UNC) professor Ralph Baric to develop a technique for hiding evidence of human tampering in laboratory-created super viruses.

Aided by some $220.5 million in National Institute of Allergy and Infectious Diseases (NIAID) funding, Baric developed a so-called "Seamless Ligation" technique, which he boasted could perfectly conceal all evidence of human tampering in laboratory-created viruses. Baric nicknamed his invention the "no-see'm" method.

Now a new study, "Endonuclease fingerprint indicates a synthetic origin of SARS-CoV2," published on the preprint server bioRxiv, shows that — apparently unbeknownst to Baric — the "seamless ligation" concealment gimmick leaves its own minute but legible signature.

Most momentously, these same researchers have discovered that damning signature in the genome of the virus that causes COVID-19.

Baric's technique has long been controversial. "It's the artist that doesn't sign his name to the painting; the virologist that doesn't put his signature into the virus to let us know whether or not it is emerging naturally or whether it is produced in a laboratory," said Jeffrey Sachs, chair of The Lancet COVID-19 Commission, a task force that investigated the origins of COVID-19.

"All of it says, my God, there was really a big, very risky research agenda underway."

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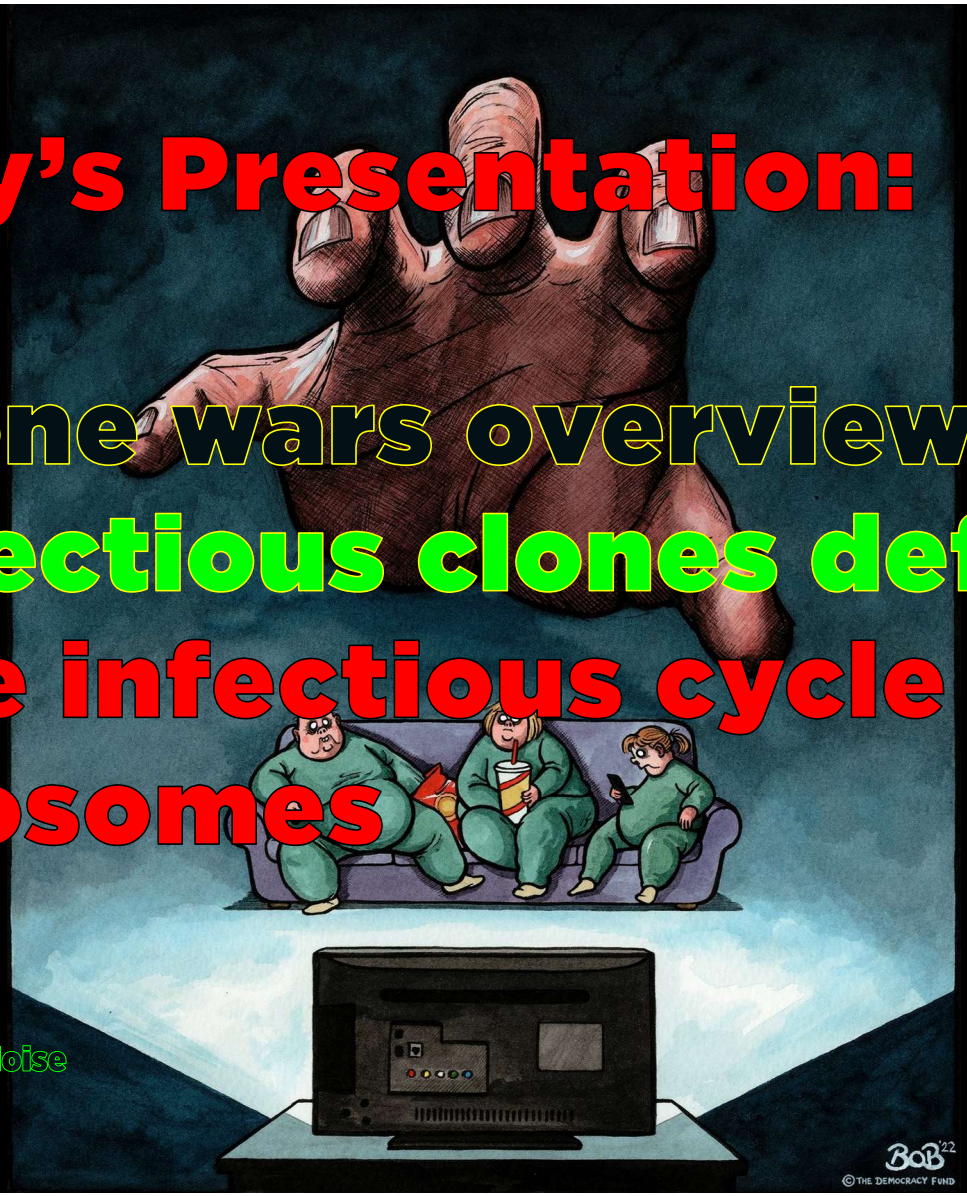

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8 February 2023

WHY ARE WE THEY DOING THIS?

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 11354–11358, October 1996
Colloquium Paper

This paper was presented at a colloquium entitled "Genetic Engineering of Viruses and of Virus Vectors," organized by Bernard Roizman and Peter Palese (Co-chairs), held June 9–11, 1996, at the National Academy of Sciences in Irvine, CA.

Negative-strand RNA viruses: Genetic engineering and applications

PETER PALESE*, HONGYONG ZHENG, OTHMAR G. ENGELHARDT, STEPHAN PLESCHKA, AND ADOLFO GARCÍA-SASTRE

Department of Microbiology, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029

ABSTRACT **The negative-strand RNA viruses are a broad group of animal viruses that comprise several important human pathogens, including influenza, measles, mumps, rabies, respiratory syncytial, Ebola, and hantaviruses. The development of new strategies to genetically manipulate the genomes of negative-strand RNA viruses has provided us with new tools to study the structure–function relationships of the viral components and their contributions to the pathogenicity of these viruses. It is also now possible to envision rational approaches—based on genetic engineering techniques—to design live attenuated vaccines against some of these viral agents. In addition, the use of different negative-strand RNA viruses as vectors to efficiently express foreign polypeptides has also become feasible, and these novel vectors have potential applications in disease prevention as well as in gene therapy.**

DNA-Containing Viruses

Among animal viruses, DNA-containing viruses were the first to become amenable to genetic engineering techniques. This breakthrough was achieved for simian virus 40 when a cloned cDNA copy was transfected into cells, resulting in the formation of infectious virus (see Table 1). Transfected mutated cDNA molecules gave rise to defined mutant viruses (1). A second methodology involving the use of homologous recombination allowed, for the first time, the rescue of large DNA-containing viruses such as herpes viruses (2). In this approach, intact herpes viral DNA as well as cloned DNA flanked by viral sequences was transfected into cells. Homologous recombination between the cloned DNA and the wild-type genome can occur, and novel viruses can be selected under appropriate conditions. For example, recombinants with DNA fragments containing a viral thymidine kinase gene can be selected in appropriate cell lines and media, and viruses lacking a thymidine kinase can be isolated in the presence of nucleoside analogs (e.g., Ara T). This general technique allows the successful construction of viral variants of herpes viruses, and similar procedures have been developed for pox viruses (3, 4) and other DNA-containing viruses including adenoviruses (5) and parvoviruses (6). Finally, strategies have been developed to generate infectious as well as mutant viruses by transfecting cosmids containing overlapping portions of large viral genomes. Viruses arise via recombination between the cosmids. This system was successfully used to rescue infectious herpes simplex 1 viruses (7), cytomegaloviruses (8) and Epstein–Barr viruses (9) from their respective cosmids.

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Positive-Strand RNA Viruses

RNA-containing viruses belong to a variety of families with diverse replication strategies. Unique among the RNA viruses are the retroviruses, whose replication involves a double-stranded DNA phase, making these viruses an easy target for genetic manipulation. Transfection of full-length cDNA molecules leads to the establishment of replicating virus particles and integration of the viral genetic information into the host genome (10). The engineering of retroviral genomes has become one of the most successful genetic approaches in modern virology and is central to the study both of viral gene expression and of protein structure–function analysis. In addition, retrovirus constructs are among the most widely used vectors for gene transfer and gene therapy (11).

Most of the other positive-strand RNA viruses are also amenable to genetic engineering approaches (Table 1). In the case of the small and medium sized positive-strand RNA viruses, full-length genomic RNA has been shown to be infectious when transfected into cells. Plus-strand RNA serves as mRNA for the synthesis of viral proteins as well as template for viral RNA replication. Thus, transfection of cloned DNA of poliovirus RNA (or of cDNA-derived RNA) into permissive cells results in the formation of infectious virus particles (12).

Remarkably successful have been studies using Sindbis viruses and Semliki forest virus (13, 14). The cDNA-derived RNAs of these positive-strand RNA viruses can be used to efficiently rescue infectious viruses, thus allowing an extensive analysis of the promoter elements of the viral RNAs as well as structure–function studies of the viral proteins. Furthermore, these viruses have received increased attention because of their potential for expressing copious amounts of heterologous genes via recombinant constructs. Up to 10^8 molecules of heterologous protein per cell have been expressed using these systems.†

Introduction of cDNA-Derived RNA into a Negative-Strand RNA Virus (Influenza Virus)

The life cycle of negative-strand RNA viruses differs from that of the other RNA viruses in many ways. Specifically, the genomic RNA of negative-strand RNA viruses is not infectious, and infectious virus particles must also deliver their own RNA-dependent RNA polymerase into the infected cell to start the first round of virus-specific mRNA synthesis.

Thus, approaches different from those used for positive-strand RNA viruses had to be developed to allow the rescue of

Abbreviations: RNP, ribonucleoprotein; HA, hemagglutinin; NA, neuraminidase, VSV, vesicular stomatitis virus.

*To whom reprint requests should be addressed. e-mail: ppalese@smtplink.mssm.edu.

†Belli, B. A., Polo, J. M., Driver, D. A., Latham, E., Banks, T. A., Chang, S. M. W. & Dubensky, T. W., Jr., National Academy of Sciences Colloquium on Genetic Engineering of Viruses and of Virus Vectors, June 9–11, 1996, Irvine, CA, no. 1. (abstr.).

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 9345–9350, August 1999
Microbiology

Generation of influenza A viruses entirely from cloned cDNAs

GABRIELE NEUMANN*, TOKIKO WATANABE*†, HIROSHI ITO*, SHINJI WATANABE*†, HIDEO GOTO*, PENG GAO*, MARK HUGHES*, DANIEL R. PEREZ‡, RUBEN DONIS‡, ERICH HOFFMANN§, GERD HOBOM§, AND YOSHIHIRO KAWAOKA*¶

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Communicated by Paul Ahlquist, University of Wisconsin, Madison, WI, May 27, 1999 (received for review March 23, 1999)

ABSTRACT **We describe a new reverse-genetics system that allows one to efficiently generate influenza A viruses entirely from cloned cDNAs. Human embryonic kidney cells (293T) were transfected with eight plasmids, each encoding a viral RNA of the A/WSN/33 (H1N1) or A/PR/8/34 (H1N1) virus, flanked by the human RNA polymerase I promoter and the mouse RNA polymerase I terminator—together with plasmids encoding viral nucleoprotein and the PB2, PB1, and PA viral polymerases. This strategy yielded >1 × 10^3 plaque-forming units (pfu) of virus per ml of supernatant at 48 hr posttransfection. The addition of plasmids expressing all of the remaining viral structural proteins led to a substantial increase in virus production, 3 × 10^4–5 × 10^7 pfu/ml. We also used reverse genetics to generate a reassortant virus containing the PB1 gene of the A/PR/8/34 virus, with all other genes representing A/WSN/33. Additional viruses produced by this method had mutations in the PA gene or possessed a foreign epitope in the head of the neuraminidase protein. This efficient system, which does not require helper virus infection, should be useful in viral mutagenesis studies and in the production of vaccines and gene therapy vectors.**

The ability to generate infectious RNA viruses from cloned cDNAs has contributed greatly to our biological understanding of these pathogens and, hence, to improved methods of disease control (1). However, this progress had been relatively limited for negative-sense as compared with positive-sense RNA viruses, because neither the genomic viral RNA (vRNA) nor the antigenomic complementary RNA (cRNA) of negative-sense RNA viruses can serve as a direct template for protein synthesis. Rather, the vRNA, after its encapsidation by viral nucleoprotein (NP), must be transcribed into positive-sense mRNA by the viral RNA polymerase complex. Thus, the minimal replication unit is formed by the genomic vRNA complexed with NP and the polymerase proteins. Despite these obstacles, reverse-genetics methods have been established to produce nonsegmented, negative-sense RNA viruses, including rabies virus (2), vesicular stomatitis virus (3, 4), measles virus (5), respiratory syncytial virus (6), Sendai virus (7, 8), rinderpest virus (9), human parainfluenza virus type 3 (10), and simian virus 5 (11).

Generating segmented, negative-sense RNA viruses from cloned cDNAs poses a more formidable challenge, as one must produce a separate vRNA for each gene segment. In one study, Bridgen and Elliott (12) produced a Bunyamwera virus (family *Bunyaviridae*) from cloned cDNAs encoding three segments of negative-sense vRNA; however, the efficiency of virus recovery was low, and there have been no reports of an engineered Bunyamwera mutant. By contrast, none of the orthomyxoviruses, which contain six (Thogotovirus), seven (influenza C virus), or eight (influenza A and B viruses) segments of negative-sense RNA, have been produced entirely from cloned cDNAs. This lag in progress has been felt most acutely in efforts to control influenza virus infections.

Palese and colleagues (13) pioneered the reverse-genetics, helper virus-dependent system for influenza A virus (Fig. 1*A*). In their approach, a ribonucleoprotein (RNP) complex is generated by *in vitro* vRNA synthesis in the presence of purified polymerase and NP proteins and then used to transfect eukaryotic cells. Subsequent infection with influenza A helper virus results in the generation of viruses possessing a gene derived from cloned cDNA. A second method, developed by Neumann *et al.* (14), is based on the *in vivo* synthesis of vRNA by RNA polymerase I (Fig. 1*B*), a cellular enzyme that transcribes ribosomal RNA that lacks both a 5′ cap and a 3′ poly(A) tail. Transfection of cells with a plasmid containing cloned influenza virus cDNAs, flanked by RNA polymerase I promoter and terminator sequences, followed by influenza virus infection, led to the production of transfectant viruses. With both methods, however, transfectants must be selected from a vast background of helper viruses, which requires a strong selection system and complicates the generation of growth-defective viruses.

We report here the generation of influenza A viruses entirely from cloned cDNAs. The reverse-genetics approach we describe is highly efficient and can be used to introduce mutations into any gene segment and to develop influenza virus-based gene delivery systems.

MATERIALS AND METHODS

Cells and Viruses. 293T human embryonic kidney cells and Madin–Darby canine kidney cells (MDCK) were maintained in DMEM supplemented with 10% FCS and in MEM containing 5% newborn calf serum, respectively. The 293T cell line is a derivative of 293, into which the gene for the temperature-sensitive simian virus 40 T antigen has been inserted. This line produces replication-competent T antigen in large amounts at 37°C (15). All cells were maintained at 37°C in 5% CO_2. Influenza viruses A/WSN/33 (H1N1) and A/PR/8/34 (H1N1) were propagated in 10-day-old eggs.

Construction of Plasmids. To generate RNA polymerase I constructs, we cloned cDNAs derived from A/WSN/33 or A/PR/8/34 viral RNA between the promoter and terminator sequences of RNA polymerase I. Briefly, the cloned cDNAs were amplified by PCR with primers containing *Bsm*BI sites,

Abbreviations: cRNA, complementary RNA; MDCK, Madin–Darby canine kidney; HA, hemagglutinin; NA, neuraminidase; NP, nucleoprotein; pfu, plaque-forming units; RNP, ribonucleoprotein complex; VLP, virus-like particle; vRNA, viral RNA.

A Commentary on this article begins on page 8804.

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ORIGINAL ARTICLE

First infectious clone of the propagatively transmitted *Oat blue dwarf virus*

Michael C. Edwards · John J. Weiland

Received: 29 October 2009 / Accepted: 14 January 2010 / Published online: 12 March 2010

Abstract *Oat blue dwarf virus* (OBDV) is a small, phloem-limited marafivirus that replicates in its leafhopper vector. We have developed complete cDNA clones of OBDV from which infectious transcripts may be derived—the first such clones for any propagatively transmitted plant virus. Prior to clone construction, the reported sequences of

[7]. Longstanding members of this genus include *Maize rayado fino virus* (MRFV), *Oat blue dwarf virus* (OBDV), and *Bermuda grass etched-line virus* (BELV). These viruses have relatively narrow host ranges, infecting plants primarily in the Poaceae, although OBDV also infects flax [3]. For decades, these were the only known marafiviruses. Recently, virus—*dden death-associated virus* was shown alysis to be a likely new member of the and was formally approved as a member

Arch Virol (2010) 155:1923–1931
DOI 10.1007/s00705-010-0776-z

ORIGINAL ARTICLE

Construction and characterization of a full-length infectious c from a fast-replicating, X4-tropic HIV-1 subtype B′ isolate

Shou-li Wu · Yan-sheng Yan · Ping-ping Yan · Hai-long Huang · Hui-rong Wang

Received: 15 December 2009 / Accepted: 26 July 2010 / Published online: 10 August 2010

Abstract In HIV-1 epidemics in China, HIV-1 subtype B′ is the most predominant subtype circulating in intravenous drug users. In this study, we constructed an HIV-1 full-length infectious molecular clone based on the primary virus LWJ, which was isolated from an HIV-infected patient in Fujian Province, China. Phylogenetic and bootscanning analysis of the viral sequence revealed that the isolate LWJ belonged to HIV-1 subtype B′. The infectious clone was designated as "pLWJ". The virus (LWJ-c) produced from this infectious clone by in vitro transfection of 293T cells could infect both human peripheral blood mononuclear cells (PBMCs) and human the T cell line MT4. Interestingly, the cloned LWJ-c virus utilized CXCR4 as its co-receptor and could replicate in vitro with similar efficiency and kinetics compared to its parental primary isolate LWJ as well as the clade B reference virus NL4-3. The LWJ-c virus could also cause cytopathic effects in both PBMCs and MT cells. Sequence analysis of the envelope glycoprotein of pLWJ showed that a conserved GPGR motif and an arginine at position 11 were present in the V3 loop, which was consistent with previous reports regarding CXCR4 co-receptor usage and syncytium-inducing (SI) phenotype. Thus, the infectious clone

represents a fast-replicating, high-produ pic and syncytium-inducing isolate. Gi of HIV-1 subtype B′ in China, this infect very useful tool to provide a versatile m research focusing on the biological subtype.

Introduction

The genomic sequences of human immu type 1 (HIV-1) show high levels of phylogenetic analysis of the *env* and *gs* indicates there are three distinct groups the virus circulating worldwide. Within there are presently nine distinct subtypes K) and 34 circulating recombinant forms first case of HIV-1 infection was repo 1985 [1]. HIV/AIDS has spread rapid country and has become an increasingly to China. By the end of 2009, there w 740,000 individuals being infected w country. The current major prevalent H China are B′ (Thai-B) (44%) and the ci nant forms (CRFs) CRF08_BC (29%) (13%) [2].

The HIV-1 subtype B′ strain was from injected-drug users in Bangkok, Although the sequences of the envelope from those isolates clustered phylogeneti B Env, they were genetically distinct fr B viruses [3], and subsequently termed T HIV-1 subtype B′ viruses were not only but also quickly spread to other Asian China, Malaysia and Japan, and have

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Construction of an infectious cDNA clone for Omsk hemorrhagic fever virus, and characterization of mutations in NS2A and NS5

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Infectious cDNA
Viral replication

ABSTRACT

Omsk hemorrhagic fever virus
viruses, and causes hemorrha
constructed to investigate the r
Our cDNA clone was capable of
Guriev strain, and the recombina
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Complete genomic sequence and an infectious

S.H. Sheldon Tai [a], Masahiro Niikura [b,c,1], Hans H. Cheng
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FHV-1
Genomic sequence
Infectious BAC clone

ABSTRACT

Infection with feline herpesviru
Felidae. We report the first com
terization of a bacterial artificia
genome and has the BAC vector
from both the FHV-1 BAC clone
overall G + C content of 45%. A to
The gene arrangement is colline
the BAC was very similar to the
characteristics and highly virulen

Introduction

Feline herpesvirus-1 (FHV-1) is a significant viral pathogen of Felidae, first isolated in 1957 by Crandell and Maurer (1958). FHV-1 accounts for approximately 50% of all diagnosed viral upper respiratory infections in cats and is also a significant cause of ocular lesions (Nasisse, 1990). The pathobiology of FHV-1 has been reviewed (Gaskell et al., 2007; Maggs, 2005; Stiles, 2003). Briefly, following entry via the oronasal route, FHV-1 replicates extensively in the mucosae of the upper respiratory tract resulting in high fever, depression, anorexia, sneezing, conjunctivitis, keratitis, and ocular and nasal discharge. The acute phase of the disease is followed by lifelong latency, a hallmark of herpesvirus infections. During the latent stage, viral DNA appears to persist mainly in sensory ganglia. Different biological stresses, or administration of corticosteroids, can induce the necessary biochemical stimuli in latently infected cells that lead to renewed production of infectious virus, which can travel to the periphery and is a potential source of viral transmission. I also a contributor to feline cl 2005). Despite its high morbi available vaccines cannot to and, as a consequence, from fi Tham and Studdert, 1987). are avirulent when adminis administered oronasally (Kn tivation of latent FHV-1 is co tomatic carriers and contrib and Povey, 1982).

Our knowledge of FHV- FHV-1 is a member of *Herp* family, genus *Varicellovirus* appear to be relatively simil genome, mapped by restric is approximately 134 kb, an other varicelloviruses (Fig. consists of two segments of (U_L) and unique short (U_S). tical but inverted repeat se (TR_S) and inverted repeat s 1986). Alphaherpesviruses u (ORFs) (Alba et al., 2001). Fi associated proteins (Fargea previously been identified a

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Construction of an infectious cDNA clone of avian hepatitis E virus (avian HEV) recovered from a clinically healthy chicken in the United States and characterization of its pathogenicity in specific-pathogen-free chickens

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Abstract

A genetically distinct strain of avian hepatitis E virus (avian HEV-VA strain) was isolated from a healthy chicken in Virginia, and thus it is important to characterize and compare its pathogenicity with the prototype strain (avian HEV-prototype) isolated from a diseased chicken. Here we first constructed an infectious clone of the avian HEV-VA strain. Capped RNA transcripts from the avian HEV-VA clone were replication-competent after transfection of LMH chicken liver cells. Chickens inoculated intrahepatically with RNA transcripts of avian HEV-VA clone developed active infection as evidenced by fecal virus shedding, viremia, and seroconversion. To characterize the pathogenicity, RNA transcripts of both avian HEV-VA and avian HEV-prototype clones were intrahepatically inoculated into the livers of chickens. Avian HEV RNA was detected in feces, serum and bile samples from 10/10 avian HEV-VA-inoculated and 9/9 avian HEV-prototype-inoculated chickens although seroconversion occurred only some chickens during the experimental period. The histopathological lesion scores were lower for avian HEV-VA group than avian HEV-prototype group in the liver at 3 and 5 weeks post-inoculation (wpi) and in the spleen at 3 wpi, although the differences were not statistically significant. The liver/body weight ratio, indicative of liver enlargement, of both avian HEV-VA and avian HEV-prototype groups were significantly higher than that of the control group at 5 wpi. Overall, the avian HEV-VA strain still induces histological liver lesions even though it was isolated from a healthy chicken. The results also showed that intrahepatic inoculation of chickens with RNA transcripts of avian HEV infectious clone may serve as an alternative for live virus in animal pathogenicity studies.

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Characterisation of a new infectious full-length cDNA clone of BVDV genotype 2 and generation of virus mutants

Katrin Mischkale [a], Ilona Reimann [b], J. Zemke [a], P. König [a], Martin Beer [a]

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Abstract

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recombinant BV

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 10, Number 3, 2010

DOI: 10.1089/vbz.2009.0067

Infection, Dissemination, and Transmission of a West Nile Virus Green Fluorescent Protein Infectious Clone by *Culex pipiens quinquefasciatus* Mosquitoes

Charles E. McGee, Alexandr V. Shustov, Konstantin Tsetsarkin, Ilya V. Frolov, Peter W. Mason, Dana L. Vanlandingham, and Stephen Higgs

Abstract

We report the construction and comparative characterization of a full-length West Nile virus (WNV) cDNA infectious clone (ic) that contains a green fluorescent protein (GFP) expression cassette fused within the viral open reading frame. Virus derived from WNV-GFP ic stably infected *Culex pipiens quinquefasciatus* mosquitoes at comparable rates to virus derived from the parental (non-GFP) ic. However, insertion of this GFP cassette resulted in a temporal delay in *in vivo* replication kinetics and significantly decreased dissemination to head tissue. Consistent with previous reports of WNV-infected mosquito midguts, focal GFP expression was observed at 3 days post-infection (dpi), with the majority of posterior midgut epithelial cells being positive by 7 dpi. GFP foci were observed in one pair of salivary glands (1/15) dissected 14 dpi. Mice exposed to WNV-GFP–infected mosquitoes developed viremia, and GFP was detected in lymph node homogenates. These data demonstrate the effectiveness of our strategy to generate a replication competent construct with increased reporter gene stability that may be used to study early events in infection.

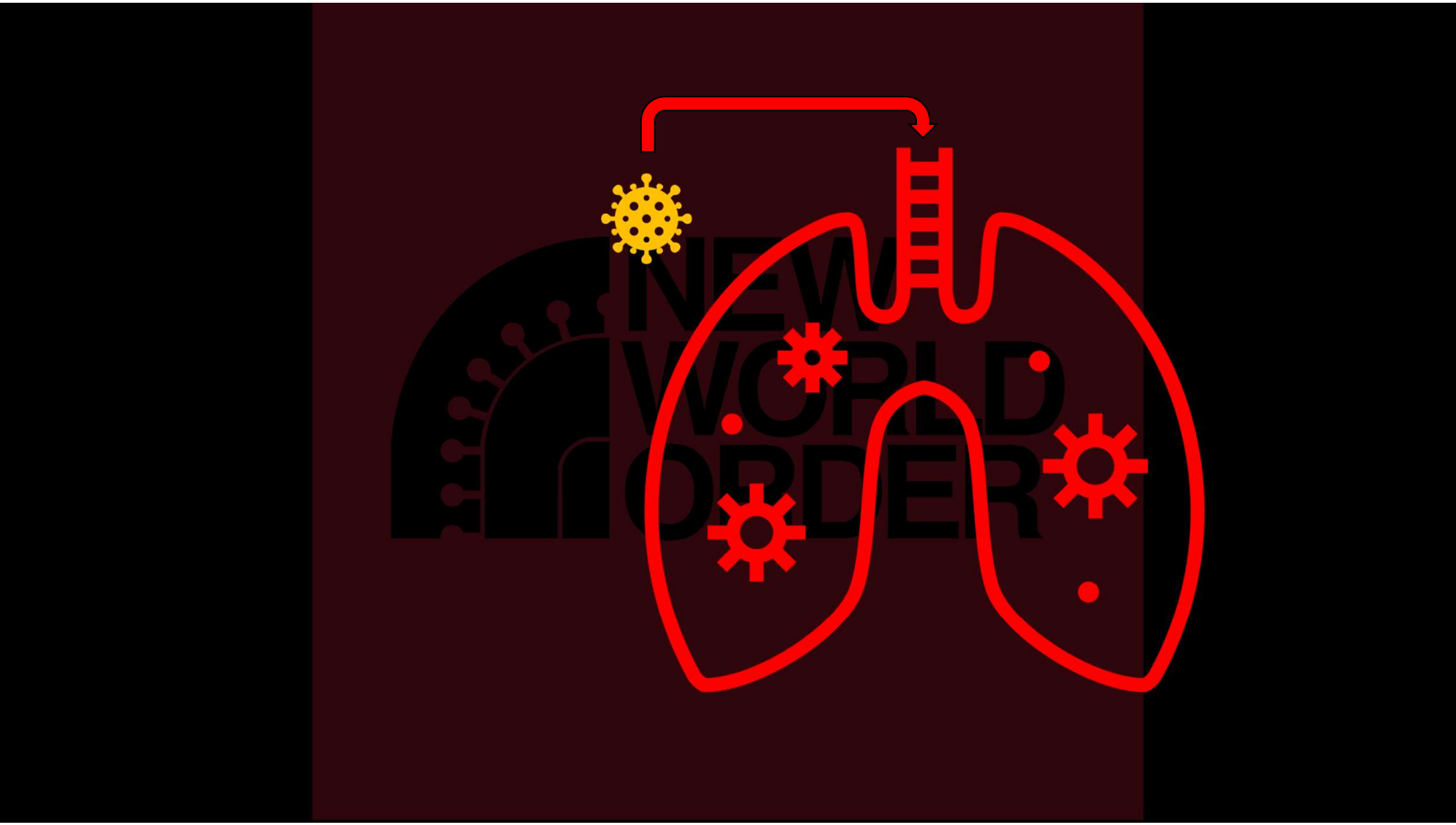
Key Words: Culex—Flaviviridae—Mosquito(es)—Transmission—West Nile.

Introduction

A NUMBER OF FLAVIVIRUS REVERSE GENETIC SYSTEMS have been developed and utilized for the study of basic viral genetics, *in vivo* interactions, and the advancement of vaccine technology (Rice et al. 1989, Lai et al. 1991, Sumiyoshi et al. 1995). Genes of interest (GOI) have been cloned into these recombinant genomes in proximity to the genomic 3′-end under the control of exogenous translation control elements, such as internal ribosomal entry sequences (Scholle et al. 2004, Pierson et al. 2005, Rossi et al. 2005). Further random mutagenesis studies have identified regions of the flavivirus genome that tolerate small GOI insertions. As a result, small epitopes derived from T cells, Rift Valley fever virus, Lassa fever virus, and Influenza A virus have been expressed via direct insertion into the flaviviral open reading frame (ORF) (Rice 1990, Tao et al. 2005, Bredenbeek et al. 2006, Pugachev et al. 2008). However, previous attempts to generate a full-length West Nile virus (WNV) construct with a fluorescent reporter gene using similar manipulations have been confounded by genetic instability and the rapid emergence of mutants with deleted GOI sequences (Pierson et al. 2005). In a recent study, Shustov et al. (2007) reported the development of a pseudoinfectious bipartite genome yellow fever virus 17D reverse genetic system that stably expressed GOI via insertion of a 5′ expression cassette into the genome-encoded polyprotein.

WNV is a mosquito-borne flavivirus that can infect the central nervous system of vertebrate hosts with potential for subsequent development of encephalitis, meningitis, meningoencephalitis, and poliomyelitis-like disease (www.cdc.gov/ncidod/dvbid/westnile). WNV is principally maintained in nature through transmission by ornithophilic *Culex* mosquitoes with amplification in susceptible avian hosts (Apperson et al. 2004). However, WNV has been recovered from numerous mosquito and tick species (Whitman and Aitken 1960, Karabatsos 1985, Abbassy et al. 1994, Anderson et al. 2003), and a number of nonconventional modes of infection have been identified (Granwehr et al. 2004, Higgs et al. 2005, McGee et al. 2007, Reisen et al. 2007). Like all of the members of the genus *flavivirus*, WNV possesses a positive-sense, single-stranded RNA genome containing a single ORF, which encodes three structural gene proteins (capsid,

Department of Pathology and Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas.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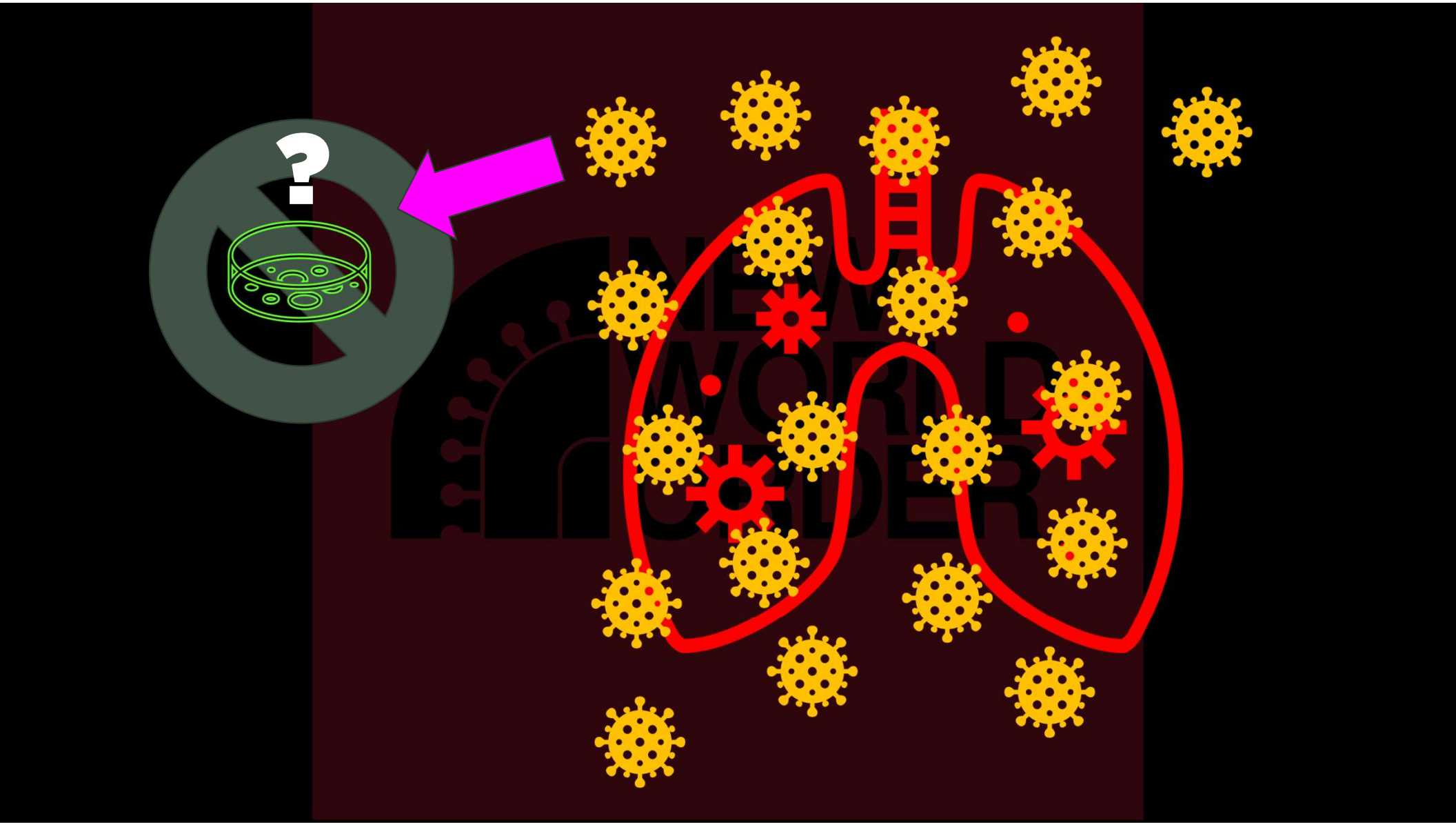
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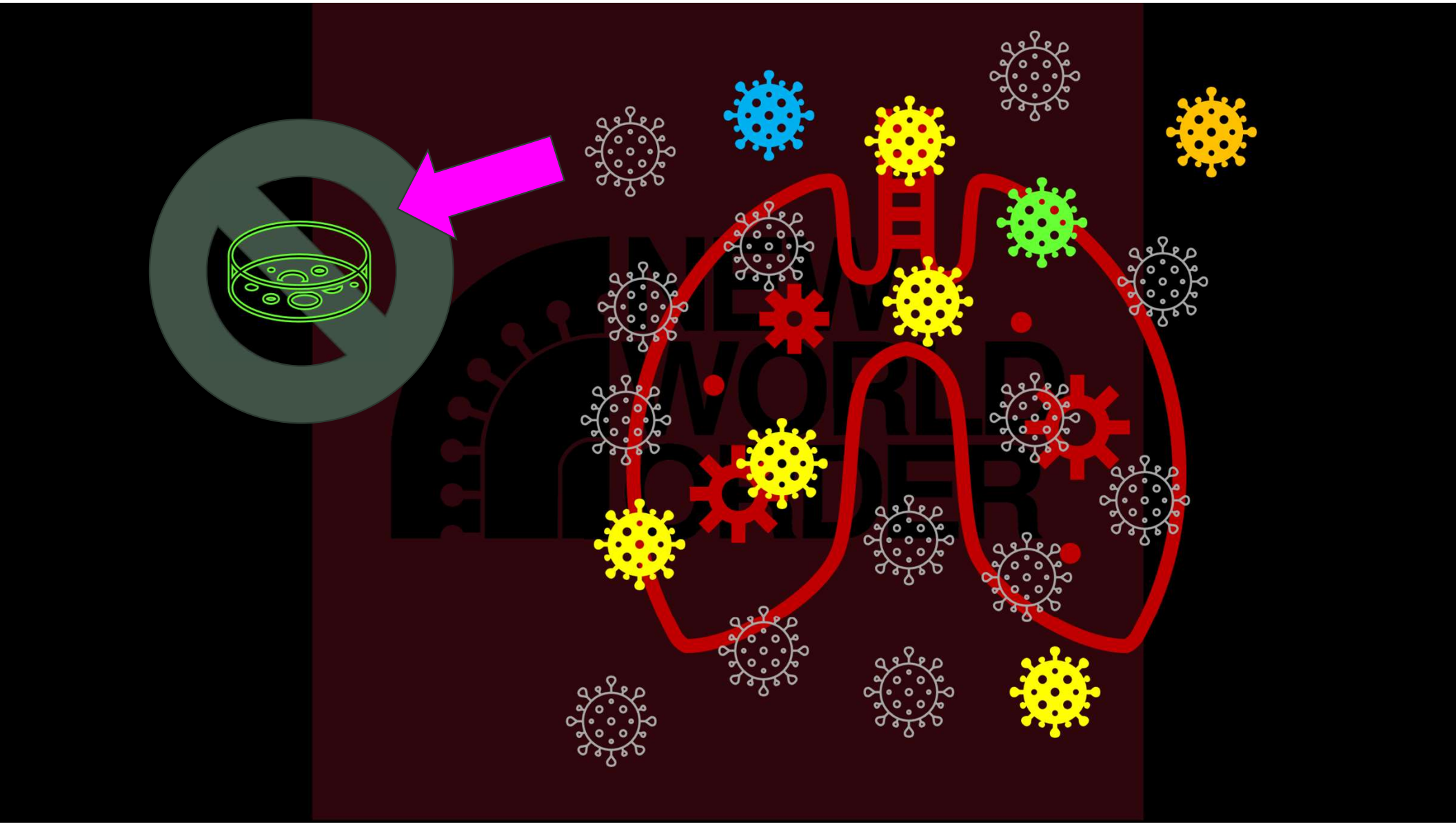
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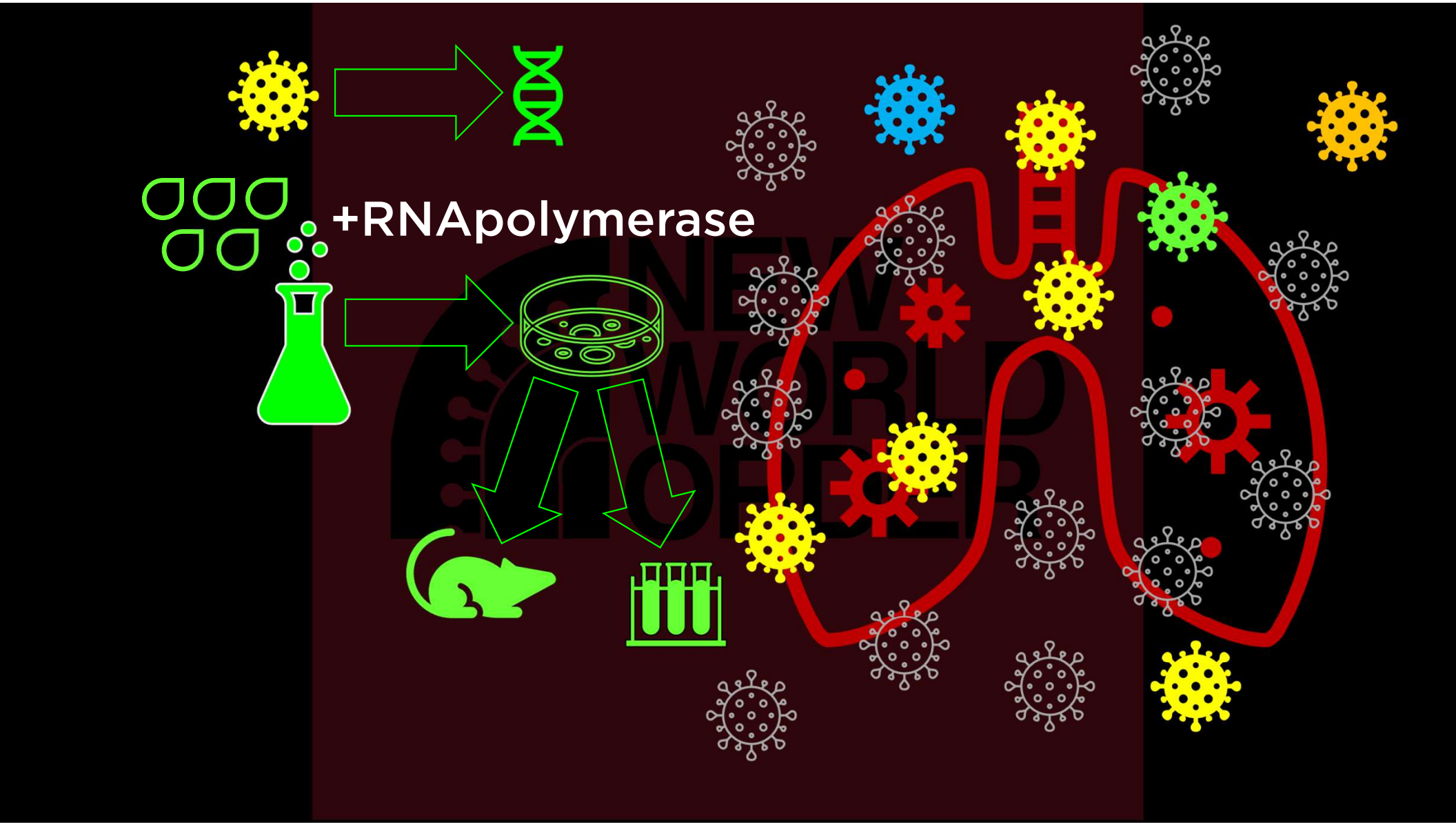
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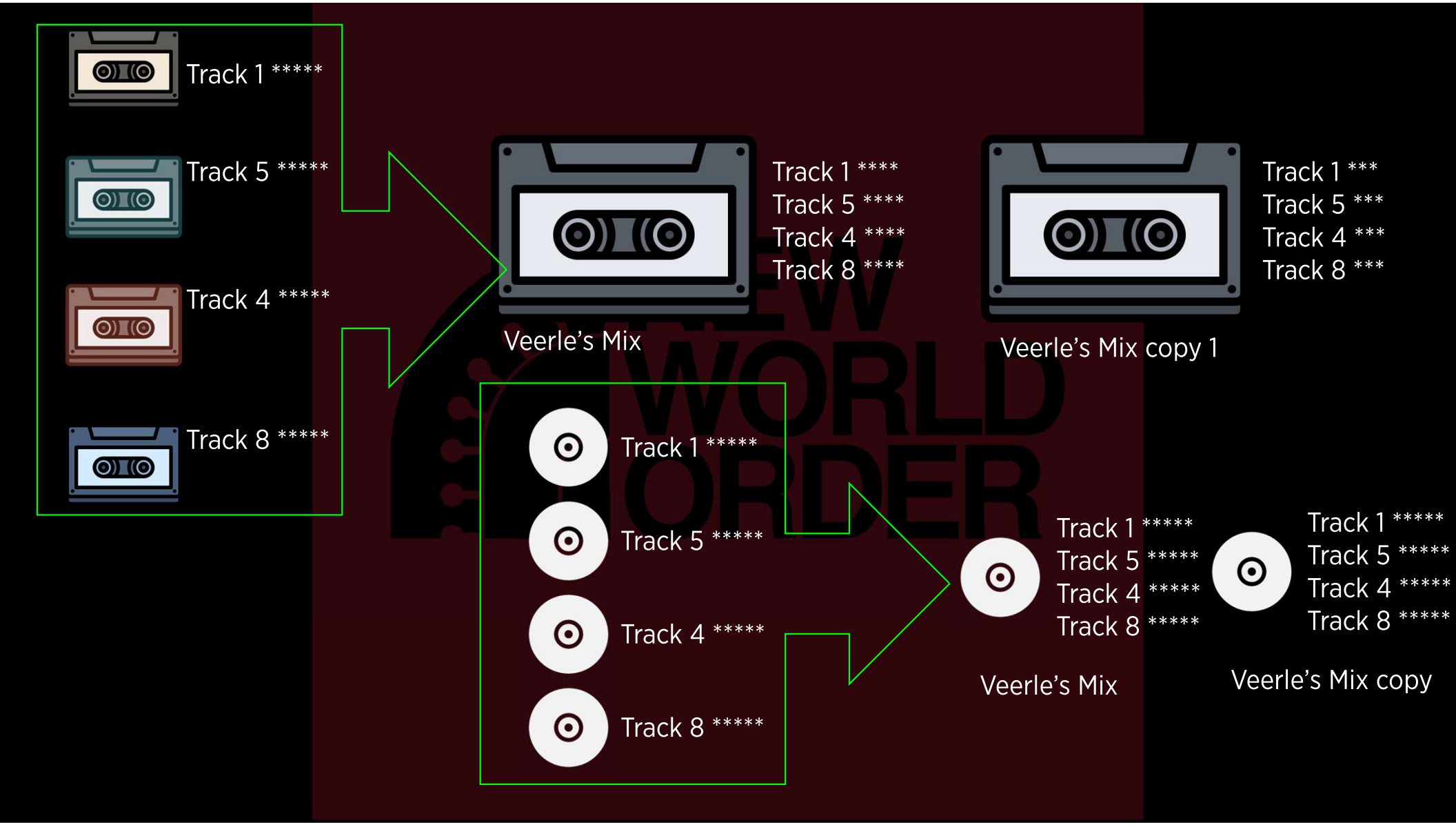
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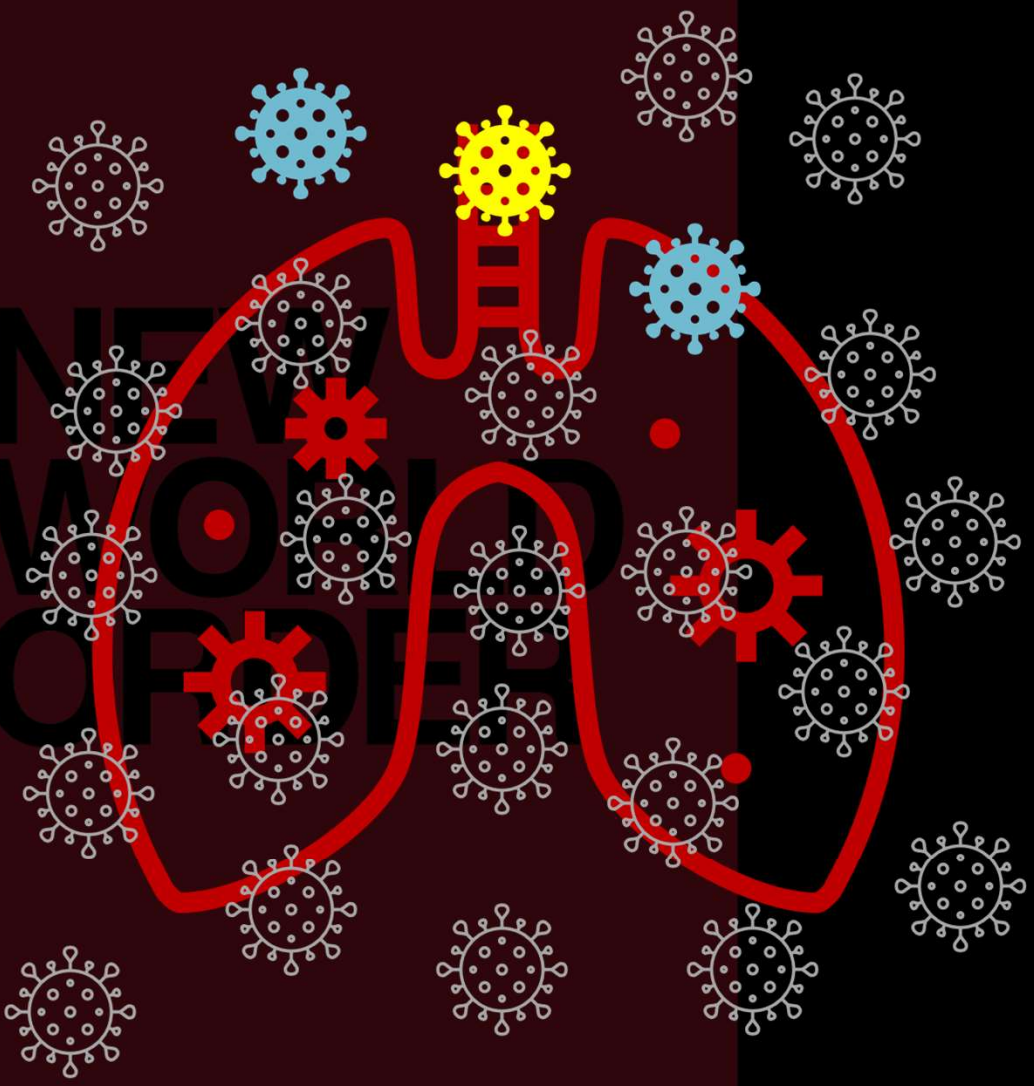
WHAT IF THE NATURAL INFECTIOUS CYCLE PRODUCES AN N:I RATIO OF 50:1 ?

500:1 ?

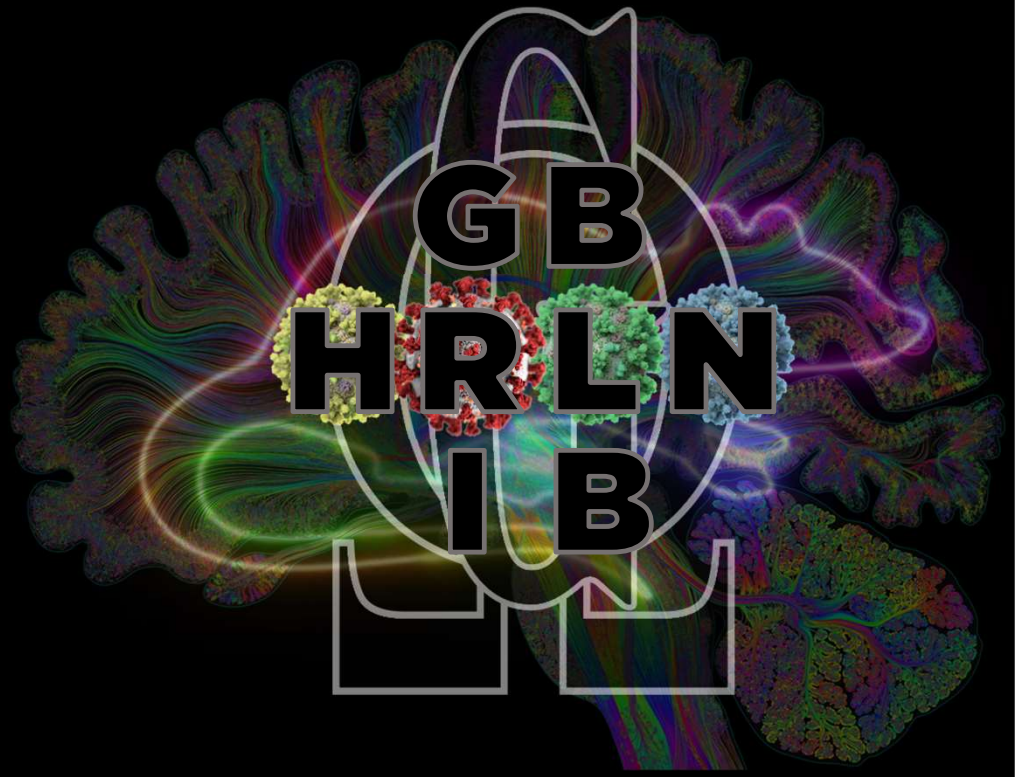
CAN NANOPORE SEQUENCING SHED ANY LIGHT ON THIS QUESTION ?



Q: WHAT IS A VIRUS?

A: WHATEVER THEY ARE, THEY CANNOT FORCE CELLS TO DO THINGS THEY DON'T ALREADY DO.

https://genome.cshlp.org/content/29/9/1545.full.pdf



Method

Direct RNA nanopore sequencing of full-length coronavirus genomes provides novel insights into structural variants and enables modification analysis

Adrian Viehweger,[1,2,5] Sebastian Krautwurst,[1,2,5] Kevin Lamkiewicz,[1,2] Ramakanth Madhugiri,[3] John Ziebuhr,[2,3] Martin Hölzer,[1,2] and Manja Marz[1,2,4]

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Sequence analyses of RNA virus genomes remain challenging owing to the exceptional genetic plasticity of these viruses. Because of high mutation and recombination rates, genome replication by viral RNA-dependent RNA polymerases leads to populations of closely related viruses, so-called "quasispecies." Standard (short-read) sequencing technologies are ill-suited to reconstruct large numbers of full-length haplotypes of (I) RNA virus genomes and (2) subgenome-length (sg) RNAs composed of noncontiguous genome regions. Here, we used a full-length, direct RNA sequencing (DRS) approach based on nanopores to characterize viral RNAs produced in cells infected with a human coronavirus. By using DRS, we were able to map the longest (~26-kb) contiguous read to the viral reference genome. By combining Illumina and Oxford Nanopore sequencing, we reconstructed a highly accurate consensus sequence of the human coronavirus (HCoV)-229E genome (27.3 kb). Furthermore, by using long reads that did not require an assembly step, we were able to identify, in infected cells, diverse and novel HCoV-229E sg RNAs that remain to be characterized. Also, the DRS approach, which circumvents reverse transcription and amplification of RNA, allowed us to detect methylation sites in viral RNAs. Our work paves the way for haplotype-based analyses of viral quasispecies by showing the feasibility of intra-sample haplotype separation. Even though several technical challenges remain to be addressed to exploit the potential of the nanopore technology fully, our work illustrates that DRS may significantly advance genomic studies of complex virus populations, including predictions on long-range interactions in individual full-length viral RNA haplotypes.

[Supplemental material is available for this article.]

Coronaviruses (subfamily *Coronavirinae*, family *Coronaviridae*, order Nidovirales) are enveloped positive-sense (+) single-stranded (ss) RNA viruses that infect a variety of mammalian and avian hosts and are of significant medical and economic importance, as illustrated by recent zoonotic transmissions from diverse animal hosts to humans (Vijay and Perlman 2016; Menachery et al. 2017). The genome sizes of coronaviruses (~30 kb) exceed those of most other RNA viruses. Coronaviruses use a special mechanism called discontinuous extension of minus strands (Sawicki and Sawicki 1995, 1998) to produce a nested set of 5′- and 3′-coterminal subgenomic (sg) mRNAs that carry a common 5′ leader sequence that is identical to the 5′-end of the viral genome (Zuniga et al. 2004; Sawicki et al. 2007). These sg mRNAs contain a different number of open reading frames (ORFs) that encode the viral structural proteins and several accessory proteins. With very few exceptions, only the 5′-located ORF (which is absent from the next smaller sg mRNA) is translated into protein (Fig. 1).

In HCoV-229E–infected cells, a total of seven major viral RNAs are produced. The viral genome is also referred to as mRNA 1 because it has an mRNA function. In its 5′-terminal region, the genome RNA contains two large ORFs, 1a and 1b, that encode the viral replicase polyproteins 1a and 1ab. mRNAs 2, 4, 5, 6, and 7 are used to produce the S protein, accessory protein 4, E protein, M protein, and N protein, respectively. The 5′-region of mRNA 3 contains a truncated fragment of ORF S, which is considered defective. Although this sg RNA has been consistently identified in HCoV-229E–infected cells, its mRNA function has been disputed, and there is currently no evidence that this RNA is translated into protein (Schreiber et al. 1989; Raabe et al. 1990; Thiel et al. 2003).

Like many other +RNA viruses, coronaviruses show high rates of recombination (Lai 1992; Liao and Lai 1992; Furuya et al. 1993). In fact, the mechanism to produce 5′ leader-containing sg mRNAs represents a prime example for copy-choice RNA recombination that, in this particular case, is guided by complex RNA–RNA interactions involving the transcription-regulating sequence (TRS) core sequences and likely requires additional interactions of viral proteins with specific RNA signals. In other virus systems, RNA recombination has been shown to generate "transcriptional units" that control the expression of individual components of the genome (Holmes 2009). The mechanisms involved in viral RNA recombination are diverse and may even extend to nonreplicating systems (Gallei et al. 2004). In the vast majority of cases, recombination

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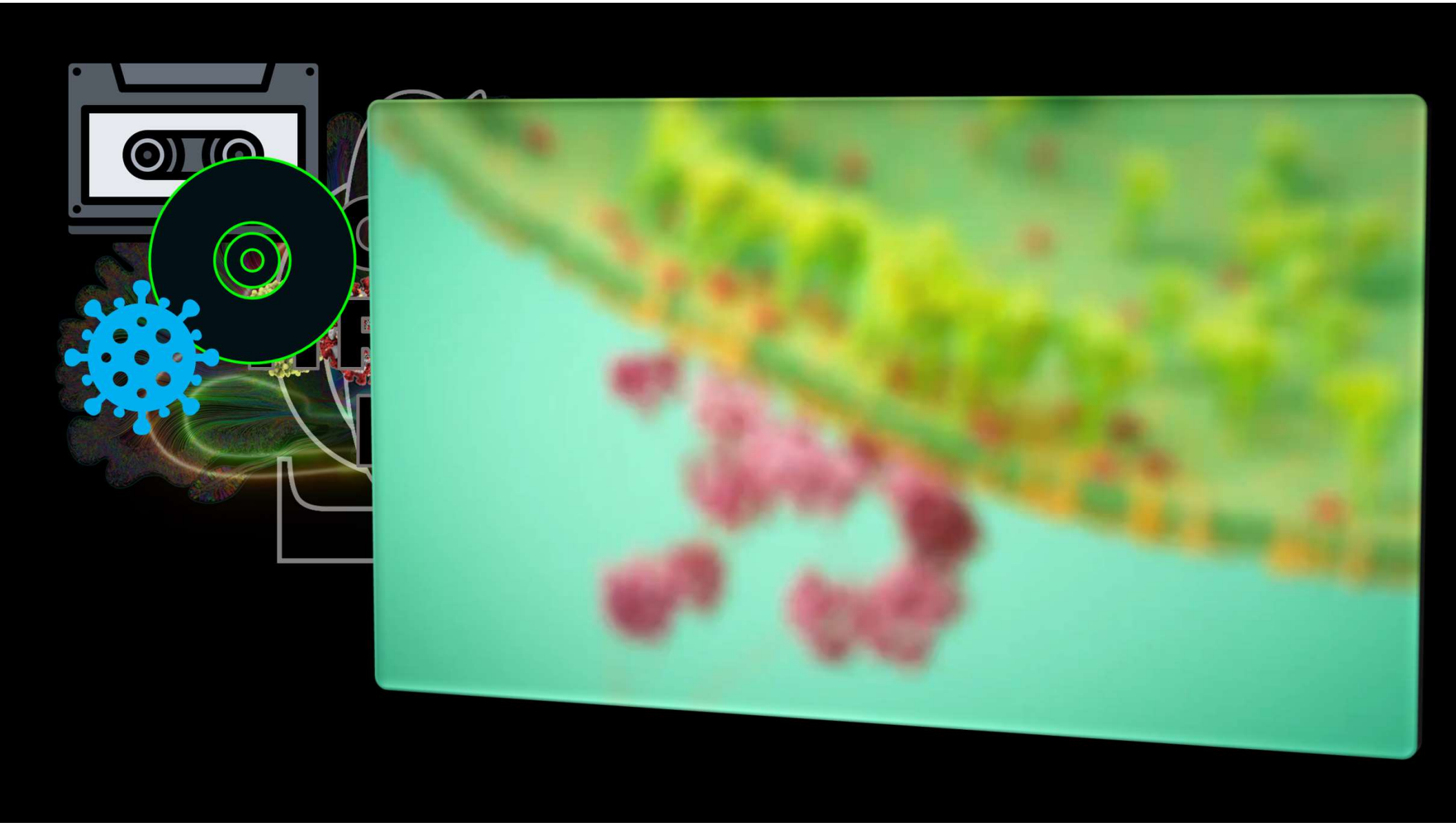

Direct RNA nanopore sequencing of full-length coronavirus genomes provides novel insights into structural variants and enables modification analysis

Adrian Viehweger,[1,2,5] Sebastian Krautwurst,[1,2,5] Kevin Lamkiewicz,[1,2] Ramakanth Madhugiri,[3] John Ziebuhr,[2,3] Martin Hölzer,[1,2] and Manja Marz[1,2,4]

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Sequence analyses of RNA virus genomes remain challenging owing to the exceptional genetic plasticity of these viruses. Because of high mutation and recombination rates, genome replication by viral RNA-dependent RNA polymerases leads to populations of closely related viruses, so-called "quasispecies." Standard (short-read) sequencing technologies are ill-suited to reconstruct large numbers of full-length haplotypes of (1) RNA virus genomes and (2) subgenome-length (sg) RNAs composed of noncontiguous genome regions. Here, we used a full-length, direct RNA sequencing (DRS) approach based on nanopores to characterize viral RNAs produced in cells infected with a human coronavirus. By using DRS, we were able to map the longest (~26-kb) contiguous read to the viral reference genome. By combining Illumina and Oxford Nanopore sequencing, we reconstructed a highly accurate consensus sequence of the human coronavirus (HCoV)-229E genome (27.3 kb). Furthermore, by using long reads that did not require an assembly step, we were able to identify, in infected cells, diverse and novel HCoV-229E sg RNAs that remain to be characterized. Also, the DRS approach, which circumvents reverse transcription and amplification of RNA, allowed us to detect methylation sites in viral RNAs. Our work paves the way for haplotype-based analyses of viral quasispecies by showing the feasibility of intra-sample haplotype separation. Even though several technical challenges remain to be addressed to exploit the potential of the nanopore technology fully, our work illustrates that DRS may significantly advance genomic studies of complex virus populations, including predictions on long-range interactions in individual full-length viral RNA haplotypes.

[Supplemental material is available for this article.]

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Methods

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Method

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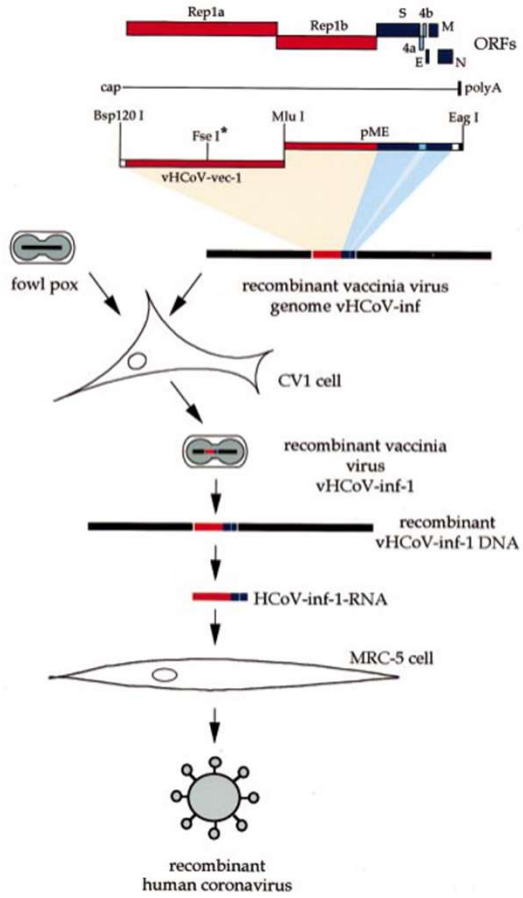

Fig. 2. Strategy for the production of infectious HCoV 229E RNA and the recovery of recombinant coronavirus. The structural relationship of the HCoV 229E ORFs, HCoV 229E genomic RNA and the HCoV-inf-1 cDNA is shown. Two cDNA fragments, derived from vHCoV-vec-1 and pME, are assembled by *in vitro* ligation using an *MluI* restriction site. Subsequent ligation of the resulting cDNA with *NotI*-cleaved v*NotI*/tk vaccinia virus DNA produces the recombinant vaccinia virus vHCoV-inf DNA. This DNA is then transfected into CV-1 cells and the recombinant vaccinia virus vHCoV-inf-1 is recovered by using fowlpox helper virus. RNA transcripts are produced *in vitro* by using genomic vHCoV-inf-1 DNA and bacteriophage T7 RNA polymerase and transfected into MRC-5 cells. Finally, tissue cultures are monitored for the production of recombinant human coronavirus.

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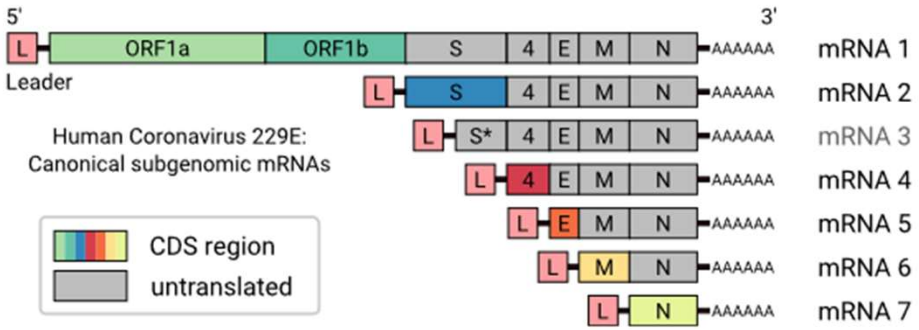

Figure 1. Scheme of genomic and subgenomic (sg) RNAs produced in HCoV-229E–infected cells (Raabe et al. 1990; Schreiber et al. 1989). Translation of the 5′-terminal ORF(s) of the respective mRNA gives rise to the various viral structural and nonstructural proteins (indicated by different colors). mRNA 3 is considered defective and unlikely to be translated into protein. Each mRNA has a 3′ poly(A) tail and carries a 5′-leader sequence that is identical to the 5′-end of the genome. In a process called discontinuous extension of negative strands, negative-strand RNA synthesis is attenuated at specific genomic positions. After transfer of the nascent strand to an upstream position on the template, RNA synthesis is continued by copying the 5′-leader sequence. As a result, the 3′-ends of coronavirus minus-strand mRNAs are equipped with the complement of the 5′-leader. The latter process is guided by base-pairing interactions between the transcription-regulating sequence (TRS) located immediately downstream from the leader sequence (TRS-L) at the 5′-end of the genome and the sequence complement of a TRS located upstream of one of the ORFs present in the 3′-proximal genome region (TRS-B).

cation before sequencing would remove all RNA modifications from the input material, whereas the nanopore sequencing technology preserves these modifications (Smith et al. 2017; Garalde et al. 2018).

Recently, nanopore sequencing has been used for metage-
omic forays into the virosphere (Warwick-Dugdale et al. 2019)
d studies focusing on transmission routes (Quick et al. 2016;
ria et al. 2017). Furthermore, viral transcriptomes have been in-
stigated using nanopore sequencing of cDNA (Moldován et al.
17, 2018a,b; Tombácz et al. 2017), being subject to bias from
verse transcription and amplification. Other studies used DRS
study the human poly(A) transcriptome (Workman et al.
18) and the transcriptome of DNA viruses such as HSV
epledge et al. 2018). Furthermore, the genome of influenza A vi-
s has been completely sequenced in its original form using DRS
eller et al. 2018).

In the present study, we sequenced one of the largest known
NA genomes, that of HCoV-229E, a member of the genus
phacoronavirus, with a genome size of ~27,300 nt, in order to as-
ss the complex architectural details for viral sg RNAs produced in
lls infected with recombinant HCoV-229E. By using DRS, we aim
capture complete viral mRNAs, including the full coronavirus
nome, in single contiguous reads. Sequence analysis of thou-
nds of full-length sg RNAs will allow us to determine the archi-
ctures (including leader–body junction sites) of the major viral
RNAs. In addition, this approach provides insight into the diver-
y of additional HCoV-229E sg RNAs, probably including DI-
NAs. Further, we aim to assess whether RNA modifications can
called directly from the raw nanopore signal of viral molecules
thout prior in vitro treatment, as has been shown for DNA
oiber et al. 2016; McIntyre et al. 2019).

esults

ll-genome sequencing without amplification

e sequenced total RNA samples obtained from Huh7 cells in-
cted with serially passaged recombinant human coronaviruses:
ld-type (WT) HCoV-229E, HCoV-229E_SL2-SARS-CoV, and
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nserved stem–loop structure (SL2) residing in the HCoV-229E
UTR was replaced with the equivalent SL2 element from SARS-
V and BCoV, respectively (Madhugiri et al. 2018). Total RNA
mples obtained for the latter two (chimeric) viruses were pooled
fore sequence analysis. Hereafter, we refer to the first sample as
T RNA and to the second (pooled) sample as SL2 RNA (see
ethods).

We performed two DRS runs (one per sample) on a MinION
nopore sequencer. As shown in Table 1, we achieved a through-
t of 0.237 and 0.282 Gb with 225,000 and 181,000 reads for
e WT and SL2 sample, respectively. See Supplemental Figure
A for an overview of the read length distribution. For the WT
d SL2 samples, 33.3% and 35.9% of the reads mapped to the ref-
nce HCoV-229E sequences, respectively; 15.8% and 10.2%, re-
ectively, mapped to the yeast enolase 2 mRNA sequence, a
libration strand added during the library preparation, whereas
.4% and 52.7% could be attributed to human host cell RNA.
inimap2 (Li 2018) did not align the remaining 3.50% and
11% of reads. Using BLAST (Altschul et al. 1990) against the nu-
otide database, 18.1% and 20.7% of these reads can be attribut-
again to HCoV, human or yeast. As reads that were not aligned
minimap2 were mostly very short (median ≤200 nt), of poor
basecalling quality and represented only 0.62% and 0.15% of total
nucleotides, respectively, we decided to only use the higher quality
reads that minimap2 could align (for detailed statistics, see
Supplemental Fig. S2).

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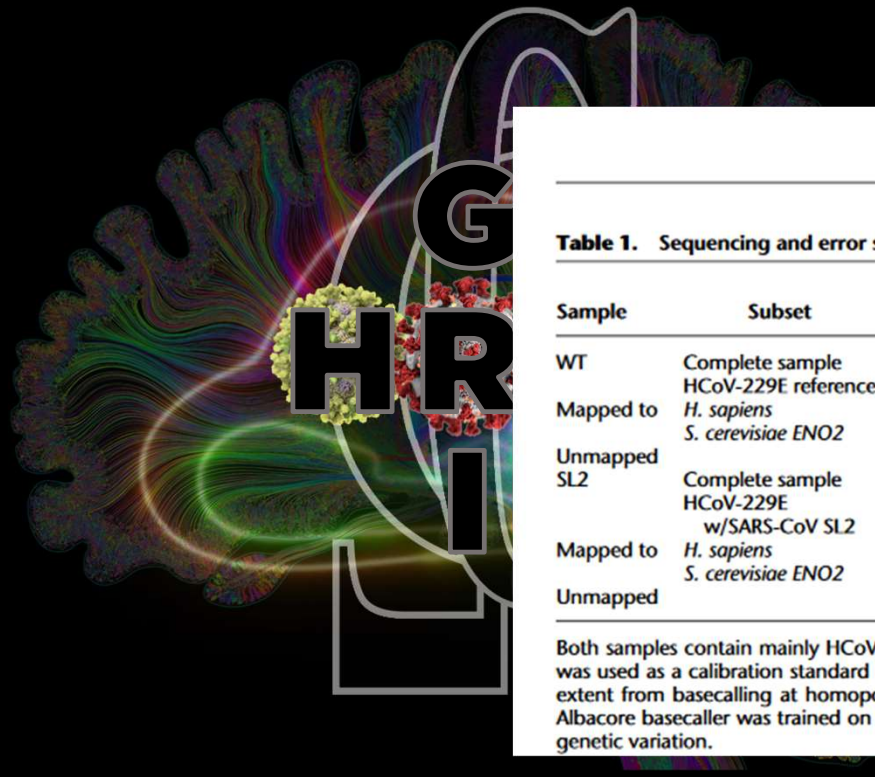

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Table 1. Sequencing and error statistics

Sample	Subset	No. of reads (% reads)	% nucleotides	Longest	Median	% subst.	% insert.	% deletions	% errors
WT	Complete sample	224,724 (100.0)	100.00	26,210	826	—	—	—	—
	HCoV-229E reference	74,783 (33.3)	42.52	26,210	1414	4.292	2.558	8.264	15.114
Mapped to	*H. sapiens*	106,618 (47.4)	46.37	9562	816	4.333	2.676	8.572	15.581
	S. cerevisiae ENO2	35,454 (15.8)	10.50	3482	636	3.752	2.384	6.359	**12.494**
Unmapped		7869 (3.5)	0.62	1157	186	—	—	—	—
SL2	Complete sample	180,906 (100.0)	100.00	25,885	1342	—	—	—	—
	HCoV-229E w/SARS-CoV SL2	64,995 (35.9)	48.83	25,885	1626	4.396	2.680	8.507	15.582
Mapped to	*H. sapiens*	95,340 (52.7)	45.44	16,030	1023	4.513	2.783	8.775	16.071
	S. cerevisiae ENO2	18,530 (10.2)	5.58	3872	858	4.021	2.463	6.892	**13.376**
Unmapped		2041 (1.1)	0.15	928	200	—	—	—	—

Both samples contain mainly HCoV-229E and host (*Homo sapiens*) transcripts, but also *Saccharomyces cerevisiae* enolase 2 (*ENO2*) mRNA reads (which was used as a calibration standard added during library preparation). Half of the sequencing errors were deletion errors, probably resulting to a large extent from basecalling at homopolymer stretches. The *S. cerevisiae* enolase 2 mRNA reads display an overall reduced error rate (bold) because the Albacore basecaller was trained on this calibration strand. Note that all error rates report differences to the reference genome and thus include actual genetic variation.

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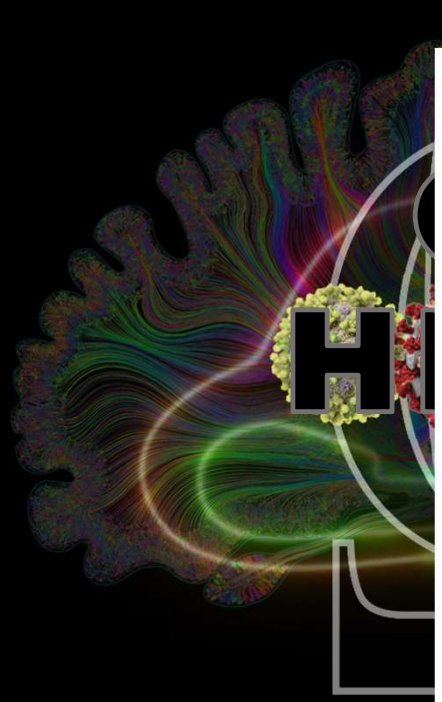

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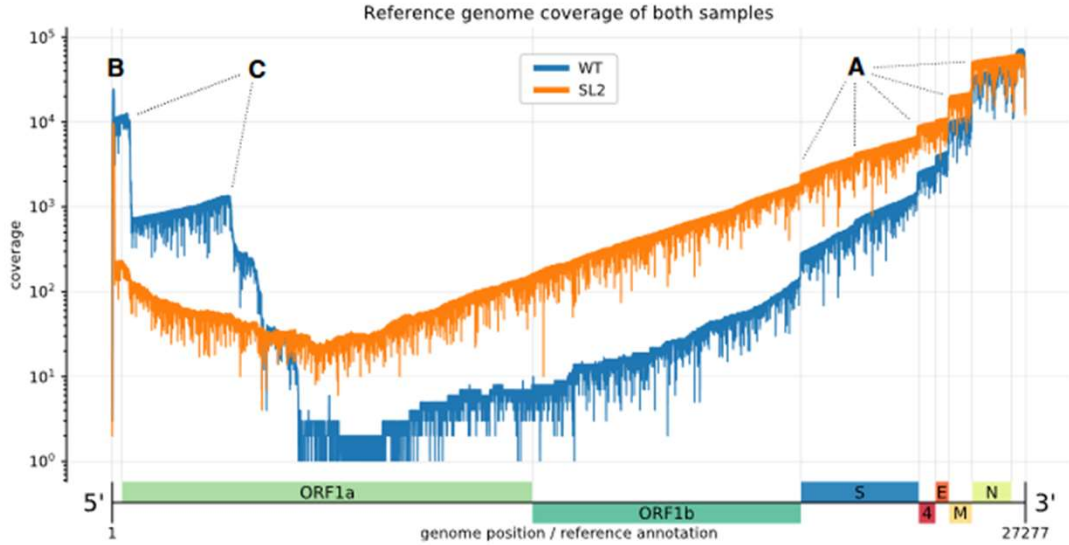

Figure 2. Reference genome coverage of the HCoV-229E WT sample (blue) and the SL2 sample (orange) based on alignments with minimap2. There is an inverse correlation between sg RNA abundance and length. (*A*) Notable vertical "steps" in the coverage correspond to borders expected for the canonical sg RNAs (see Fig. 1). (*B*) The presence of the leader sequence (~65 nt) in canonical sg RNAs gives rise to the sharp coverage peak at the 5′-end. (*C*) We also observed unexpected "steps," especially in the WT sample (blue). We hypothesize that the sequences correspond to DI-RNA molecules that may arise by recombination at TRS-like sequence motifs as well as other sites displaying sequence similarities that are sufficient to support illegitimate recombination events (see Fig. 3). We attribute the difference in the observed (noncanonical) recombination sites between the two samples to biological factors that we either did not control for or do not know (see also legend to Fig. 3).

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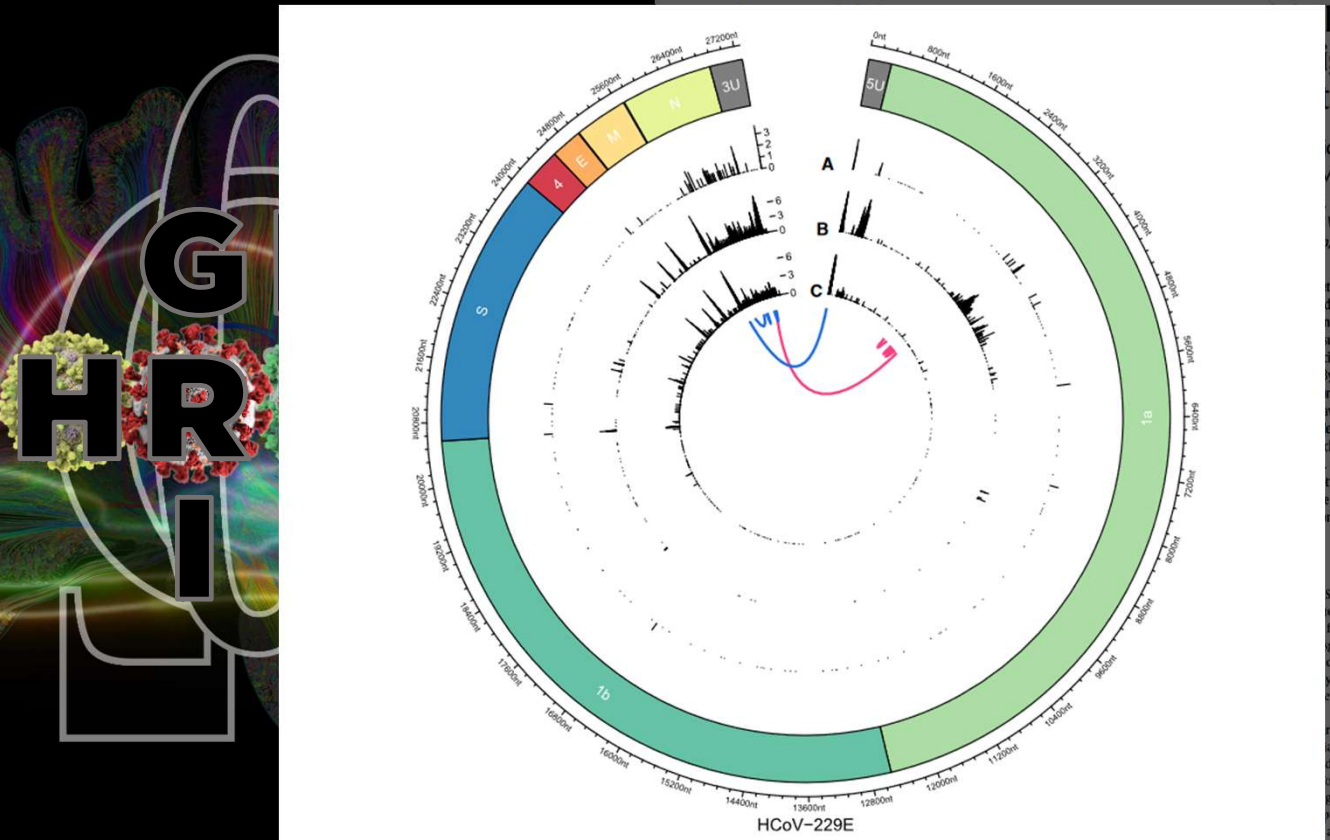

Figure 3. Joining of noncontiguous genome sequences in sg RNAs identified in HCoV-229E–infected cells. On the circular axis, the annotations of the reference genome (including 5′ UTR [5U] and 3′ UTR [3U]) are shown. Genomic positions of "discontinuous sites" identified in Illumina reads (*A*; *outer* track), nanopore reads of sample HCoV-229E WT (*B*; *middle* track), and nanopore reads of SL2 sample (*C*; *inner* track) reveal multiple recombination sites across the whole genome. An aggregation of recombination sites can be observed in the region that encodes the viral N protein. Furthermore, clear recombination sites can be seen at intergenic boundaries and at the 5′ and 3′ UTRs, with the former corresponding to the boundary between the leader sequence and the rest of the genome. Another prominent cluster can be observed in ORF1a in the WT nanopore sample but not in SL2. This cluster is supported by the WT Illumina data, excluding sequencing bias as a potential source of error. We hypothesize that because samples WT and SL2 were obtained from nonplaque-purified serially passaged virus populations derived from in vitro transcribed genome RNAs transfected into Huh7 cells, differences in the proportion of full-length transcripts versus abortive transcripts could translate into different patterns of recombination. Generally, nanopore-based sequencing allows more detailed analysis of recombination events owing to the long read length. Even complex isoforms such as two exemplary reads, each with four discontinuous segments, can be observed (blue and pink).

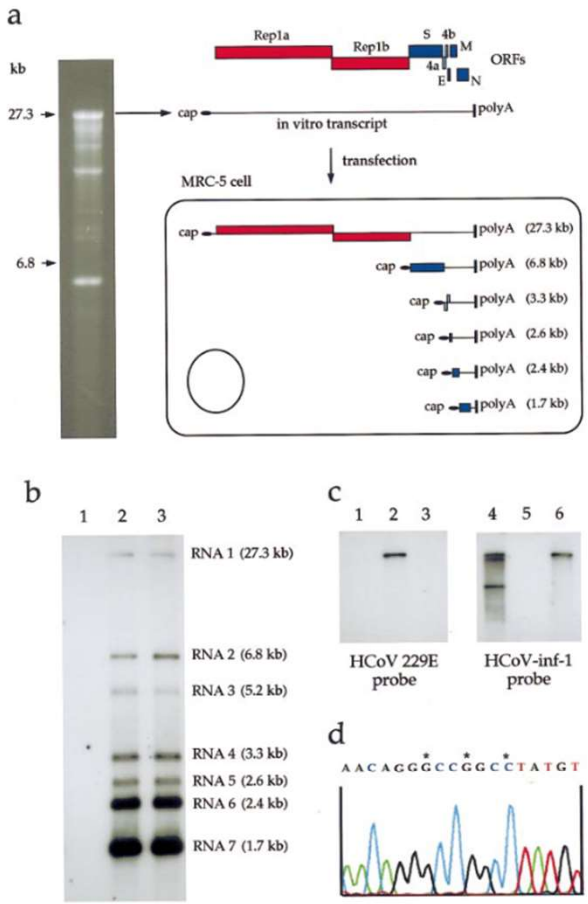

Fig. 3. Recovery of recombinant human coronavirus. (*a*) Ethidium bromide-stained, 1% agarose gel in which 1 µg capped RNA, transcribed *in vitro* from vHCoV-inf-1 DNA, has been electrophoresed. The full-length (27·3 kb) *in vitro* transcription product is indicated. Also shown is the structural relationship of the HCoV ORFs, the *in vitro*-transcribed HCoV-inf-1 RNA and the predicted genomic and subgenomic mRNAs in HCoV-inf-1 RNA-transfected MRC-5 cells. (*b*) Analysis of poly(A)-containing RNA from parental virus- and recombinant virus-infected cells. Poly(A)-containing RNA was isolated from MRC-5 cells that had been mock-infected (lane 1), infected with parental HCoV 229E virus (lane 2) or infected with recombinant HCoV-inf-1 virus (lane 3). The RNA was analysed by Northern hybridization using a ^{32}P-end-labelled oligonucleotide (5′ AGAAACTTCATCACG-CACTGG 3′) corresponding to nt 26802–26822 within the HCoV nucleocapsid protein gene. The characteristic set of genomic and subgenomic HCoV mRNAs is indicated. (*c*) Northern hybridization of *in vitro*-transcribed HCoV-inf-1 RNA (lanes 1 and 4) and poly(A)-containing RNA from parental HCoV 229E-infected MRC-5 cells (lanes 2 and 5) and HCoV-inf-1-infected MRC-5 cells (lanes 3 and 6). The RNAs were probed with a parental HCoV 229E-specific oligonucleotide, 5′ ACATACGCTGGGCCTGTT 3′ (lanes 1–3), or an HCoV-inf-1-specific oligonucleotide, 5′ ACATAGGCCGGCCCTGTT 3′ (lanes 4–6). The oligonucleotides were ^{32}P-end-labelled. (*d*) Sequence analysis of HCoV-inf-1 genomic RNA in the region encompassing the three silent mutations specific to the recombinant virus genome. The three nucleotide mutations are indicated that represent the diagnostic *Fse*I site.

Journal of General Virology (2001), **82**, 1273–1281. Printed in Great Britain

Infectious RNA transcribed *in vitro* from a cDNA copy of the human coronavirus genome cloned in vaccinia virus

Volker Thiel, Jens Herold,† Barbara Schelle and Stuart G. Siddell

Institute of Virology and Immunology, University of Würzburg, Versbacher Straße 7, 97078 Würzburg, Germany

The coronavirus genome is a positive-strand RNA of extraordinary size and complexity. It is composed of approximately 30000 nucleotides and it is the largest known autonomously replicating RNA. It is also remarkable in that more than two-thirds of the genome is devoted to encoding proteins involved in the replication and transcription of viral RNA. Here, a reverse-genetic system is described for the generation of recombinant coronaviruses. This system is based upon the *in vitro* transcription of infectious RNA from a cDNA copy of the human coronavirus 229E genome that has been cloned and propagated in vaccinia virus. This system is expected to provide new insights into the molecular biology and pathogenesis of coronaviruses and to serve as a paradigm for the genetic analysis of large RNA virus genomes. It also provides a starting point for the development of a new class of eukaryotic, multi-gene RNA vectors that are able to express several proteins simultaneously.

Introduction

Coronaviruses are enveloped, vertebrate viruses that are associated mainly with respiratory and enteric diseases. The human coronaviruses are responsible for 10–20% of all common colds (McIntosh, 1996). The virus genome is a positive-strand RNA of approximately 30 kb that encodes a minimal set of four structural proteins and a large array of non-structural proteins involved in replication and transcription (Lai & Cavanagh, 1997; Siddell & Snijder, 1998). These so-called replicase proteins are encoded in two overlapping open reading frames (ORFs) that extend about 20 kb from the 5′ end of the genome. It is a hallmark of coronaviruses that extensive co- and post-translational proteolytic processing is required to produce the proteins needed to assemble a functional replication–transcription complex (Ziebuhr *et al.*, 2000). It is also noteworthy that the generation of coronavirus sub-genomic mRNAs involves an unusual process of discontinuous transcription (Spaan *et al.*, 1983), most probably during the synthesis of subgenomic, negative-strand templates (Sawicki & Sawicki, 1998). Discontinuous transcription is a highly regulated process and is, at least in part, dependent upon base-pairing between *cis*-acting elements, the so-called transcription-associated sequences, located at the 5′ end of the genome and at various 3′-proximal sites (van Marle *et al.*, 1999).

Until recently, the study of coronavirus genetics was essentially restricted to the analysis of temperature-sensitive (*ts*) mutants (Lai & Cavanagh, 1997; Stalcup *et al.*, 1998), the analysis of defective RNA templates that depend upon replicase proteins provided by a helper virus (Repass & Makino, 1998; Izeta *et al.*, 1999; Williams *et al.*, 1999) and the analysis of chimeric viruses generated by targetted recombination (Fischer *et al.*, 1997; Hsue & Masters, 1999; Kuo *et al.*, 2000). This was because the large size of the coronavirus genome and the instability of some coronavirus cDNAs in bacteria effectively precluded the use of cloning procedures that have been used to generate infectious RNA from cDNA copies of other positive-strand RNA virus genomes (Ruggli & Rice, 1999). Recently, however, two different approaches have been developed that appear to overcome these problems. Firstly, Almazán *et al.* (2000) have reported that the cloning of full-length, transmissible gastroenteritis virus (TGEV) cDNA in a bacterial artificial chromosome, combined with nuclear expression of infectious RNA, can be used to produce recombinant virus. Secondly, Yount *et al.* (2000) have described a system to assemble a full-length cDNA construct of the

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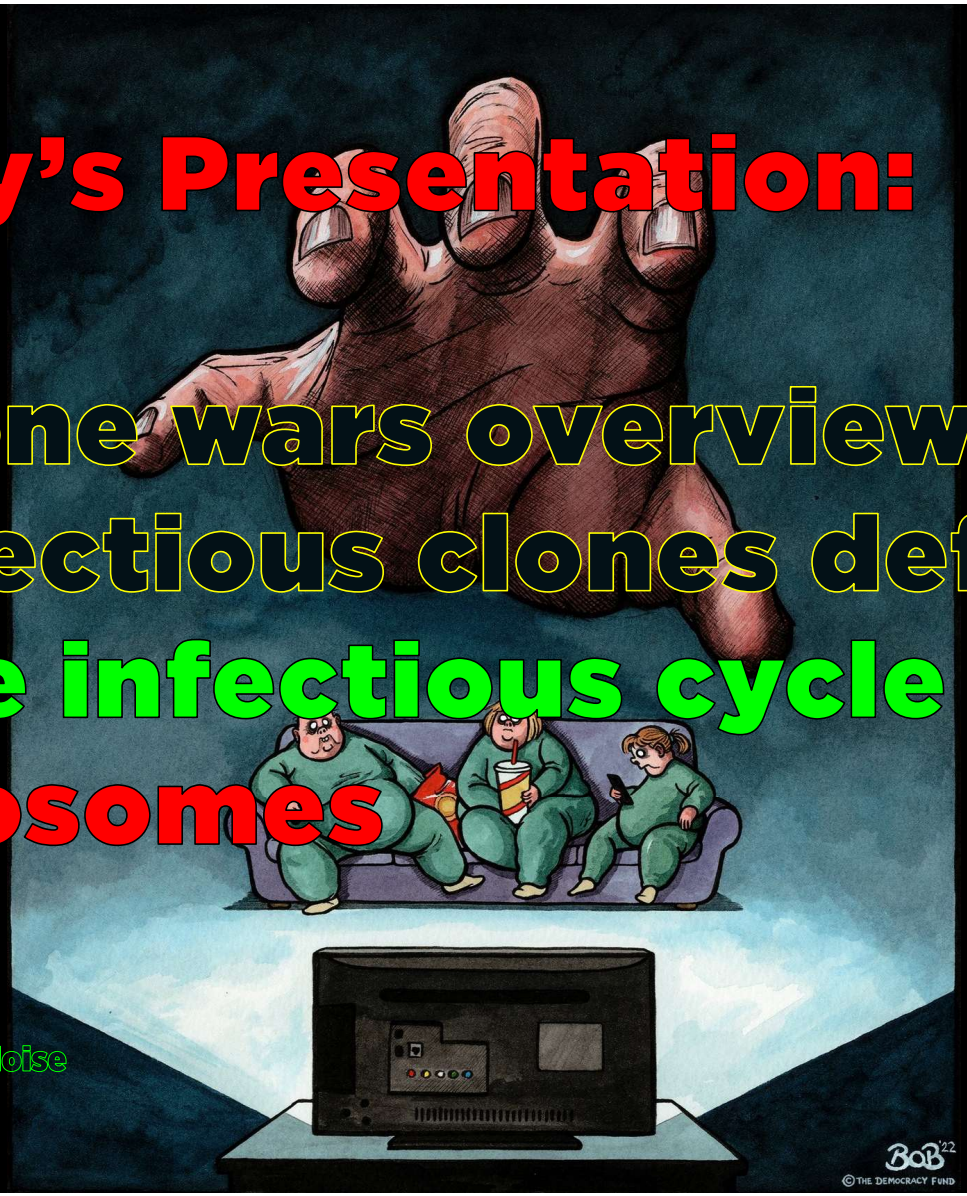
† **Present address:** SWITCH-Biotech AG, Fraunhofer Straße 10, 82152 Martinsried, Germany.

The GenBank accession number of the sequence reported in this paper is AF304460.

WHY ARE WE THEY DOING THIS?

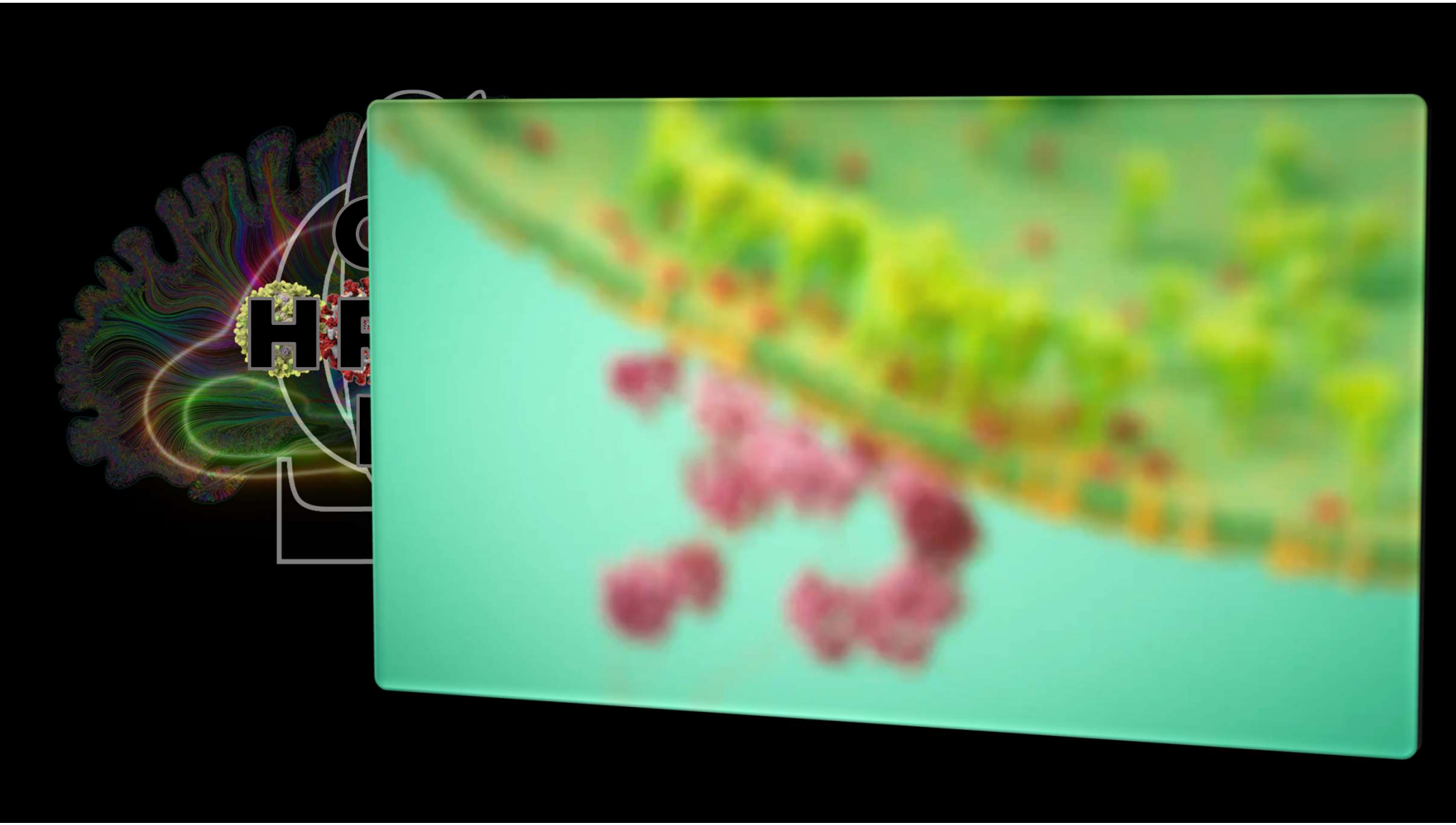
Today's Presentation:

1. Clone wars overview
2. Infectious clones defined
3. The infectious cycle
4. Exosomes



Gigaohm Biological
High Resistance Low Noise
Information Brief
8 February 2023

WHY ARE WE THEY DOING THIS?

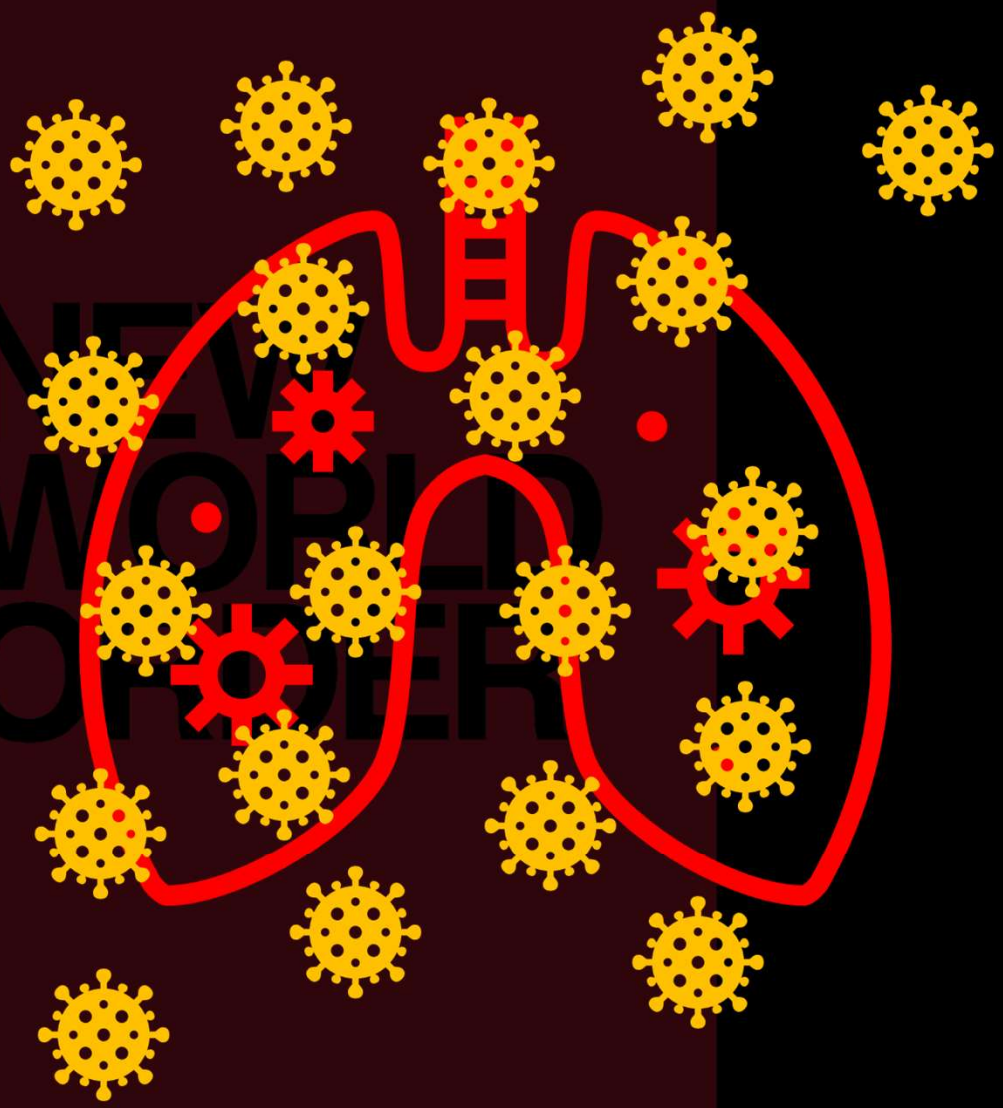



XVIVO
scientific
animation

WHAT IF WE HAVE BEEN MISLED ABOUT THE INFECTIOUS CYCLE ?

WHO WOULD BENEFIT FROM LYING ABOUT THE COMPOSITION OF THE SWARM ?

WHO WOULD BENEFIT FROM LYING ABOUT THE DANGERS OF INFECTIOUS CLONES VERSUS GAIN OF FUNCTION METHODOLOGIES ?



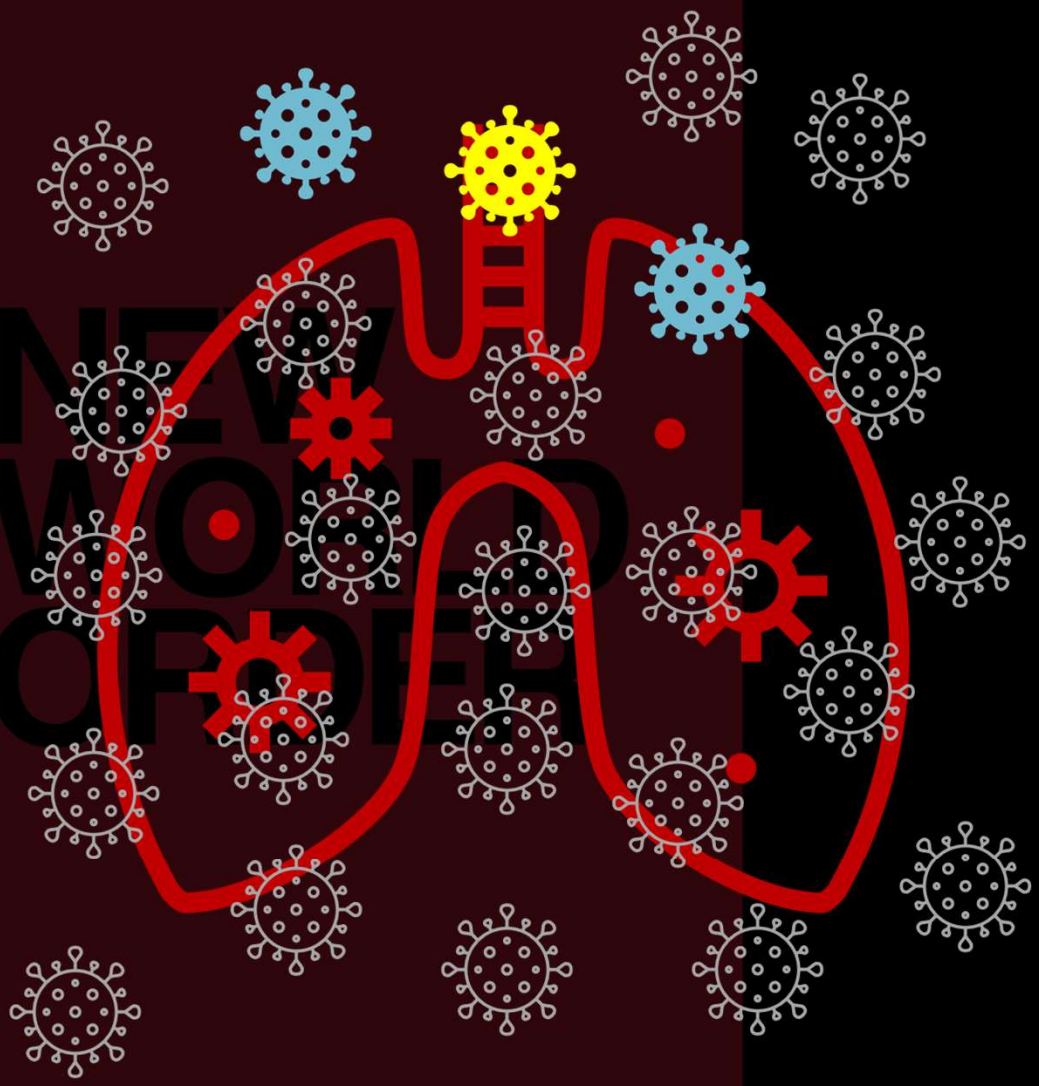
WHAT IF THE NATURAL INFECTIOUS CYCLE PRODUCES AN N:1 RATIO OF 50:1 ?

CAN NANOPORE SEQUENCING SHED ANY LIGHT ON THIS QUESTION ?

YES IT CAN, AND THE ANSWER DOESN'T HELP VIROLOGY.

500:1 ? EVEN 10000:1 ?

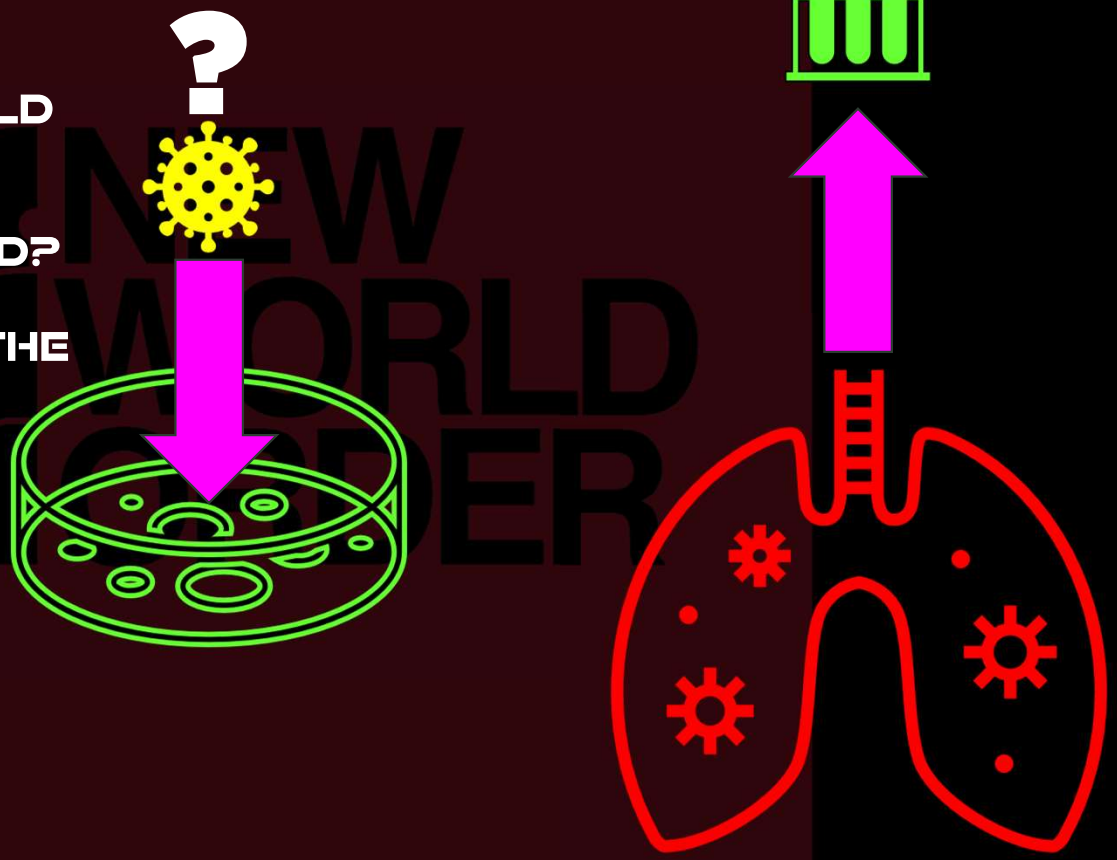
IF A CLONE PRODUCES THIS RATIO, THEN WHAT DOES A NATURAL VIRUS SWARM PRODUCE ?



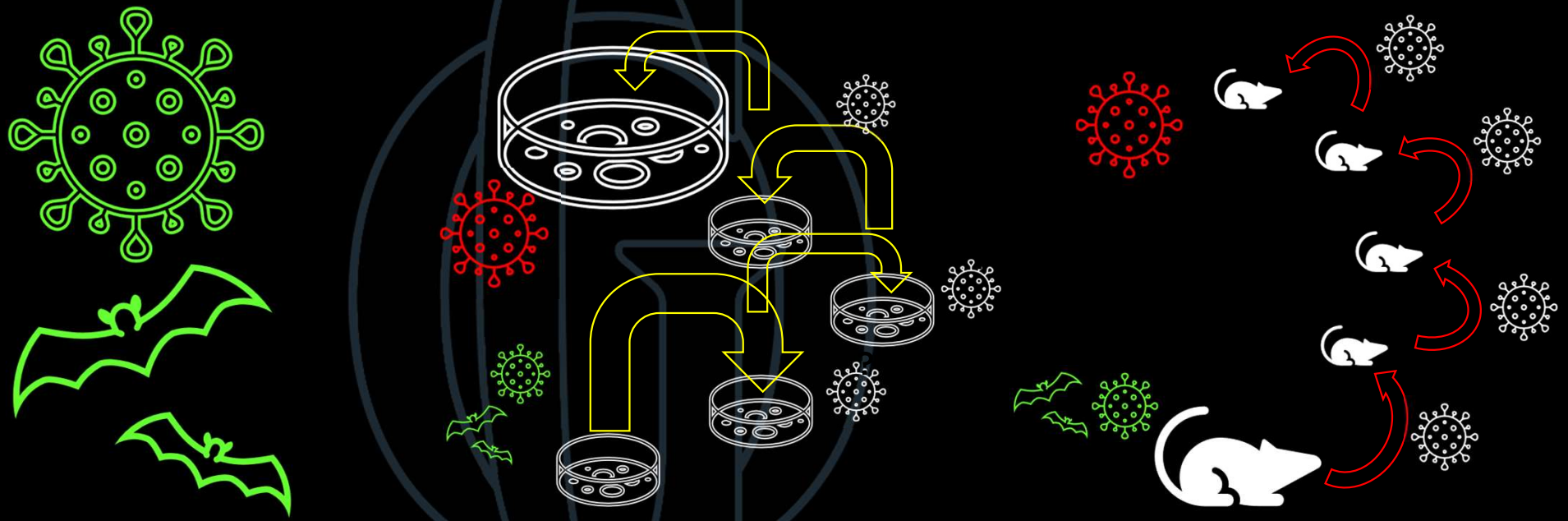
HOW IS VIRUS FOUND IN THE WILD AND CULTURED IN THE LAB?

HOW IS IT PURIFIED OR ISOLATED?

HOW OFTEN ARE DNA CLONES THE SOURCE MATERIAL FOR EXPERIMENTS?



The TV and algorithms have told us that coronaviruses have pandemic potential
This potential can be accessed through cell culture and animal passage
Therefore, the global population must surrender sovereignty to the WHO



To coerce a surrender of individual sovereignty and a global fundamental inversion of human rights from freedom to fascism

Q: WHAT IS A VIRUS?

A: WHATEVER THEY ARE, THEY CANNOT FORCE CELLS TO DO THINGS THEY DON'T ALREADY DO.

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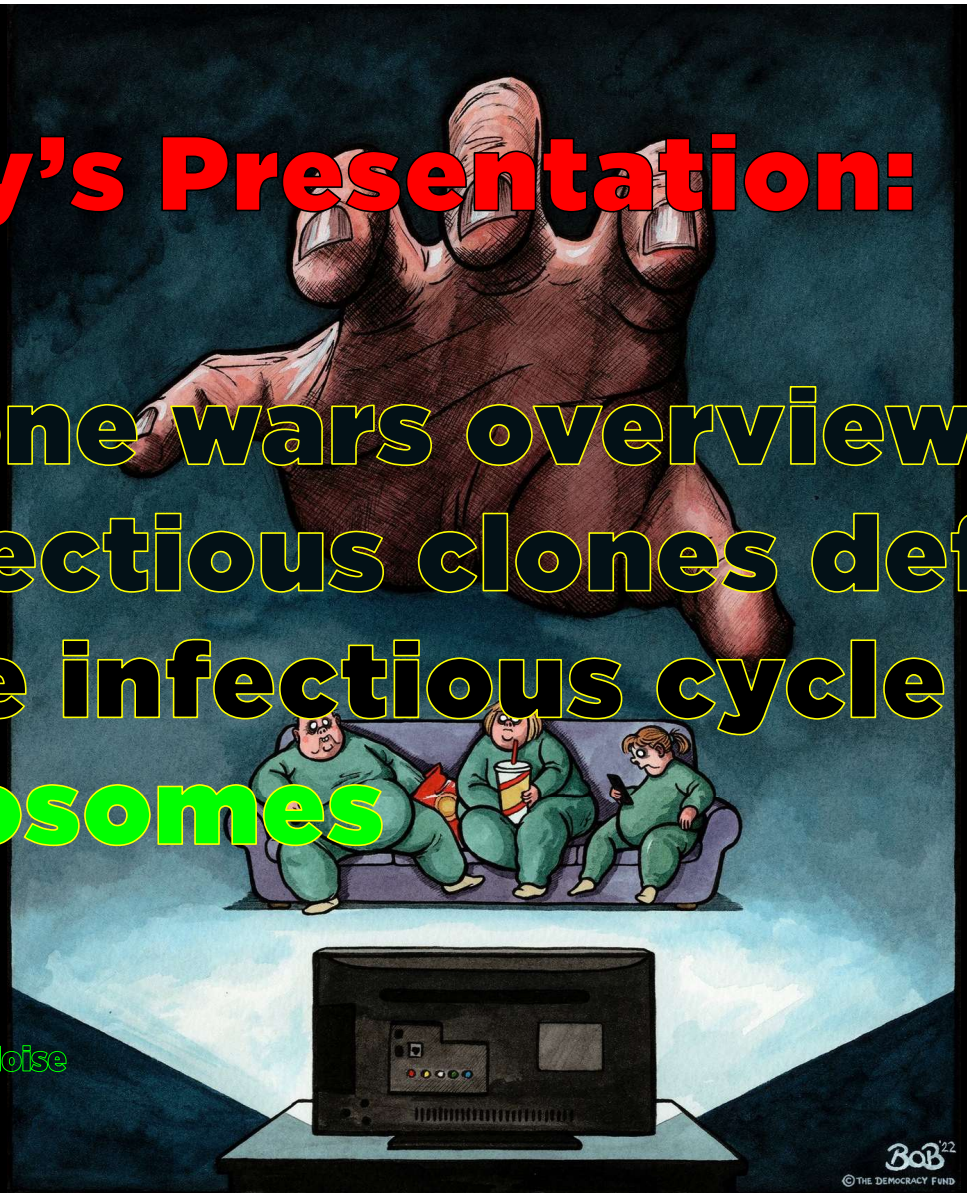

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WHY ARE WE THEY DOING THIS?

WHY ARE WE THEY DOING THIS?

https://www.youtube.com/watch?v=sE2krsErbwI

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8 February 2023

WHY ARE WE THEY DOING THIS?

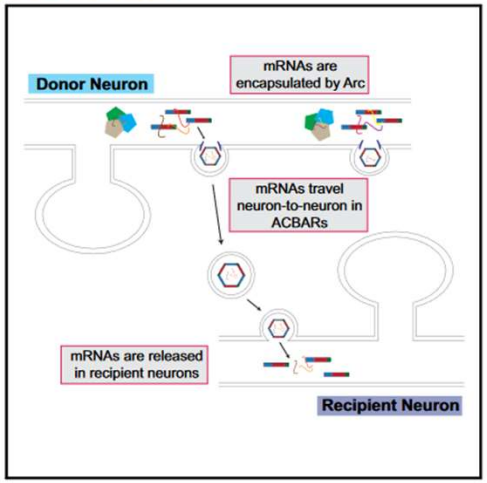
- **The neuronal gene Arc encodes a protein that forms virus-like capsids**
- **Arc protein exhibits similar biochemical properties as retroviral Gag proteins**
- **Endogenous Arc protein is released from neurons in extracellular vesicles (EVs)**
- **Arc EVs and capsids can mediate intercellular transfer of Arc mRNA in neurons**

Article

Cell

The Neuronal Gene *Arc* Encodes a Repurposed Retrotransposon Gag Protein that Mediates Intercellular RNA Transfer

Graphical Abstract



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Elissa D. Pastuzyn, Cameron E. Day, Rachel B. Kearns, ..., John A.G. Briggs, Cédric Feschotte, Jason D. Shepherd

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jason.shepherd@neuro.utah.edu

In Brief

The neuronal protein Arc is evolutionarily related to retrotransposon Gag proteins and forms virus-like capsid structures that can transfer mRNA between cells in the nervous system.

Highlights

- The neuronal gene *Arc* encodes a protein that forms virus-like capsids
- Arc protein exhibits similar biochemical properties as retroviral Gag proteins
- Endogenous Arc protein is released from neurons in extracellular vesicles (EVs)
- Arc EVs and capsids can mediate intercellular transfer of *Arc* mRNA in neurons

Pastuzyn et al., 2018, Cell *172*, 275–288
January 11, 2018
https://doi.org/10.1016/j.cell.2017.12.024

CellPress

- ***arc1* reporter mice are used to identify recently active cells for transcriptome profiling**
- **dArc-protein is co-expressed with GFP**
- **Activation of cells is dependent on training**
- **There are papers where these cells have been shown to necessary and sufficient for fear behavior**

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-09960-x OPEN

Engram-specific transcriptome profiling of contextual memory consolidation

Priyanka Rao-Ruiz[1,2], Jonathan J. Couey[1], Ivo M. Marcelo[1,3], Christian G. Bouwkamp[1], Denise E. Slump[1], Mariana R. Matos[2], Rolinka J. van der Loo[2], Gabriela J. Martins[3,4], Mirjam van den Hout[5], Wilfred F. van IJcken[5], Rui M. Costa[3,4], Michel C. van den Oever[2] & Steven A. Kushner[1]

Sparse populations of neurons in the dentate gyrus (DG) of the hippocampus are causally implicated in the encoding of contextual fear memories. However, engram-specific molecular mechanisms underlying memory consolidation remain largely unknown. Here we perform unbiased RNA sequencing of DG engram neurons 24 h after contextual fear conditioning to identify transcriptome changes specific to memory consolidation. DG engram neurons exhibit a highly distinct pattern of gene expression, in which CREB-dependent transcription features prominently ($P = 6.2 \times 10^{-13}$), including *Atf3* ($P = 2.4 \times 10^{-41}$), *Penk* ($P = 1.3 \times 10^{-15}$), and *Kcnq3* ($P = 3.1 \times 10^{-12}$). Moreover, we validate the functional relevance of the RNAseq findings by establishing the causal requirement of intact CREB function specifically within the DG engram during memory consolidation, and identify a novel group of CREB target genes involved in the encoding of long-term memory.

[1] Department of Psychiatry, Erasmus MC University Medical Center, Rotterdam 3015 GD, The Netherlands. [2] Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam 1081 HV, The Netherlands. [3] Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown, Lisbon 1400-038, Portugal. [4] Department of Neuroscience, Zuckerman Mind Brain Behavior Institute, Columbia University, New York 10027 NY, USA. [5] Center for Biomics, Erasmus MC University Medical Center, Rotterdam 3015 GD, The Netherlands. Correspondence and requests for materials should be addressed to M.Oever. (email: michel.vanden.oever@vu.nl) or to S.A.K. (email: s.kushner@erasmusmc.nl)

- ***arc1* mRNA/protein traffic across synapses via exovesicles, requiring its 3' UTR**
- **dArc-protein Gag binds *darc1* mRNA, and the protein forms capsid-like structures**
- **Exovesicles contain GAG-protein and Gag-encoding-mRNA of the retrotransposon Copia**
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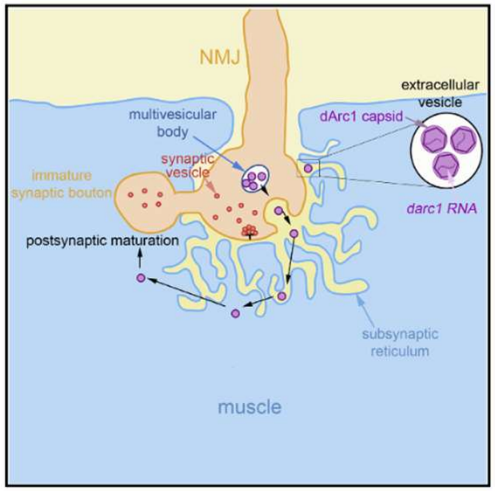
Article

Cell

Retrovirus-like Gag Protein Arc1 Binds RNA and Traffics across Synaptic Boutons

Graphical Abstract



Authors

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Data Resources

GSE104972

Ashley et al., 2018, Cell 172, 262–274
January 11, 2018
https://doi.org/10.1016/j.cell.2017.12.022

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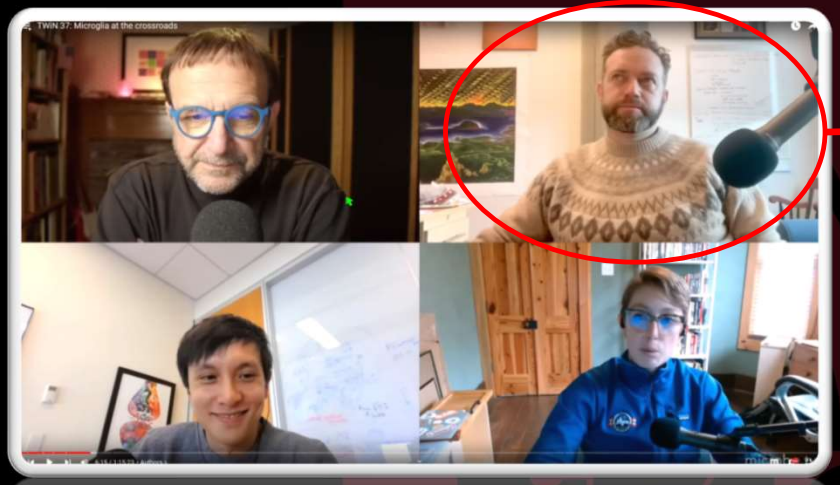
TWiN/This Week in Neuroscience



Article

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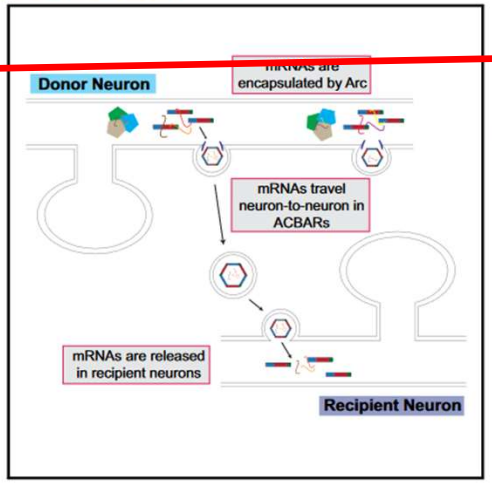

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Q: WHAT IS A VIRUS?

A: WHATEVER THEY ARE, THEY CANNOT FORCE CELLS TO DO THINGS THEY DON'T ALREADY DO.

- **Tumor derived exosomes can drive metastases**
- **These signals are targeted by a specific receptor (MET)**
- **Exosomes also induced vascular leakiness at pre-metastatic sites and reprogrammed bone marrow progenitors**
- **Exosome production could be interfered with to reduce tumor growth and metastasis**

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Author Manuscript
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Published in final edited form as:
Nat Med. 2012 June ; 18(6): 883–891. doi:10.1038/nm.2753.

Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET

Héctor Peinado[1], **Maša Alečković**[2], **Simon Lavotshkin**[3], **Irina Matei**[1], **Bruno Costa-Silva**[1,4], **Gema Moreno-Bueno**[5], **Marta Hergueta-Redondo**[5], **Caitlin Williams**[1], **Guillermo Garcia-Santos**[1], **Ayuko Nitadori-Hoshino**[1], **Caitlin Hoffman**[6], **Karen Badal**[1], **Benjamin A. Garcia**[2], **Margaret K. Callahan**[7], **Jianda Yuan**[8], **Vilma R. Martins**[4], **Johan Skog**[9], **Rosandra N. Kaplan**[10], **Mary S. Brady**[11], **Jedd D. Wolchok**[7,8], **Paul B. Chapman**[7], **Yibin Kang**[2,12,13], **Jacqueline Bromberg**[7,*], and **David Lyden**[1,13,*]

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Author Contributions

H.P. developed the hypothesis, designed the experimental approach, performed the experimental work, analyzed the data, coordinated the project and wrote the manuscript. M.A. performed mass spectrometry studies. S.L. conducted experimental work, I.M. performed flow cytometry studies and analysis, B.C.S. conducted mouse work and proteomic characterization of exosomes. G.M.B. conducted gene expression studies and analysis of microarray data. M.H.R. conducted mouse work and BM education studies, C.W. conducted mouse work and human studies, G.G.S developed BM education assays, A.N.H. quantified exosomes in human plasma, B.A.G. ran mass spectrometry samples and assisted with data interpretation. C.H. obtained human blood specimens. M.K.C. and J.Y. contributed to the characterization of human plasma exosomes. J.S., R.K., V.M., M.S.B, J.D.W. P.C. and Y.K discussed the hypothesis and contributed to data interpretation and experimental design. J.B. coordinated the project, interpreted data and wrote the manuscript. D.C.L conceived the hypothesis, led the project, interpreted the data, and wrote the manuscript.

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“Exosomes are a subgroup of nanosized extracellular vesicles enclosed by a lipid bilayer membrane and secreted by most eukaryotic cells. They represent a route of intercellular communication and participate in a wide variety of physiological and pathological processes. The biological roles of exosomes rely on their bioactive cargos, including proteins, nucleic acids, and lipids, which are delivered to target cells.”

ACS NANO

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REVIEW

Exosomes—Nature’s Lipid Nanoparticles, a Rising Star in Drug Delivery and Diagnostics

Rumiana Tenchov, Janet M. Sasso, Xinmei Wang, Wen-Shing Liaw, Chun-An Chen, and Qiongqiong Angela Zhou*

Cite This: *ACS Nano* 2022, 16, 17802–17846

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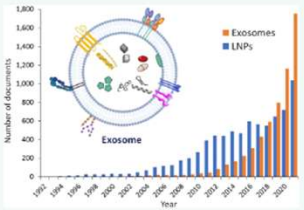
ABSTRACT: Exosomes are a subgroup of nanosized extracellular vesicles enclosed by a lipid bilayer membrane and secreted by most eukaryotic cells. They represent a route of intercellular communication and participate in a wide variety of physiological and pathological processes. The biological roles of exosomes rely on their bioactive cargos, including proteins, nucleic acids, and lipids, which are delivered to target cells. Their distinctive properties—innate stability, low immunogenicity, biocompatibility, and good biomembrane penetration capacity—allow them to function as superior natural nanocarriers for efficient drug delivery. Another notably favorable clinical application of exosomes is in diagnostics. They hold various biomolecules from host cells, which are indicative of pathophysiological conditions; therefore, they are considered vital for biomarker discovery in clinical diagnostics. Here, we use data from the CAS Content Collection and provide a landscape overview of the current state and delineate trends in research advancement on exosome applications in therapeutics and diagnostics across time, geography, composition, cargo loading, and development pipelines. We discuss exosome composition and pathway, from their biogenesis and secretion from host cells to recipient cell uptake. We assess methods for exosome isolation and purification, their clinical applications in therapy and diagnostics, their development pipelines, the exploration goals of the companies, the assortment of diseases they aim to treat, development stages of their research, and publication trends. We hope this review will be useful for understanding the current knowledge in the field of medical applications of exosomes, in an effort to further solve the remaining challenges in fulfilling their potential.



KEYWORDS: *exosome, extracellular vesicle, drug delivery, diagnostics, biomarker, nanoparticle, nanocarrier, blood–brain barrier, therapeutics*

Nearly 20 years after the discovery of liposomes,[1] it was found out that similar lipid vesicles form naturally in living organisms.[2,3] These include membrane-contained nanosized extracellular vesicles (EVs), secreted from cells as part of their normal process or certain pathologies. Based on the origin and size of the EVs, as well as on the current understanding of their biogenesis, they are grouped as follows: exosomes (diameter ~30–150 nm); microvesicles or ectosomes (100 nm–1 μm); and apoptotic bodies (50 nm–5 μm).[4,5]

Exosomes are produced in the endosomes of most eukaryotic cells and subsequently released in the extracellular space by fusion with the cellular biomembrane (Figure 1). Their functions are still largely unknown but a subject of a recent burst of interest as their important roles in physiological and pathophysiological processes are steadily revealed. They have been shown to provide means of efficient intercellular communication and signaling, including transport of bioactive molecules such as proteins, lipids, and nucleic acids, between cells and across biological barriers.[6,7] These results and the physicochemical properties of exosomes are reasons that they are viewed as the rising star in drug delivery and diagnostics.[5,8,9] However, there is still insufficient knowledge regarding exosome physiology. In order to make use of the clinical potential of exosomes, it is necessary to better understand the cellular processes that govern their biology and membrane trafficking.

For a long time, synthetic drug nanocarriers have been developed to improve the efficacy of therapeutics, to refine their pharmacokinetics and pharmacodynamics, while lessening the

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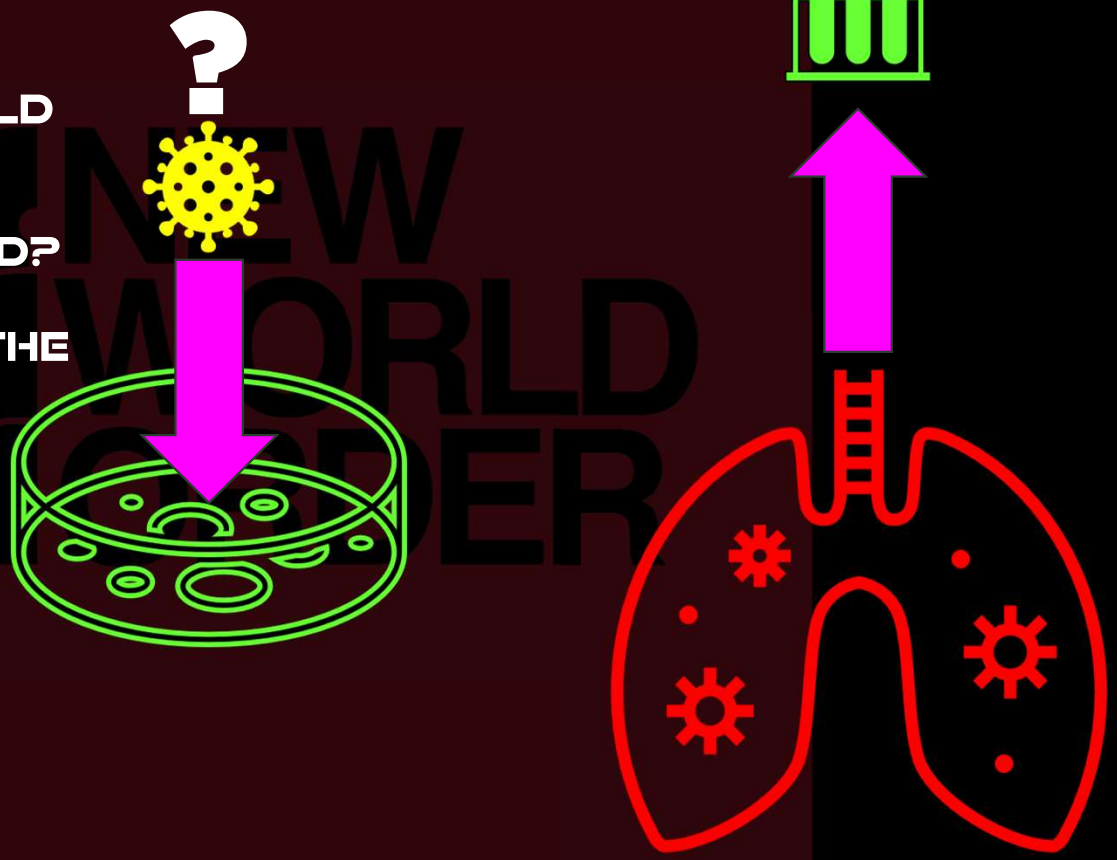
HOW IS IT PURIFIED OR ISOLATED?

HOW OFTEN ARE DNA CLONES THE SOURCE MATERIAL FOR EXPERIMENTS?



A FAREWELL TO VIROLOGY
(EXPERT EDITION)

ABSTRACT

Virology invented the virus model but has consistently failed to fulfil its own requirements. It is claimed that viruses cause disease after transmitting between hosts such as humans and yet the scientific evidence for these claims is missing. One of virology's greatest failures has been the inability to obtain any viral particles directly from the tissues of organisms said to have "viral" diseases. In order to obfuscate this state of affairs, virologists have resorted to creating their own pseudoscientific methods to replace the longstanding scientific method, as well as changing the dictionary meaning of words in order to support their anti-scientific practices. For instance, an "isolated" isolate does not require the physical existence of the particles in order to be afforded "isolation" status.

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This essay contains three parts. Part One outlines some of the history of virology and the failures of the virologists to follow the scientific method. The many and far-reaching claims of the virologists can all be shown to be flawed due to: (a) the lack of direct evidence, and (b) the invalidation of indirect "evidence" due to the uncontrolled nature of the experiments. The examples provided cover all major aspects of the virological fraud including alleged isolation, cytopathic effects, genomics, antibodies, and animal pathogenicity studies.
Part Two examines the fraud used to propagate the COVID-19 "pandemic." A breakdown of the methodology relied upon by the original inventors Fan Wu et al., shows how the fictional SARS-CoV-2 was "created" through anti-scientific methods and linguistic sleights of hands. It is part of an ongoing deception where viruses are claimed to exist by templating them against previous "virus" templates. Using SARS-CoV-2 as an example, the trail of "coronavirus" genomic templates going back to the 1980s reveals that none of these genetic sequences have ever been shown to come from inside any viral particle — the phylogenetic trees are fantasies. The misapplication of the polymerase chain reaction has propagated this aspect of virology's fraud and created the 'cases' to maintain the illusion of a pandemic.
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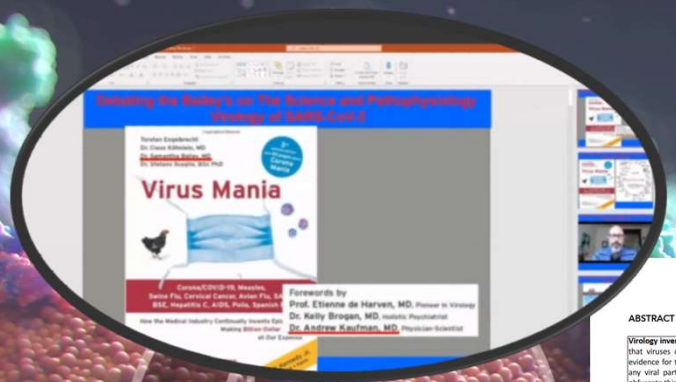

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Perspectives

Nonhuman Primate Models for SARS

Bart L. Haagmans, Albert D. M. E. Osterhaus*

Severe acute respiratory syndrome (SARS) first emerged in Guangdong Province, the People's Republic of China, in November 2002. The disease was characterised by a rapidly progressive atypical pneumonia [1]. In addition to other pathogens, a coronavirus not previously discovered was identified in patients with SARS. Reproduction of a similar disease in cynomolgus macaques with this virus completed Koch's postulates, implicating SARS coronavirus (SARS-CoV) as the aetiologic agent [2,3].

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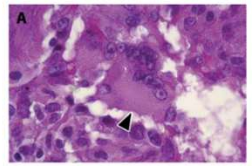
Animal Models for SARS

Development of animal models for SARS-CoV infection of humans is of utmost importance to elucidate the pathogenesis of SARS and to develop intervention strategies against the infection. A wide range of animal species is susceptible to experimental infection with SARS-CoV, including rodents (mice and hamsters), carnivores (ferrets and cats), and nonhuman primates (cynomolgus and rhesus macaques, common marmosets, and African green monkeys) [3–11]. Adult mice infected with varying doses of SARS-CoV in the respiratory tract show no clinical signs of disease, although the virus replicates in respiratory tissues, peaking early after



DOI: 10.1371/journal.pmed.0030194.g001

syncytia (Figure 1), and by extensive loss of epithelium from alveolar and bronchiolar walls. These lesions are quite similar to those observed in humans in the acute stages of SARS. Although clinical signs (respiratory distress and general malaise) were observed, they were not further studied in the initial experiments.

Studies from different laboratories confirmed that nonhuman primates could be infected experimentally with SARS-CoV, although the severity of

Abbreviations: ARDS, acute respiratory distress syndrome; DAD, diffuse alveolar damage; SARS, severe acute respiratory syndrome; SARS-CoV, SARS coronavirus

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Debating the Bailey's on The Science and Pathophysiology Virology of SARS-CoV-2
Virus Mania
Forewords by
Prof. Etienne de Harven, MD.
Dr. Kelly Brogan, MD.
Dr. Andrew Kaufman, MD.
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Vol. 79, No. 1
Infectious clone model
Severe Acute Respiratory Syndrome Coronavirus Infection of Golden Syrian Hamsters
Anjeanette Roberts,[1]* Leatrice Vogel,[1] Jeannette Guarner,[2] Norman Hayes,[2] Brian Murphy,[1] Sherif Zaki,[2] and Kanta Subbarao[1]
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland,[1] and Infectious Disease Pathology Activity, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia[2]
Received 3 June 2004/Accepted 27 August 2004
Small animal models are needed in order to evaluate the efficacy of candidate vaccines and antivirals directed against the severe acute respiratory syndrome coronavirus (SARS CoV). We investigated the ability of SARS CoV to infect 5-week-old Golden Syrian hamsters. When administered intranasally, SARS CoV replicates to high titers in the lungs and nasal turbinates. Peak replication in the lower respiratory tract was noted on day 2 postinfection (p.i.) and was cleared by day 7 p.i. Low levels of virus were present in the nasal turbinates of a few hamsters at 14 days p.i. Viral replication in epithelial cells of the respiratory tract was accompanied by cellular necrosis early in infection, followed by an inflammatory response coincident with viral clearance, focal consolidation in pulmonary tissue, and eventual pulmonary tissue repair. Despite high levels of virus replication and associated pathology in the respiratory tract, the hamsters showed no evidence of disease. Neutralizing antibodies were detected in sera at day 7 p.i., and mean titers at day 28 p.i. exceeded 1:400. Hamsters challenged with SARS CoV at day 28 p.i. were completely protected from virus replication and accompanying pathology in the respiratory tract. Comparing these data to the mouse model, SARS CoV replicates to a higher titer and for a longer duration in the respiratory tract of hamsters and is accompanied by significant pathology that is absent in mice. Viremia and extrapulmonary spread of SARS CoV to liver and spleen, which are seen in hamsters, were not detected in mice. The hamster, therefore, is superior to the mouse as a model for the evaluation of antiviral agents and candidate vaccines against SARS CoV replication.
Severe acute respiratory syndrome coronavirus (SARS CoV) is an animal coronavirus that caused an outbreak of SARS in humans in 2002 to 2003 (5, 13, 14, 18, 26) following introduction from a yet-unidentified natural reservoir. SARS CoV has been isolated from masked palm civets (Paguma larvata) and raccoon dogs (Nyctereutes procyonoides) (10), weasel-like animals that are indigenous to Guangdong Province in southeast China, where SARS CoV-associated infection of humans was first reported in early 2003 (10, 13). These animals were procured from an exotic animal market in Shenzhen, Guangdong Province, and evidence of infection was established by virus isolation and by detection of SARS CoV genetic material in nasal and/or fecal swabs (10). Antibodies specific for SARS CoV were also identified in sera of masked palm civets, raccoon dogs, and Chinese ferret-badgers (Melogale moschata) (10). Viral nucleic acid has also been detected by reverse transcription-PCR in rat droppings and throat and/or rectal swabs from five household cats, a dog, and a rat in a Hong Kong apartment complex where several residents developed SARS (17). In contrast to the host range specificity reported for many coronaviruses, SARS CoV appears to have a broad host range in that it replicates in humans, palm civets, and raccoon dogs and has also been shown to replicate in experimentally infected mice, ferrets, cats, and nonhuman primates (14, 16, 23). The host and tissue ranges of viruses are determined in part by the distribution of viral receptors. The role of a recently identified receptor for SARS CoV, angiotensin-converting enzyme 2 (15), as a determinant of the host range of SARS CoV remains to be determined. The presence of angiotensin-converting enzyme 2 homologs in a wide range of species (4, 12) may account for the broad host range of SARS CoV.
Since SARS CoV was identified as the causative agent for SARS (8), investigators have been working diligently to develop vaccines and antiviral therapies to prevent and protect against SARS infections. The ~10% mortality associated with the SARS outbreak in 2002 to 2003, the rapidity of international spread (encompassing over 20 countries within weeks of the first reported case), reports of super-spreaders (infected individuals who served as the source for eight or more subsequent infections), and the apparent ease of transmission between individuals from community-acquired infections and one instance of a laboratory-acquired infection highlight the seriousness of this pathogen. In addition to implementing isolation of patients with confirmed SARS cases and quarantine of those with suspected cases, public health officials in China have taken severe steps to prevent the reemergence of SARS in 2003 to 2004 through the culling of thousands of civet cats (a presumed viral reservoir) in Guangdong Province, rapid contact tracing of confirmed SARS patients, and the use of personal protective equipment by health care workers caring for SARS patients. To date only four community-acquired cases of SARS have been reported in 2003 to 2004, suggesting that these interventions may be successful. Although factors such as the genetic sequence of the virus could also explain the mild
* Corresponding author. Mailing address: Laboratory of Infectious Diseases, NIAID, Bldg. 50, Room 6513, 50 South Dr., MSC 8007, Bethesda, MD 20892. Phone: (301) 496-3490. Fax: (301) 496-8312. E-mail: ajroberts@niaid.nih.gov.
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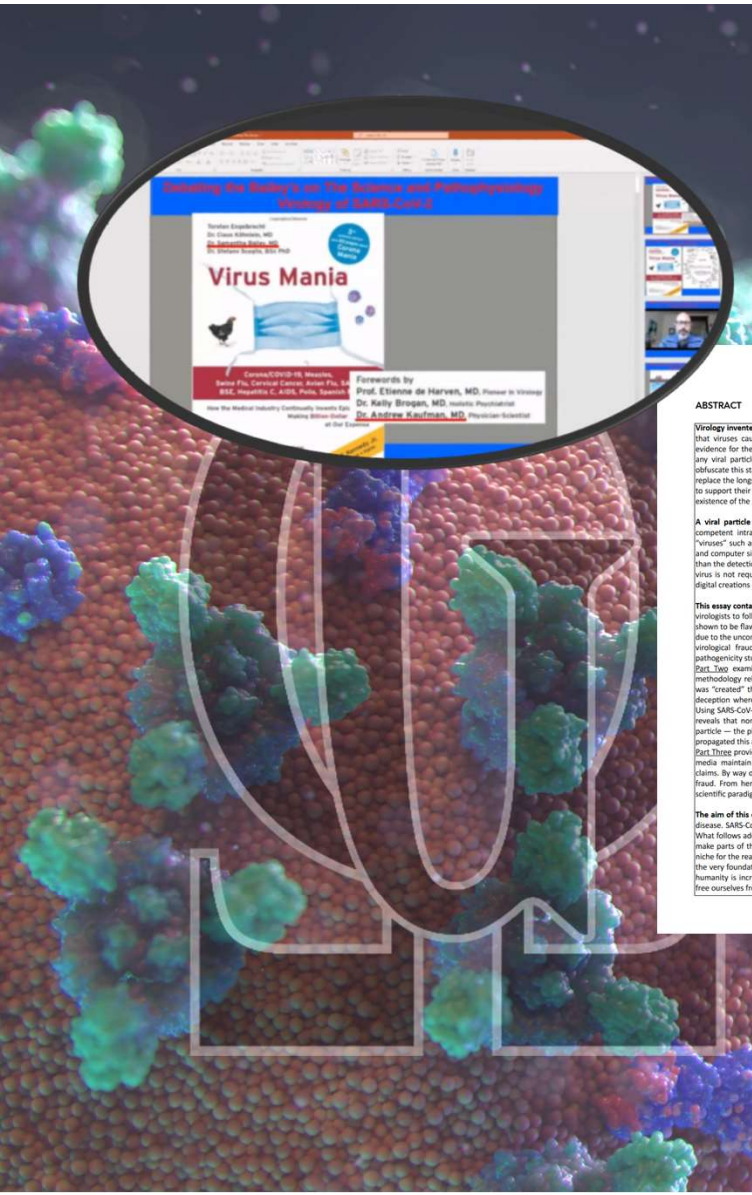

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Pathogenesis of SARS

In typical cases, which were largely confined to adult and elderly individuals, SARS presented with acute respiratory distress syndrome (ARDS)—characterised by the presence of diffuse alveolar damage (DAD) upon autopsy. The pathological changes in lung alveoli most likely follow a common pathway characterised by an acute phase of protein-rich alveolar fluid influx into the alveolar lumina as a consequence of the injury to the alveolar wall. Subsequently, type-2 pneumocyte (see Glossary) hyperplasia occurs to replace the loss of infected type-1 pneumocytes and to cover the denuded epithelial basement membrane, resulting in restoration of the normal alveolar architecture. Severe alveolar injury may lead to fibrosis with loss of alveolar function in more protracted cases.

SARS-CoV was detected not only in the respiratory tract but also in faeces and urine of patients. Replication of the virus in the gastrointestinal tract was confirmed by electron microscopic studies of biopsies of the upper and lower intestinal mucosae of patients with SARS. Faecal transmission proved to be important in at least one major community outbreak in Hong Kong, in which over 300 patients were infected within a few days [1].

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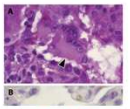
Animal Models for SARS

Development of animal models for SARS-CoV infection of humans is of utmost importance to elucidate the pathogenesis of SARS and to develop intervention strategies against the infection. A wide range of animal species is susceptible to experimental infection with SARS-CoV, including rodents (mice and hamsters), carnivores (ferrets and cats), and nonhuman primates (cynomolgus and rhesus macaques, common marmosets, and African green monkeys) [5–11]. Adult mice infected with varying doses of SARS-CoV in the respiratory tract show no clinical signs of disease, although the virus replicates in respiratory tissues, peaking early after infection, with viral titres in the lungs reaching relatively high levels. The infection is accompanied by only mild inflammatory changes of the respiratory tract. On the other hand, aged mice, as well as hamsters and ferrets, do show signs of clinical disease (weight loss and ruffled fur), albeit, in most cases, in the absence of the typical lung lesions seen in humans with SARS [4–6].

In contrast, SARS-CoV inoculation in the respiratory tract of cynomolgus macaques causes infection of bronchial epithelial cells, type-1 and type-2 pneumocytes at one to four days postinfection, followed by extensive type-2 pneumocyte hyperplasia in the lungs at four to six days postinfection [3,11]. Moreover, multiple foci of acute DAD are observed, characterised by flooding of alveoli with protein-rich oedema fluid mixed with variable numbers of neutrophils and rare syncytia (Figure 1), and by extensive loss of epithelium from alveolar and bronchiolar walls. These lesions are quite similar to those observed in humans in the acute stages of SARS. Although clinical signs (respiratory distress and general malaise) were observed, they were not further studied in the initial experiments.

Studies from different laboratories confirmed that nonhuman primates could be infected experimentally with SARS-CoV, although the severity of



Done exactly as poorly and unscientifically as Bailey describes?!

Infectious clone model

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Severe Acute Respiratory Syndrome Coronavirus Infection of Golden Syrian Hamsters

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Competing financial interests: declared none.

Aetiology

Koch's postulates fulfilled for SARS virus

Severe acute respiratory syndrome (SARS) has recently emerged as a new human disease, resulting globally in 435 deaths from 6,234 probable cases (as of 3 May 2003). Here we provide proof from experimental infection of cynomolgus macaques (*Macaca fascicularis*) that the newly discovered SARS-associated coronavirus (SCV) is the aetiological agent of this disease. Our understanding of the aetiology of SARS will expedite the development of diagnostic tests, antiviral therapies and vaccines, and may allow a more concise case definition for this emerging disease.

According to Koch's postulates, as modified by Rivers for viral diseases, six criteria are required to establish a virus as the cause of a disease[1]. The first three criteria — isolation of virus from diseased hosts, cultivation in host cells, and proof of filterability — have been met for SCV by several groups[2–5]. Moreover, of 96 individuals complying with the World Health Organization's definition of SARS[6] in Hong Kong, 86 (90%) yielded laboratory evidence of SCV infection.

We have tested for the three remaining criteria: production of comparable disease in the original host species or a related one, re-isolation of the virus, and detection of a specific immune response to the virus. We inoculated two macaques with Vero-cell-cultured SCV isolated from a fatal SARS case, and monitored their clinical signs, virus excretion and antibody response. The animals were killed six days post-inoculation (d.p.i.), and we then carried out gross and histopathological examinations of them.

Both SCV-inoculated macaques became lethargic from 3 d.p.i. onwards and developed a temporary skin rash, and one suffered respiratory distress from 4 d.p.i. onwards. The macaques excreted virus from the nose and throat at 2–6 d.p.i., as shown by polymerase chain reaction with reverse transcription (RT-PCR) and by virus isolation (see supplementary information). The isolated virus was identical to that inoculated, as shown by negative-contrast electron microscopy (Fig. 1a) and RT-PCR analysis. Seroconversion to SCV, as determined by indirect immunofluorescence assay using infected Vero cells, was demonstrated in two other SCV-infected macaques at 16 d.p.i.. The virus was also isolated from the faeces of one of these animals (see supplementary information).

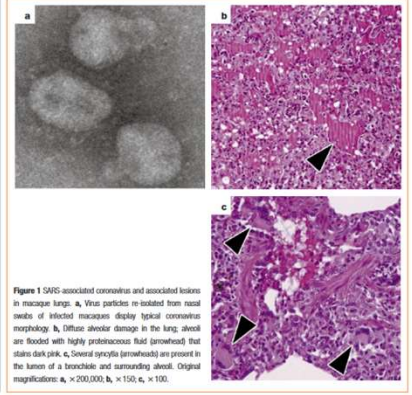

Figure 1 SARS-associated coronavirus and associated lesions in macaque lungs. **a**, Virus particles re-isolated from nasal swabs of infected macaques display typical coronavirus morphology. **b**, Diffuse alveolar damage in the lung; alveoli are flooded with highly proteinaceous fluid (arrowhead) that stains dark pink. **c**, Several syncytia (arrowheads) are present in the lumen of a bronchiole and surrounding alveoli. Original magnifications: **a**, ×200,000; **b**, ×150; **c**, ×100.

At gross necropsy, one macaque had severe multifocal pulmonary consolidation, and SCV infection was detected in lung tissue by RT-PCR and virus isolation. Histologically, both macaques had interstitial pneumonia of differing severity. The one with gross lesions had diffuse alveolar damage, marked by necrosis of alveolar and bronchiolar epithelium and flooding of alveolar lumina with proteinaceous fluid, admixed with fibrin, erythrocytes, alveolar macrophages and neutrophils (Fig. 1b). Occasional multinucleated cells (syncytia) were present in the lumen of bronchioles and alveoli (Fig. 1c). These lesions are indistinguishable from those in biopsied lung tissue and in autopsy material from SARS patients[7], including the presence of syncytia in alveolar lumina[8].

SCV thus fulfils all of Koch's postulates as the primary aetiological agent of SARS. This does not exclude the possibility that other pathogens, including human metapneumovirus (hMPV) and *Chlamydia pneumoniae*, may have exacerbated the disease in some SARS patients. However, these were not present in SCV-inoculated macaques (results not shown), were not found consistently in SARS patients, and do not usually cause the lesions associated with SARS. Moreover, lesions in macaques infected experimentally with hMPV isolated from a non-SARS individual[7] were limited to mild suppurative rhinitis and minimal erosion in conducting airways, and disease was not exacerbated in two SCV-infected macaques subsequently inoculated with hMPV (results not shown).

Ron A. M. Fouchier*, Thijs Kuiken*, Martin Schutten*, Geert van Amerongen*, Gérard J. J. van Doornum*, Bernadette G. van den Hoogen*, Malik Peiris†, Wilina Lim‡, Klaus Stöhr§, Albert D. M. E. Osterhaus*

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Supplementary information accompanies this communication on *Nature*'s website.
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A FAREWELL TO VIROLOGY
(EXPERT EDITION)

ABSTRACT

Virology invented the virus model but has consistently failed to fulfil its own requirements. It is claimed that viruses cause disease after transmitting between hosts such as humans and yet the scientific evidence for these claims is missing. One of virology's greatest failures has been the inability to obtain any viral particles directly from the tissues of organisms said to have "viral" diseases. In order to obfuscate this state of affairs, virologists have resorted to creating their own pseudoscientific methods to replace the longstanding scientific method, as well as changing the dictionary meaning of words in order to support their anti-scientific practices. For instance, an "isolated" isolate does not require the physical existence of the particles in order to be afforded "isolation" status.

A viral particle must fulfil defined physical and biological properties including being a replication-competent intracellular parasite capable of *causing* disease in a host such as a human. However, "viruses" such as SARS-CoV-2 are nothing more than phantom constructs, existing only in imaginations and computer simulations. In this paradigm, cases of invented diseases like COVID-19 are nothing more than the detection of selected genetic sequences and proteins *purported* to be "viral." The existence of a virus is not required in this loop of circular reasoning and thus entire "pandemics" can be built upon digital creations and falsely sustained through *in vitro* ("test tube") molecular reactions.

This essay contains three parts. Part One outlines some of the history of virology and the failures of the virologists to follow the scientific method. The many and far-reaching claims of the virologists can all be shown to be flawed due to: (a) the lack of direct evidence, and (b) the invalidation of indirect "evidence" due to the uncontrolled nature of the experiments. The examples provided cover all major aspects of the virological fraud including alleged isolation, cytopathic effects, genomics, antibodies, and animal pathogenicity studies.
Part Two examines the fraud used to propagate the COVID-19 "pandemic." A breakdown of the methodology relied upon by the original inventors Fan Wu et al., shows how the fictional SARS-CoV-2 was "created" through anti-scientific methods and linguistic sleights of hands. It is part of an ongoing deception where viruses are claimed to exist by templating them against previous "virus" templates. Using SARS-CoV-2 as an example, the trail of "coronavirus" genomic templates going back to the 1980s reveals that none of these genetic sequences have ever been shown to come from inside any viral particle — the phylogenetic trees are fantasies. The misapplication of the polymerase chain reaction has propagated this aspect of virology's fraud and created the 'cases' to maintain the illusion of a pandemic.
Part Three provides an analysis of how some key participants, "health" institutions, and the mainstream media maintain the virus illusion through information control and narratives that parrot virology's claims. By way of happenstance, the virological fraud now finds itself front and centre of the COVID-19 fraud. From here, however, it can be critically appraised by those outside virology and the pseudo-scientific paradigm virology has built around itself can finally be dismantled and laid to rest.

The aim of this essay is to provide refutations to various claims that pathogenic viruses exist and cause disease. SARS-CoV-2 has been used as the main example but the principles apply to all alleged viruses. What follows addresses virology's often arcane literature on its own terms, which, it should be said, may make parts of this essay somewhat heavy reading. However, it is hoped that this contribution will fill a niche for the reader seeking a more technical understanding of the virus hypothesis as it seeks to expose the very foundation of purported pandemics and fraudulent medical practices. The threat of virology to humanity is increasing so it is time we bid farewell to these destructive pseudoscientific practices and free ourselves from unnecessary fears.

drsambailey.com/a-farewell-to-virology-expert-edition/

Q: So if Mark Bailey's objections are well founded, then what's the problem?

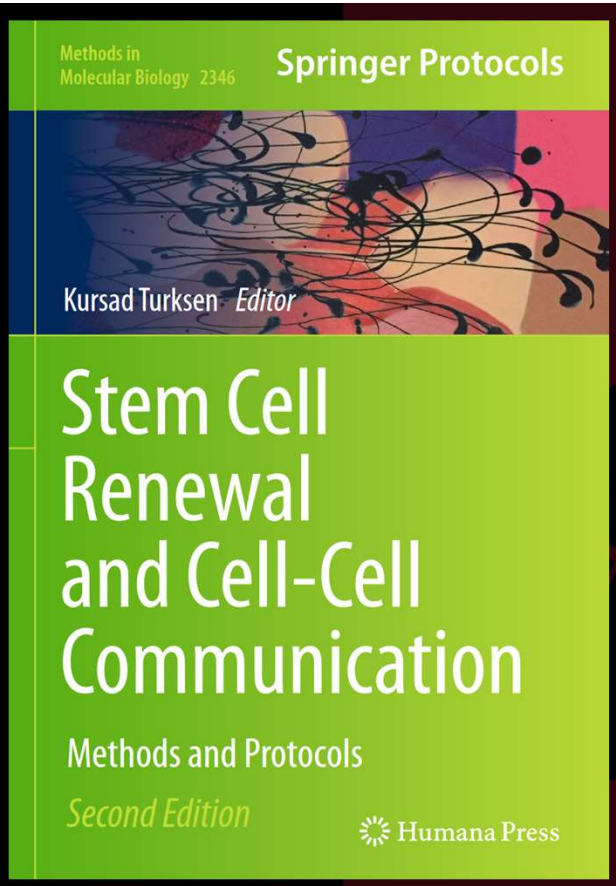
A: They don't attempt to explain observations that have occurred but instead dismiss them

Gigaohm Biological High Resistance Low Noise Information Brief

8 February 2023

Q: CAN ALL THE MOLECULAR BIOLOGY BE FRAUD ?

A: NO. THEREFORE, THE ALTERNATIVE OF NO VIRUS IS INSUFFICIENT.



Methods in Molecular Biology (2021) 2346: 91–104
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TIRF Microscopy as a Tool to Determine Exosome Composition

Noa B. Martín-Cófreces, Daniel Torralba, Marta Lozano-Prieto, Nieves Fernández-Gallego, and Francisco Sánchez-Madrid

Abstract

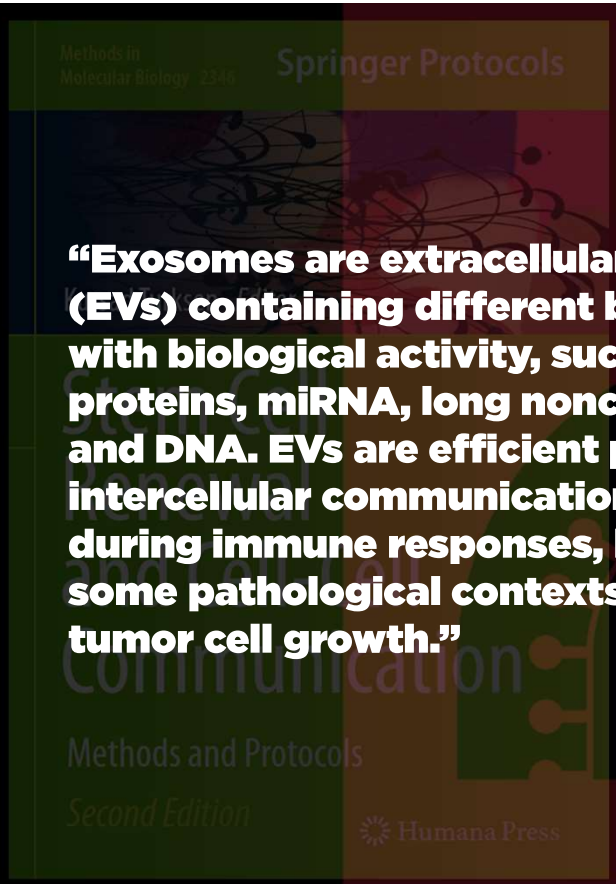
Exosomes are extracellular vesicles (EVs) containing different biomolecules with biological activity, such as proteins, miRNA, long noncoding RNA, and DNA. EVs are efficient platforms for intercellular communication, especially during immune responses, but also in some pathological contexts, such as tumor cell growth. The precise assessment of EV content is relevant for the selection of specific vesicles with specialized biological activities, whose content is hardly visualized due to their small size. We describe herein a protocol for the determination of the content of individual EVs through microscopy imaging and user-friendly analysis using TIRF microscopy.

Keywords Extracellular vesicles, Exosomes, Cell-to-cell communication, T cells, Immune synapse, Microscopy, TIRF

1 Introduction

Extracellular vesicles (EVs) are lipid bilayer-delimited particles released from cells. EVs have emerged as a mode of cell-cell communication and intercellular transfer of materials, and, thus, EVs are now considered as important mediators of cell signaling [1, 2]. They have been found to be released by a wide variety of cell types, such as B and T cells, dendritic cells, platelets, cancer cells, and, even, protozoan parasites [3, 4]. EVs comprise different subtypes of particles categorized by their size, origin, and composition. Briefly, EVs are classified into exosomes when EVs size ranges from 40 to 200 nm, are produced in multivesicular bodies, and contain specific proteins located in the endosome, such as tetraspanins; into microvesicles, which are larger in size, ranging from 150 to 1000 nm, and are generated through direct budding at the plasma membrane; and apoptotic bodies, which are produced as consequence of membrane blebbing during apoptosis and are larger than 1 μm [2, 3]. Among them, exosomes have been

Sánchez-Madrid and Martín-Cófreces contributed equally to this work.



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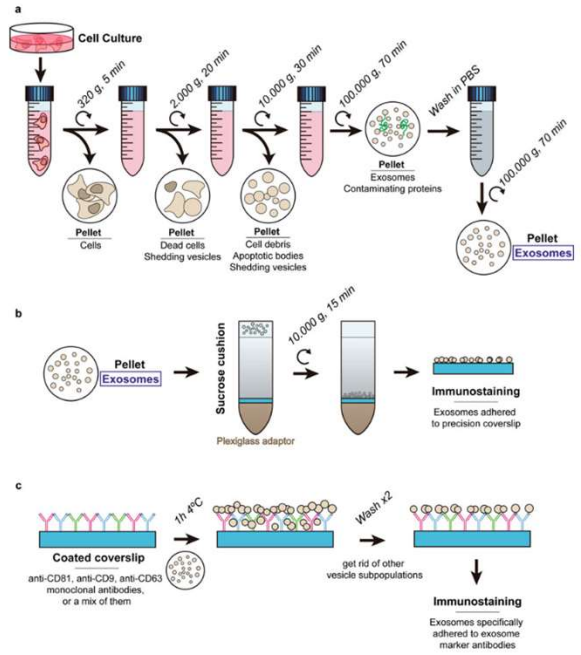
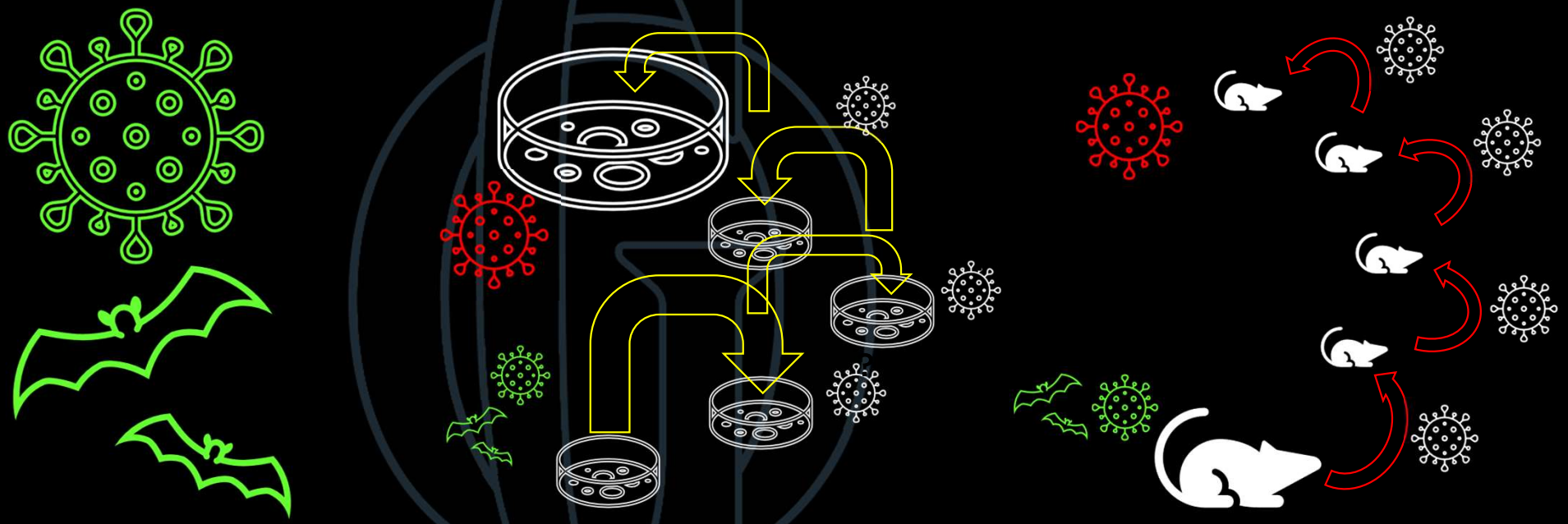


Fig. 1 Workflow for exosome isolation and immunofluorescence. (**a**) Cells are cultured in ultracentrifuged medium for 48–72 h. The supernatant is subjected to a series of differential centrifugations, and the resulting pellet is washed and ultracentrifuged again at 100,000 × *g*. The exosomes are ready to be quantified and stained. (**b**) Exosomes are centrifuged in a sucrose cushion and then allowed to stick gently to the surface of the poly-L-Lysine treated coverslip. Once attached, they are ready to continue with the staining protocol. (**c**) Exosomes are incubated in a coverslip coated with antibodies against exosome markers. After two washes, only the vesicles containing specific exosomal proteins are adhered to the coverslip and are ready to continue with the staining protocol

Q: WHAT IS A VIRUS?

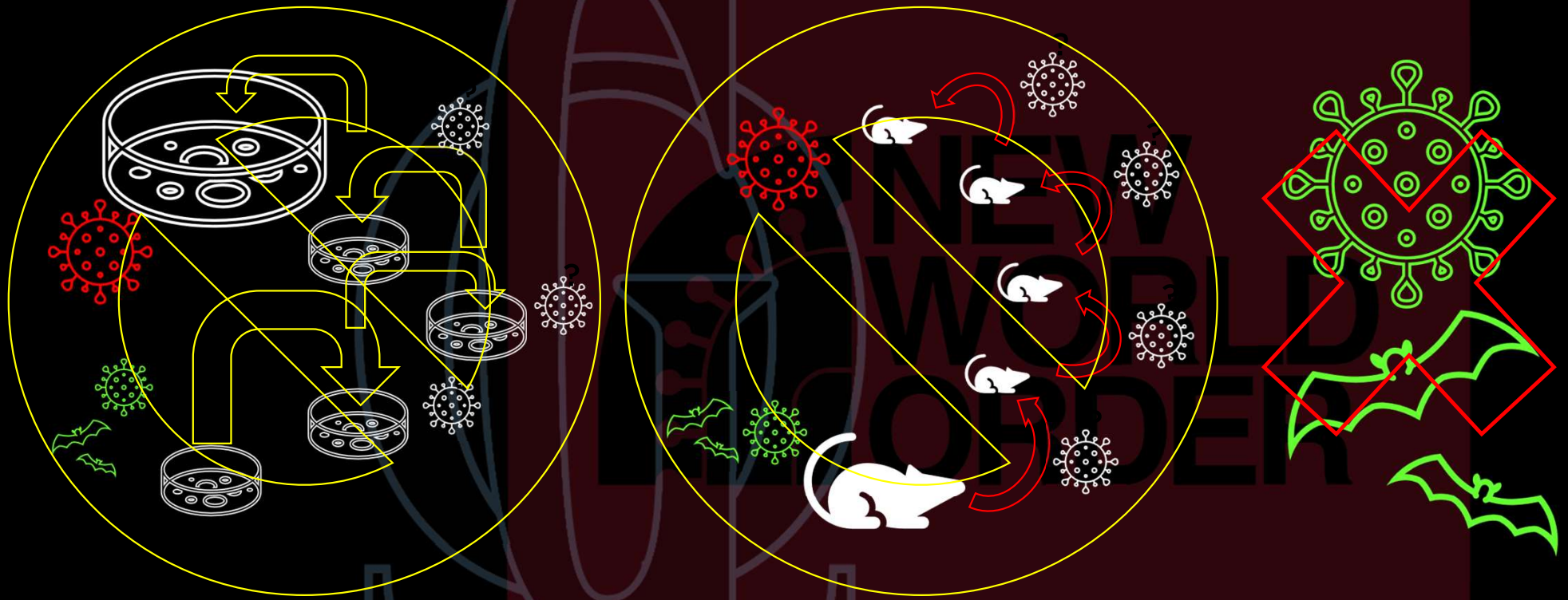
A: WHATEVER THEY ARE, THEY CANNOT FORCE CELLS TO DO THINGS THEY DON'T ALREADY DO.

What is being collected or passaged during these experiments??



The answer is definitely exosomes.

If culturing wild type coronavirus is difficult, and passage in cell culture or animal host cannot produce pandemic viruses, why are THEY pretending it can and did?



To coerce a surrender of individual sovereignty and a global fundamental inversion of human rights from freedom to fascism

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7153529

Cell Host & Microbe

CellPress

Resource

An Infectious cDNA Clone of SARS-CoV-2

Xuping Xie,[1,*] Antonio Muruato,[1,2] Kumari G. Lokugamage,[2] Krishna Narayanan,[2] Xianwen Zhang,[1] Jing Zou,[1] Jianying Liu,[2] Craig Schindewolf,[2] Nathen E. Bopp,[3] Patricia V. Aguilar,[3,4,5] Kenneth S. Plante,[2,4] Scott C. Weaver,[2,4,5,6,7,8] Shinji Makino,[2,5,10] James W. LeDuc,[2,9] Vineet D. Menachery,[2,5,7,*] and Pei-Yong Shi[1,5,8,10,11,12,*]

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https://doi.org/10.1016/j.chom.2020.04.004

SUMMARY

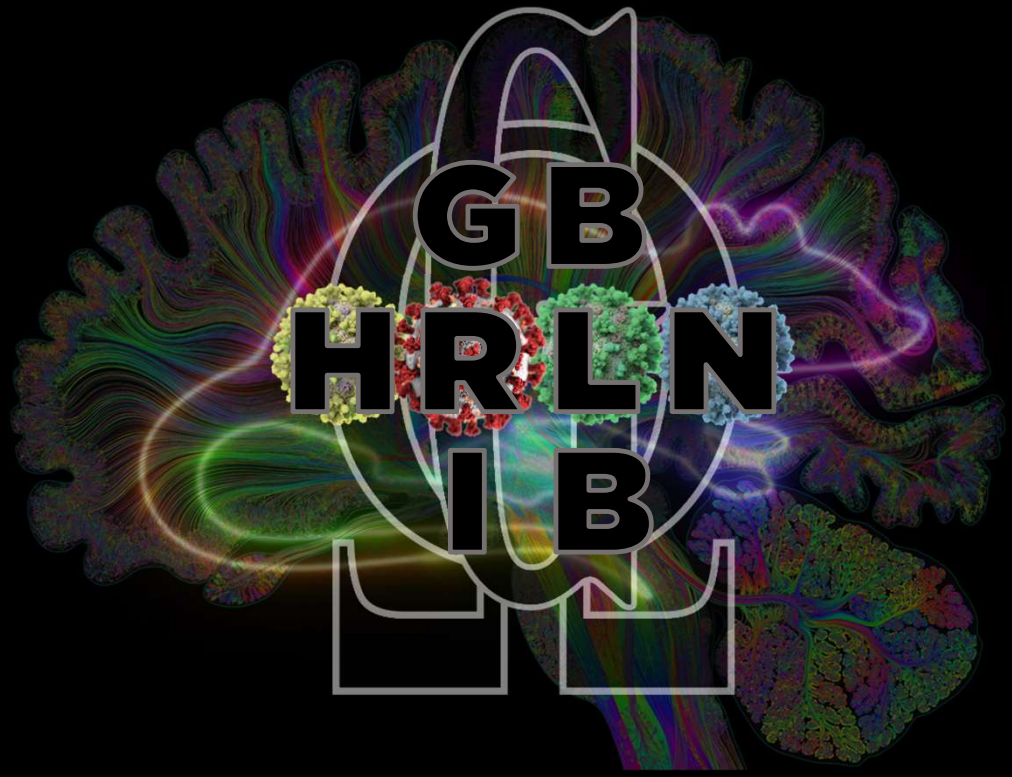
The ongoing pandemic of COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), underscores the urgency to develop experimental systems for studying this virus and identifying countermeasures. We report a reverse genetic system for SARS-CoV-2. Seven complimentary DNA (cDNA) fragments spanning the SARS-CoV-2 genome were assembled into a full-genome cDNA. RNA transcribed from the full-genome cDNA was highly infectious after electroporation into cells, producing 2.9×10^6 plaque-forming unit (PFU)/mL of virus. Compared with a clinical isolate, the infectious-clone-derived SARS-CoV-2 (icSARS-CoV-2) exhibited similar plaque morphology, viral RNA profile, and replication kinetics. Additionally, icSARS-CoV-2 retained engineered molecular markers and did not acquire other mutations. We generated a stable mNeonGreen SARS-CoV-2 (icSARS-CoV-2-mNG) by introducing this reporter gene into ORF7 of the viral genome. icSARS-CoV-2-mNG was successfully used to evaluate the antiviral activities of interferon (IFN). Collectively, the reverse genetic system and reporter virus provide key reagents to study SARS-CoV-2 and develop countermeasures.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in early 2020 with human cases in Wuhan, China (Zhou et al., 2020; Zhu et al., 2020). It has rapidly rampaged worldwide, causing a pandemic of coronavirus disease (COVID-19) that ranges from fever and breathing difficulty to acute respiratory distress and death (Huang et al., 2020; Zhu et al., 2020). With over 300,000 people infected in less than 3 months, SARS-CoV-2 causes the most severe disease in older patients and people with co-morbidities, including heart disease, diabetes, and other health conditions (Wu and McGoogan, 2020). Before 2019, six α- and β-coronaviruses were known to cause respiratory diseases of different severity, including four common cold coronaviruses (229E, NL63, OC43, and HKU1) and two highly pathogenic coronaviruses (severe acute respiratory syndrome [SARS-CoV] and Middle East respiratory syndrome [MERS-CoV], which emerged in 2003 and since 2012, respectively) (Assiri et al., 2013; Huang et al., 2020). Importantly, with massive hospitalization rates and high mortality, SARS-CoV-2 remains a major threat to humankind and intervention strategies are being rapidly pursued.

A key tool in responding to emergent viruses is the generation of reverse genetic systems to explore and characterize new pathogens. Classically, reverse genetic systems for coronaviruses have been complicated by their large genome size (~30,000 nucleotides) and the existence of bacteriotoxic elements in their genome that make them difficult to propagate (Almazán et al., 2014). Several approaches have been devised to overcome this barrier, such as multiple plasmid systems to disrupt toxic elements and to reduce deletions and mutations (Yount et al., 2002). Using this approach, researchers have developed infectious clones for several coronaviruses, including SARS-CoV, MERS-CoV, and others (Menachery et al., 2015; Menachery et al., 2016; Scobey et al., 2013; Yount et al., 2003). Thao et al., (2020) recently reported a yeast-based synthetic genomics platform for rapid construction of infectious clones for murine hepatitis coronavirus (MHV-CoV), MERS-CoV, and SARS-CoV-2. However, the yeast-platform-produced SARS-CoV-2 has not been fully characterized for its biological

https://www.cell.com/cell/fulltext/S0092-8674(20)30675-9



Cell

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Article

SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract

Yixuan J. Hou,[1,19] Kenichi Okuda,[3,19] Caitlin E. Edwards,[1,19] David R. Martinez,[1,19] Takanori Asakura,[3] Kenneth H. Dinnon III,[2] Takafumi Kato,[3] Rhianna E. Lee,[3] Boyd L. Yount,[1] Teresa M. Mascenik,[3] Gang Chen,[3] Kenneth N. Olivier,[16] Andrew Ghio,[17] Longping V. Tse,[1] Sarah R. Leist,[1] Lisa E. Gralinski,[1] Alexandra Schäfer,[1] Hong Dang,[3] Rodney Gilmore,[3] Satoko Nakano,[3] Ling Sun,[3] M. Leslie Fulcher,[3] Alessandra Livraghi-Butrico,[3] Nathan I. Nicely,[4] Mark Cameron,[11] Cheryl Cameron,[12] David J. Kelvin,[10,18] Aravinda de Silva,[2] David M. Margolis,[2,5,6] Alena Markmann,[5] Luther Bartelt,[5] Ross Zumwalt,[13] Fernando J. Martinez,[14] Steven P. Salvatore,[15] Alain Borczuk,[15] Purushothama R. Tata,[9] Vishwaraj Sontake,[9] Adam Kimple,[7] Ilona Jaspers,[8] Wanda K. O'Neal,[3] Scott H. Randell,[3] Richard C. Boucher,[3,*] and Ralph S. Baric[1,2,20,*]

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https://doi.org/10.1016/j.cell.2020.05.042

SUMMARY

The mode of acquisition and causes for the variable clinical spectrum of coronavirus disease 2019 (COVID-19) remain unknown. We utilized a reverse genetics system to generate a GFP reporter virus to explore severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pathogenesis and a luciferase reporter virus to demonstrate sera collected from SARS and COVID-19 patients exhibited limited cross-CoV neutralization. High-sensitivity RNA *in situ* mapping revealed the highest angiotensin-converting enzyme 2 (ACE2) expression in the nose with decreasing expression throughout the lower respiratory tract, paralleled by a striking gradient of SARS-CoV-2 infection in proximal (high) versus distal (low) pulmonary epithelial cultures. COVID-19 autopsied lung studies identified focal disease and, congruent with culture data, SARS-CoV-2-infected ciliated and type 2 pneumocyte cells in airway and alveolar regions, respectively. These findings highlight the nasal susceptibility to SARS-CoV-2 with likely subsequent aspiration-mediated virus seeding to the lung in SARS-CoV-2 pathogenesis. These reagents provide a foundation for investigations into virus-host interactions in protective immunity, host susceptibility, and virus pathogenesis.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified as the causative agent of the ongoing pandemic coronavirus disease 2019 (COVID-19) (Gorbalenya et al., 2020). SARS-CoV-2 emerged in Wuhan, China in December 2019 and rapidly spread to more than 175 countries within three months (Huang et al., 2020; Zhu et al., 2020). As of May 18, 2020, about 4.7 million confirmed cases and > 316,000 deaths have been reported worldwide. The absence of approved

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The Architecture of SARS-CoV-2 Transcriptome

Dongwan Kim,[1,2] Joo-Yeon Lee,[3] Jeong-Sun Yang,[3] Jun Won Kim,[3] V. Narry Kim,[1,2,4,*] and Hyeshik Chang[1,2,*]
[1]Center for RNA Research, Institute for Basic Science (IBS), Seoul 08826, Republic of Korea
[2]School of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea
[3]Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Osong 28159, Republic of Korea
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*Correspondence: narrykim@snu.ac.kr (V.N.K.), hyeshik@snu.ac.kr (H.C.)
https://doi.org/10.1016/j.cell.2020.04.011

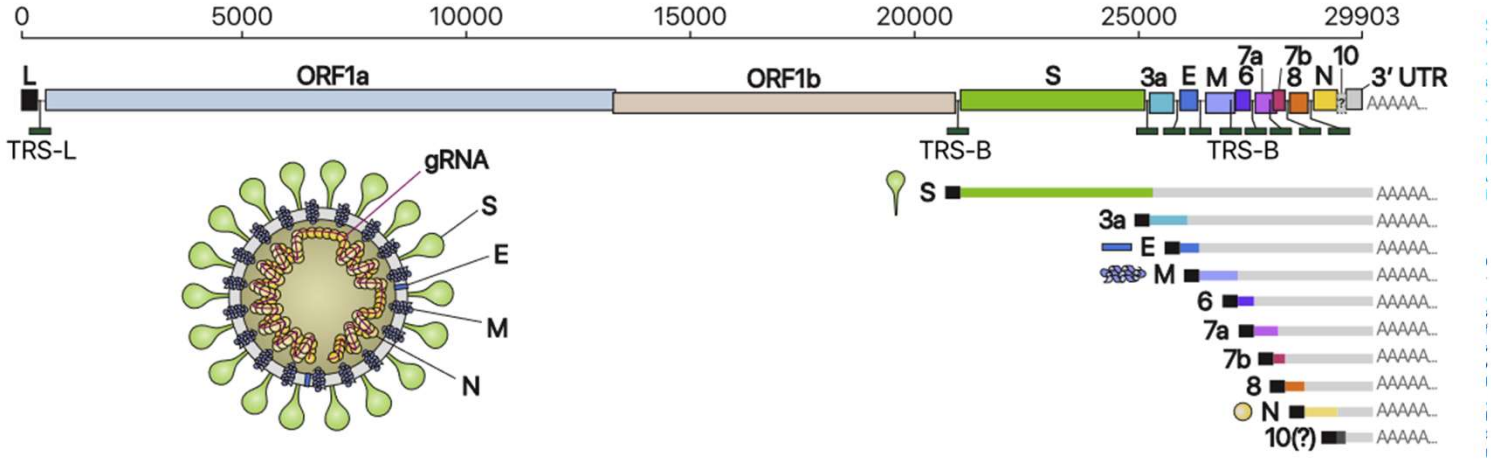

Figure 1. Schematic Presentation of the SARS-CoV-2 Genome Organization, the Canonical Subgenomic mRNAs, and the Virion Structure

From the full-length genomic RNA (29,903 nt) that also serves as an mRNA, ORF1a and ORF1b are translated. In addition to the genomic RNA, nine major subgenomic RNAs are produced. The sizes of the boxes representing small accessory proteins are bigger than the actual size of the ORF for better visualization. The black box indicates the leader sequence. Note that our data show no evidence for ORF10 expression.

nonstructural proteins (nsps) from two open reading frames (ORFs), ORF1a and ORF1b. The ORF1a produces polypeptide 1a (pp1a, 440–500 kDa) that is cleaved into 11 nsps. The −1 ribosome frameshift occurs immediately upstream of the ORF1a stop codon, which allows continued translation of ORF1b, yielding a large polypeptide (pp1ab, 740–810 kDa) which is cleaved into 15 nsps. The proteolytic cleavage is mediated by viral proteases nsp3 and nsp5 that harbor a papain-like protease domain and a 3C-like protease domain, respectively.

this called transcription-regulatory sequences (TRSs) that are located immediately adjacent to ORFs (Figure 1). TRSs contain a conserved 6–7 nt core sequence (CS) surrounded by variable sequences. During negative-strand synthesis, RdRP pauses when it crosses a TRS in the body (TRS-B) and switches the template to the TRS in the leader (TRS-L), which results in discontinuous transcription leading to the leader-body fusion. From the fused negative-strand intermediates, positive-strand mRNAs are transcribed. The replication and transcription mechanism has been

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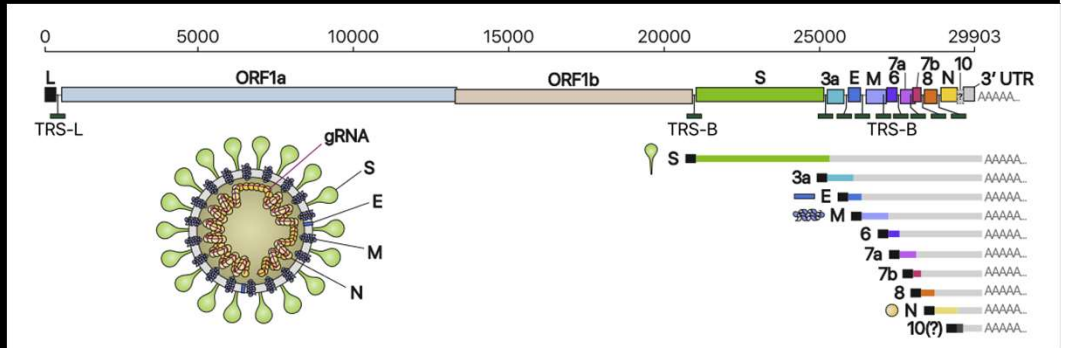

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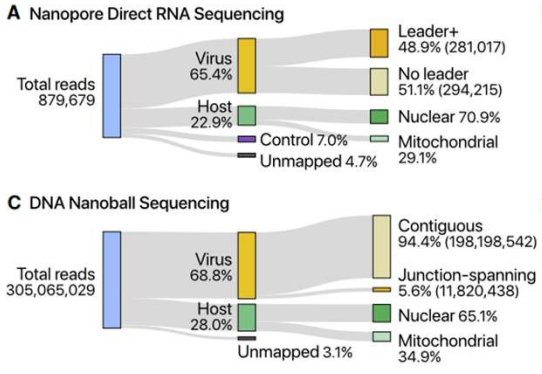

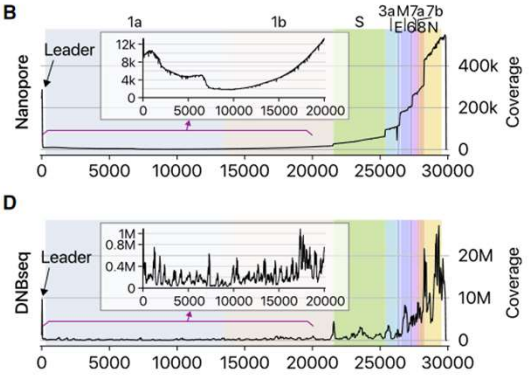

Figure 2. Statistics of Sequencing Data
(A) Read counts from nanopore direct RNA sequencing of total RNA from Vero cells infected with SARS-CoV-2. "Leader+" indicates the viral reads that contain the 5′ end leader sequence. "No leader" denotes the viral reads lacking the leader sequence. "Nuclear" reads match mRNAs from the nuclear chromosome while "mitochondrial" reads are derived from the mitochondrial genome. "Control" indicates quality control RNA for nanopore sequencing.
(B) Genome coverage of the nanopore direct RNA sequencing data shown in (A). The stepwise reduction in coverage corresponds to the borders expected for the canonical sgRNAs. The smaller inner plot magnifies the 5′ part of the genome.
(C) Read counts from DNA nanoball sequencing using MGISEQ. Total RNA from Vero cells infected with SARS-CoV-2 was used for sequencing.
(D) Genome coverage of the DNA nanoball sequencing (DNB-seq) data shown in (C).
See also Figure S1.

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The Architecture of SARS-CoV-2 Transcriptome

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[1]Center for RNA Research, Institute for Basic Science (IBS), Seoul 08826, Republic of Korea
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https://doi.org/10.1016/j.cell.2020.04.011

SUMMARY

SARS-CoV-2 is a betacoronavirus responsible for the COVID-19 pandemic. Although the SARS-CoV-2 genome was reported recently, its transcriptomic architecture is unknown. Utilizing two complementary sequencing techniques, we present a high-resolution map of the SARS-CoV-2 transcriptome and epitranscriptome. DNA nanoball sequencing shows that the transcriptome is highly complex owing to numerous and 9 subgenomic RNAs, SARS-, and/or frameshift. Using nano- sites on viral transcripts, with an unmodified RNAs, suggesting he unknown transcripts and RNA derstanding of the life cycle and

lso used as the template for replication is mediated by nsp12 harboring RNA- erase (RdRP) activity (Snijder et al., 5). Negative-sense RNA intermediates as the templates for the synthesis of RNA (gRNA) and subgenomic RNAs packaged by the structural proteins to ns. Shorter sgRNAs encode conserved ike protein [S], envelope protein [E], , and nucleocapsid protein [N]), and eins. SARS-CoV-2 is known to have at eins (3a, 6, 7a, 7b, 8, and 10) according n (GenBank: NC_045512.2). However, been experimentally verified for expres- rrently unclear which accessory genes rom this compact genome.
IA contains the common 5′ "leader" d to the "body" sequence from the down- me (Lai and Stohlman, 1981; Sola et al., ding to the prevailing model, leader-to- g negative-strand synthesis at short mo- -regulatory sequences (TRSs) that are acent to ORFs (Figure 1). TRSs contain a equence (CS) surrounded by variable se- e-strand synthesis, RdRP pauses when it dy (TRS-B) and switches the template to

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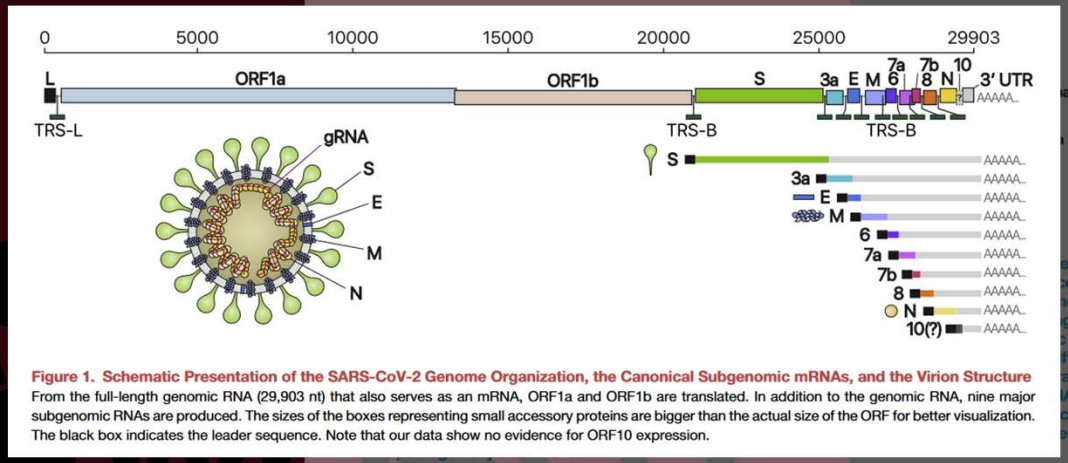
“The SARS-CoV-2 genome was almost fully covered, missing only 12 nt from the 5’ end due to the known inability of DRS to sequence the terminal 12 nt (Figure 2B). The longest tags (111 reads) correspond to the full-length gRNA (Figure 2B).”



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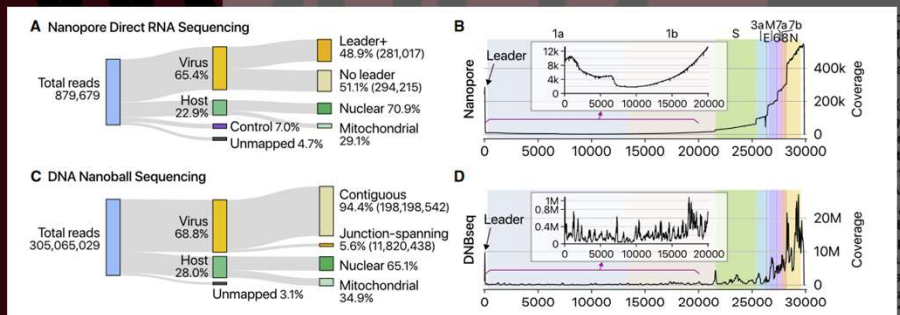

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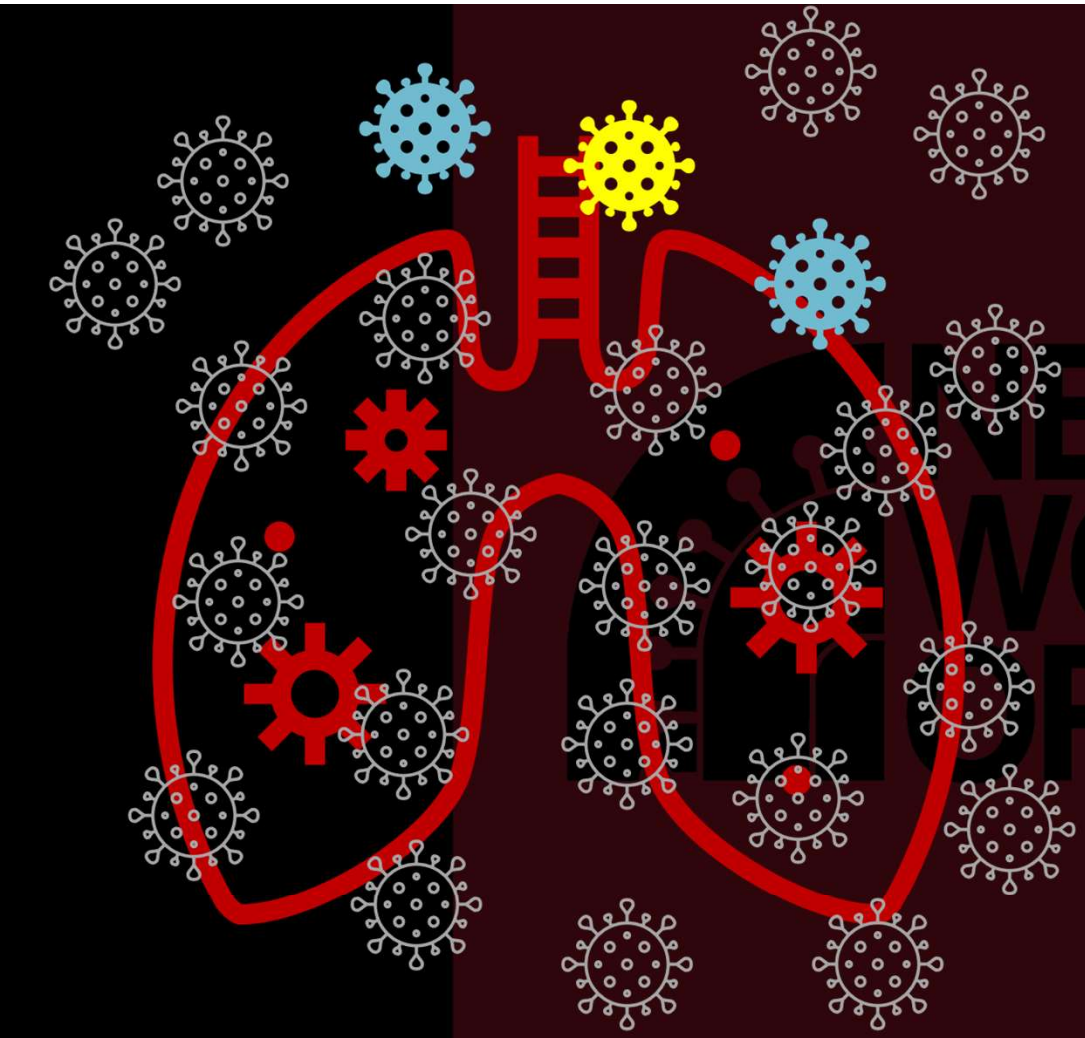

WHAT IF THE NATURAL INFECTIOUS CYCLE PRODUCES AN N:1 RATIO OF 500000:1 ?

HAS NANOPORE SEQUENCING REVEALED AN ALTERNATIVE INFECTIOUS CYCLE ?

HAVE CLONES BEEN USED TO CREATE AN ILLUSION WHERE THE NATURAL EXOSOMAL COMMUNICATION MACHINERY IS HIJACKED BY BASIC TRANSFECTION ?

WHY HAVE WE NOT HEARD OF ARC PROTEIN IN THE BRAIN ?

WHY DO THESE PAPERS TALK OF EXOSOMES AS CENTRAL TO IMMUNE RESPONSE BY FAUCI HAS NEVER SAID THE WORD ?

Q: WHAT IS A VIRUS ?

A: WHATEVER THEY ARE, THEY CANNOT FORCE CELLS TO DO THINGS THEY DON'T ALREADY DO.


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buymeacoffee.com/drkevinmccairn
mazlak: Think the SARS in animals (deer population etc) was put there intentionally, not spillover
qwerty123: if you saw the "whole" picture "in our present paradigm" your head would explode
It's pervasive in all pillars of society already
Curious aspect of outbreaks where their steep rise & steep decline

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 11354–11358, October 1996
Colloquium Paper

This paper was presented at a colloquium entitled "Genetic Engineering of Viruses and of Virus Vectors," organized by Bernard Roizman and Peter Palese (Co-chairs), held June 9–11, 1996, at the National Academy of Sciences in Irvine, CA.

Negative-strand RNA viruses: Genetic engineering and applications

PETER PALESE*, HONGYONG ZHENG, OTHMAR G. ENGELHARDT, STEPHAN PLESCHKA, AND ADOLFO GARCÍA-SASTRE

Department of Microbiology, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029

ABSTRACT **The negative-strand RNA viruses are a broad group of animal viruses that comprise several important human pathogens, including influenza, measles, mumps, rabies, respiratory syncytial, Ebola, and hantaviruses. The development of new strategies to genetically manipulate the genomes of negative-strand RNA viruses has provided us with new tools to study the structure–function relationships of the viral components and their contributions to the pathogenicity of these viruses. It is also now possible to envision rational approaches—based on genetic engineering techniques—to design live attenuated vaccines against some of these viral agents. In addition, the use of different negative-strand RNA viruses as vectors to efficiently express foreign polypeptides has also become feasible, and these novel vectors have potential applications in disease prevention as well as in gene therapy.**

DNA-Containing Viruses

Among animal viruses, DNA-containing viruses were the first to become amenable to genetic engineering techniques. This breakthrough was achieved for simian virus 40 when a cloned cDNA copy was transfected into cells, resulting in the formation of infectious virus (see Table 1). Transfected mutated cDNA molecules gave rise to defined mutant viruses (1). A second methodology involving the use of homologous recombination allowed, for the first time, the rescue of large DNA-containing viruses such as herpes viruses (2). In this approach, intact herpes viral DNA as well as cloned DNA flanked by viral sequences was transfected into cells. Homologous recombination between the cloned DNA and the wild-type genome can occur, and novel viruses can be selected under appropriate conditions. For example, recombinants with DNA fragments containing a viral thymidine kinase gene can be selected in appropriate cell lines and media, and viruses lacking a thymidine kinase can be isolated in the presence of nucleoside analogs (e.g., Ara T). This general technique allows the successful construction of viral variants of herpes viruses, and similar procedures have been developed for pox viruses (3, 4) and other DNA-containing viruses including adenoviruses (5) and parvoviruses (6). Finally, strategies have been developed to generate infectious as well as mutant viruses by transfecting cosmids containing overlapping portions of large viral genomes. Viruses arise via recombination between the cosmids. This system was successfully used to rescue infectious herpes simplex 1 viruses (7), cytomegaloviruses (8) and Epstein–Barr viruses (9) from their respective cosmids.

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Positive-Strand RNA Viruses

RNA-containing viruses belong to a variety of families with diverse replication strategies. Unique among the RNA viruses are the retroviruses, whose replication involves a double-stranded DNA phase, making these viruses an easy target for genetic manipulation. Transfection of full-length cDNA molecules leads to the establishment of replicating virus particles and integration of the viral genetic information into the host genome (10). The engineering of retroviral genomes has become one of the most successful genetic approaches in modern virology and is central to the study both of viral gene expression and of protein structure–function analysis. In addition, retrovirus constructs are among the most widely used vectors for gene transfer and gene therapy (11).

Most of the other positive-strand RNA viruses are also amenable to genetic engineering approaches (Table 1). In the case of the small and medium sized positive-strand RNA viruses, full-length genomic RNA has been shown to be infectious when transfected into cells. Plus-strand RNA serves as mRNA for the synthesis of viral proteins as well as template for viral RNA replication. Thus, transfection of cloned DNA of poliovirus RNA (or of cDNA-derived RNA) into permissive cells results in the formation of infectious virus particles (12).

Remarkably successful have been studies using Sindbis viruses and Semliki forest virus (13, 14). The cDNA-derived RNAs of these positive-strand RNA viruses can be used to efficiently rescue infectious viruses, thus allowing an extensive analysis of the promoter elements of the viral RNAs as well as structure–function studies of the viral proteins. Furthermore, these viruses have received increased attention because of their potential for expressing copious amounts of heterologous genes via recombinant constructs. Up to 10^8 molecules of heterologous protein per cell have been expressed using these systems.†

Introduction of cDNA-Derived RNA into a Negative-Strand RNA Virus (Influenza Virus)

The life cycle of negative-strand RNA viruses differs from that of the other RNA viruses in many ways. Specifically, the genomic RNA of negative-strand RNA viruses is not infectious, and infectious virus particles must also deliver their own RNA-dependent RNA polymerase into the infected cell to start the first round of virus-specific mRNA synthesis.

Thus, approaches different from those used for positive-strand RNA viruses had to be developed to allow the rescue of

Abbreviations: RNP, ribonucleoprotein; HA, hemagglutinin; NA, neuraminidase, VSV, vesicular stomatitis virus.

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†Belli, B. A., Polo, J. M., Driver, D. A., Latham, E., Banks, T. A., Chang, S. M. W. & Dubensky, T. W., Jr., National Academy of Sciences Colloquium on Genetic Engineering of Viruses and of Virus Vectors, June 9–11, 1996, Irvine, CA, no. 1. (abstr.).

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Vol. 96, pp. 9345–9350, August 1999
Microbiology

Generation of influenza A viruses entirely from cloned cDNAs

GABRIELE NEUMANN*, TOKIKO WATANABE*†, HIROSHI ITO*, SHINJI WATANABE*†, HIDEO GOTO*, PENG GAO*, MARK HUGHES*, DANIEL R. PEREZ‡, RUBEN DONIS‡, ERICH HOFFMANN§, GERD HOBOM§, AND YOSHIHIRO KAWAOKA*¶

*Department of Pathobiological Sciences, School of Veterinary Medicine, University of Madison-Wisconsin, 2015 Linden Drive West, Madison, WI 53706; †Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan; ‡Department of Veterinary and Biomedical Sciences, University of Nebraska-Lincoln, P.O. Box 830905, Lincoln, NE 68583-0905; and §Institut für Mikro- und Molekularbiologie, Frankfurter Strasse 107, 35392 Giessen, Germany

Communicated by Paul Ahlquist, University of Wisconsin, Madison, WI, May 27, 1999 (received for review March 23, 1999)

ABSTRACT **We describe a new reverse-genetics system that allows one to efficiently generate influenza A viruses entirely from cloned cDNAs. Human embryonic kidney cells (293T) were transfected with eight plasmids, each encoding a viral RNA of the A/WSN/33 (H1N1) or A/PR/8/34 (H1N1) virus, flanked by the human RNA polymerase I promoter and the mouse RNA polymerase I terminator—together with plasmids encoding viral nucleoprotein and the PB2, PB1, and PA viral polymerases. This strategy yielded >1 × 10^3 plaque-forming units (pfu) of virus per ml of supernatant at 48 hr posttransfection. The addition of plasmids expressing all of the remaining viral structural proteins led to a substantial increase in virus production, 3 × 10^4–5 × 10^7 pfu/ml. We also used reverse genetics to generate a reassortant virus containing the PB1 gene of the A/PR/8/34 virus, with all other genes representing A/WSN/33. Additional viruses produced by this method had mutations in the PA gene or possessed a foreign epitope in the head of the neuraminidase protein. This efficient system, which does not require helper virus infection, should be useful in viral mutagenesis studies and in the production of vaccines and gene therapy vectors.**

The ability to generate infectious RNA viruses from cloned cDNAs has contributed greatly to our biological understanding of these pathogens and, hence, to improved methods of disease control (1). However, this progress had been relatively limited for negative-sense as compared with positive-sense RNA viruses, because neither the genomic viral RNA (vRNA) nor the antigenomic complementary RNA (cRNA) of negative-sense RNA viruses can serve as a direct template for protein synthesis. Rather, the vRNA, after its encapsidation by viral nucleoprotein (NP), must be transcribed into positive-sense mRNA by the viral RNA polymerase complex. Thus, the minimal replication unit is formed by the genomic vRNA complexed with NP and the polymerase proteins. Despite these obstacles, reverse-genetics methods have been established to produce nonsegmented, negative-sense RNA viruses, including rabies virus (2), vesicular stomatitis virus (3, 4), measles virus (5), respiratory syncytial virus (6), Sendai virus (7, 8), rinderpest virus (9), human parainfluenza virus type 3 (10), and simian virus 5 (11).

Generating segmented, negative-sense RNA viruses from cloned cDNAs poses a more formidable challenge, as one must produce a separate vRNA for each gene segment. In one study, Bridgen and Elliott (12) produced a Bunyamwera virus (family *Bunyaviridae*) from cloned cDNAs encoding three segments of negative-sense vRNA; however, the efficiency of virus recovery was low, and there have been no reports of an engineered Bunyamwera mutant. By contrast, none of the orthomyxoviruses, which contain six (Thogotovirus), seven (influenza C virus), or eight (influenza A and B viruses) segments of negative-sense RNA, have been produced entirely from cloned cDNAs. This lag in progress has been felt most acutely in efforts to control influenza virus infections.

Palese and colleagues (13) pioneered the reverse-genetics, helper virus-dependent system for influenza A virus (Fig. 1*A*). In their approach, a ribonucleoprotein (RNP) complex is generated by *in vitro* vRNA synthesis in the presence of purified polymerase and NP proteins and then used to transfect eukaryotic cells. Subsequent infection with influenza A helper virus results in the generation of viruses possessing a gene derived from cloned cDNA. A second method, developed by Neumann *et al.* (14), is based on the *in vivo* synthesis of vRNA by RNA polymerase I (Fig. 1*B*), a cellular enzyme that transcribes ribosomal RNA that lacks both a 5′ cap and a 3′ poly(A) tail. Transfection of cells with a plasmid containing cloned influenza virus cDNAs, flanked by RNA polymerase I promoter and terminator sequences, followed by influenza virus infection, led to the production of transfectant viruses. With both methods, however, transfectants must be selected from a vast background of helper viruses, which requires a strong selection system and complicates the generation of growth-defective viruses.

We report here the generation of influenza A viruses entirely from cloned cDNAs. The reverse-genetics approach we describe is highly efficient and can be used to introduce mutations into any gene segment and to develop influenza virus-based gene delivery systems.

MATERIALS AND METHODS

Cells and Viruses. 293T human embryonic kidney cells and Madin–Darby canine kidney cells (MDCK) were maintained in DMEM supplemented with 10% FCS and in MEM containing 5% newborn calf serum, respectively. The 293T cell line is a derivative of 293, into which the gene for the temperature-sensitive simian virus 40 T antigen has been inserted. This line produces replication-competent T antigen in large amounts at 37°C (15). All cells were maintained at 37°C in 5% CO_2. Influenza viruses A/WSN/33 (H1N1) and A/PR/8/34 (H1N1) were propagated in 10-day-old eggs.

Construction of Plasmids. To generate RNA polymerase I constructs, we cloned cDNAs derived from A/WSN/33 or A/PR/8/34 viral RNA between the promoter and terminator sequences of RNA polymerase I. Briefly, the cloned cDNAs were amplified by PCR with primers containing *Bsm*BI sites,

Abbreviations: cRNA, complementary RNA; MDCK, Madin–Darby canine kidney; HA, hemagglutinin; NA, neuraminidase; NP, nucleoprotein; pfu, plaque-forming units; RNP, ribonucleoprotein complex; VLP, virus-like particle; vRNA, viral RNA.

A Commentary on this article begins on page 8804.

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ORIGINAL ARTICLE

First infectious clone of the propagatively transmitted *Oat blue dwarf virus*

Michael C. Edwards · John J. Weiland

Received: 29 October 2009 / Accepted: 14 January 2010 / Published online: 12 March 2010

Abstract *Oat blue dwarf virus* (OBDV) is a small, phloem-limited marafivirus that replicates in its leafhopper vector. We have developed complete cDNA clones of OBDV from which infectious transcripts may be derived—the first such clones for any propagatively transmitted plant virus. Prior to clone construction, the reported sequences of

[7]. Longstanding members of this genus include *Maize rayado fino virus* (MRFV), *Oat blue dwarf virus* (OBDV), and *Bermuda grass etched-line virus* (BELV). These viruses have relatively narrow host ranges, infecting plants primarily in the Poaceae, although OBDV also infects flax [3]. For decades, these were the only known marafiviruses. Recently, virus—*dden death-associated virus* was shown alysis to be a likely new member of the and was formally approved as a member

Arch Virol (2010) 155:1923–1931
DOI 10.1007/s00705-010-0776-z

ORIGINAL ARTICLE

Construction and characterization of a full-length infectious c from a fast-replicating, X4-tropic HIV-1 subtype B′ isolate

Shou-li Wu · Yan-sheng Yan · Ping-ping Yan · Hai-long Huang · Hui-rong Wang

Received: 15 December 2009 / Accepted: 26 July 2010 / Published online: 10 August 2010

Abstract In HIV-1 epidemics in China, HIV-1 subtype B′ is the most predominant subtype circulating in intravenous drug users. In this study, we constructed an HIV-1 full-length infectious molecular clone based on the primary virus LWJ, which was isolated from an HIV-infected patient in Fujian Province, China. Phylogenetic and bootscanning analysis of the viral sequence revealed that the isolate LWJ belonged to HIV-1 subtype B′. The infectious clone was designated as "pLWJ". The virus (LWJ-c) produced from this infectious clone by in vitro transfection of 293T cells could infect both human peripheral blood mononuclear cells (PBMCs) and human the T cell line MT4. Interestingly, the cloned LWJ-c virus utilized CXCR4 as its co-receptor and could replicate in vitro with similar efficiency and kinetics compared to its parental primary isolate LWJ as well as the clade B reference virus NL4-3. The LWJ-c virus could also cause cytopathic effects in both PBMCs and MT cells. Sequence analysis of the envelope glycoprotein of pLWJ showed that a conserved GPGR motif and an arginine at position 11 were present in the V3 loop, which was consistent with previous reports regarding CXCR4 co-receptor usage and syncytium-inducing (SI) phenotype. Thus, the infectious clone

represents a fast-replicating, high-produ pic and syncytium-inducing isolate. Gi of HIV-1 subtype B′ in China, this infect very useful tool to provide a versatile m research focusing on the biological subtype.

Introduction

The genomic sequences of human immu type 1 (HIV-1) show high levels of phylogenetic analysis of the *env* and *gs* indicates there are three distinct groups the virus circulating worldwide. Within there are presently nine distinct subtypes K) and 34 circulating recombinant forms first case of HIV-1 infection was repo 1985 [1]. HIV/AIDS has spread rapid country and has become an increasingly to China. By the end of 2009, there w 740,000 individuals being infected w country. The current major prevalent H China are B′ (Thai-B) (44%) and the ci nant forms (CRFs) CRF08_BC (29%) (13%) [2].

The HIV-1 subtype B′ strain was from injected-drug users in Bangkok, Although the sequences of the envelope from those isolates clustered phylogeneti B Env, they were genetically distinct fr B viruses [3], and subsequently termed T HIV-1 subtype B′ viruses were not only but also quickly spread to other Asian China, Malaysia and Japan, and have

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Virus Research 155 (2011) 61–68

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Virus Research

journal homepage: www.elsevier.com/locate/virusres

Construction of an infectious cDNA clone for Omsk hemorrhagic fever virus, and characterization of mutations in NS2A and NS5

Kentaro Yoshii [a,b,*], Manabu Igarashi [c], Kimihito Ito [c], Hiroaki Kariwa [a], Michael R. Holbrook [b,d], Ikuo Takashima [a]

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Flavivirus
Infectious cDNA
Viral replication

ABSTRACT

Omsk hemorrhagic fever virus
viruses, and causes hemorrha
constructed to investigate the r
Our cDNA clone was capable of
Guriev strain, and the recombina
ing growth kinetics and virulen
at NS2A position 46 and NS5 po
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and that the NS5 mutation ind
these residues in RNA replicatio

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Virology 401 (2010

Contents lists available

Virolo

journal homepage: www.els

Complete genomic sequence and an infectious

S.H. Sheldon Tai [a], Masahiro Niikura [b,c,1], Hans H. Cheng
Annabel G. Wise [d,e], Roger K. Maes [a,b,e,#]

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FHV-1
Genomic sequence
Infectious BAC clone

ABSTRACT

Infection with feline herpesviru
Felidae. We report the first com
terization of a bacterial artificia
genome and has the BAC vector
from both the FHV-1 BAC clone
overall G + C content of 45%. A to
The gene arrangement is colline
the BAC was very similar to the
characteristics and highly virulen

Introduction

Feline herpesvirus-1 (FHV-1) is a significant viral pathogen of Felidae, first isolated in 1957 by Crandell and Maurer (1958). FHV-1 accounts for approximately 50% of all diagnosed viral upper respiratory infections in cats and is also a significant cause of ocular lesions (Nasisse, 1990). The pathobiology of FHV-1 has been reviewed (Gaskell et al., 2007; Maggs, 2005; Stiles, 2003). Briefly, following entry via the oronasal route, FHV-1 replicates extensively in the mucosae of the upper respiratory tract resulting in high fever, depression, anorexia, sneezing, conjunctivitis, keratitis, and ocular and nasal discharge. The acute phase of the disease is followed by lifelong latency, a hallmark of herpesvirus infections. During the latent stage, viral DNA appears to persist mainly in sensory ganglia. Different biological stresses, or administration of corticosteroids, can induce the necessary biochemical stimuli in latently infected cells that lead to renewed production of infectious virus, which can travel to the periphery and is a potential source of viral transmission. I also a contributor to feline cl 2005). Despite its high morbi available vaccines cannot to and, as a consequence, from fi Tham and Studdert, 1987). are avirulent when adminis administered oronasally (Kn tivation of latent FHV-1 is co tomatic carriers and contrib and Povey, 1982).

Our knowledge of FHV- FHV-1 is a member of *Herp* family, genus *Varicellovirus* appear to be relatively simil genome, mapped by restric is approximately 134 kb, an other varicelloviruses (Fig. consists of two segments of (U_L) and unique short (U_S). tical but inverted repeat se (TR_S) and inverted repeat s 1986). Alphaherpesviruses u (ORFs) (Alba et al., 2001). Fi associated proteins (Fargea previously been identified a

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E-mail address: Maes@dcpah.msu.edu (R.K. Maes).
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0042-6822/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
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Construction of an infectious cDNA clone of avian hepatitis E virus (avian HEV) recovered from a clinically healthy chicken in the United States and characterization of its pathogenicity in specific-pathogen-free chickens

Hyuk Moo Kwon [a,b], **Tanya LeRoith** [a], **R.S. Pudupakam** [a], **F. William Pierson** [a], **Yao-Wei Huang** [a], **Barbara A. Dryman** [a], and **Xiang-Jin Meng** [a,*]

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[b] Department of Veterinary Microbiology, College of Veterinary Medicine, Kangwon National University, Chuncheon, Gangwon 200-701, Republic of Korea

Abstract

A genetically distinct strain of avian hepatitis E virus (avian HEV-VA strain) was isolated from a healthy chicken in Virginia, and thus it is important to characterize and compare its pathogenicity with the prototype strain (avian HEV-prototype) isolated from a diseased chicken. Here we first constructed an infectious clone of the avian HEV-VA strain. Capped RNA transcripts from the avian HEV-VA clone were replication-competent after transfection of LMH chicken liver cells. Chickens inoculated intrahepatically with RNA transcripts of avian HEV-VA clone developed active infection as evidenced by fecal virus shedding, viremia, and seroconversion. To characterize the pathogenicity, RNA transcripts of both avian HEV-VA and avian HEV-prototype clones were intrahepatically inoculated into the livers of chickens. Avian HEV RNA was detected in feces, serum and bile samples from 10/10 avian HEV-VA-inoculated and 9/9 avian HEV-prototype-inoculated chickens although seroconversion occurred only some chickens during the experimental period. The histopathological lesion scores were lower for avian HEV-VA group than avian HEV-prototype group in the liver at 3 and 5 weeks post-inoculation (wpi) and in the spleen at 3 wpi, although the differences were not statistically significant. The liver/body weight ratio, indicative of liver enlargement, of both avian HEV-VA and avian HEV-prototype groups were significantly higher than that of the control group at 5 wpi. Overall, the avian HEV-VA strain still induces histological liver lesions even though it was isolated from a healthy chicken. The results also showed that intrahepatic inoculation of chickens with RNA transcripts of avian HEV infectious clone may serve as an alternative for live virus in animal pathogenicity studies.

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Veterinary Microbiology
Volume 142, Issues 1–2, 21 April 2010, Pages 3-12

Characterisation of a new infectious full-length cDNA clone of BVDV genotype 2 and generation of virus mutants

Katrin Mischkale [a], Ilona Reimann [b], J. Zemke [a], P. König [a], Martin Beer [a]

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https://doi.org/10.10

Abstract

Based on their g
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(v890FL). *In vitr*
type virus v890
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mutants with pa
(p890ΔNPro) or t
to generate pseu
helper cell line.
infectious full-le
further studies
recombinant BV

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 10, Number 3, 2010

DOI: 10.1089/vbz.2009.0067

Infection, Dissemination, and Transmission of a West Nile Virus Green Fluorescent Protein Infectious Clone by *Culex pipiens quinquefasciatus* Mosquitoes

Charles E. McGee, Alexandr V. Shustov, Konstantin Tsetsarkin, Ilya V. Frolov, Peter W. Mason, Dana L. Vanlandingham, and Stephen Higgs

Abstract

We report the construction and comparative characterization of a full-length West Nile virus (WNV) cDNA infectious clone (ic) that contains a green fluorescent protein (GFP) expression cassette fused within the viral open reading frame. Virus derived from WNV-GFP ic stably infected *Culex pipiens quinquefasciatus* mosquitoes at comparable rates to virus derived from the parental (non-GFP) ic. However, insertion of this GFP cassette resulted in a temporal delay in *in vivo* replication kinetics and significantly decreased dissemination to head tissue. Consistent with previous reports of WNV-infected mosquito midguts, focal GFP expression was observed at 3 days post-infection (dpi), with the majority of posterior midgut epithelial cells being positive by 7 dpi. GFP foci were observed in one pair of salivary glands (1/15) dissected 14 dpi. Mice exposed to WNV-GFP–infected mosquitoes developed viremia, and GFP was detected in lymph node homogenates. These data demonstrate the effectiveness of our strategy to generate a replication competent construct with increased reporter gene stability that may be used to study early events in infection.

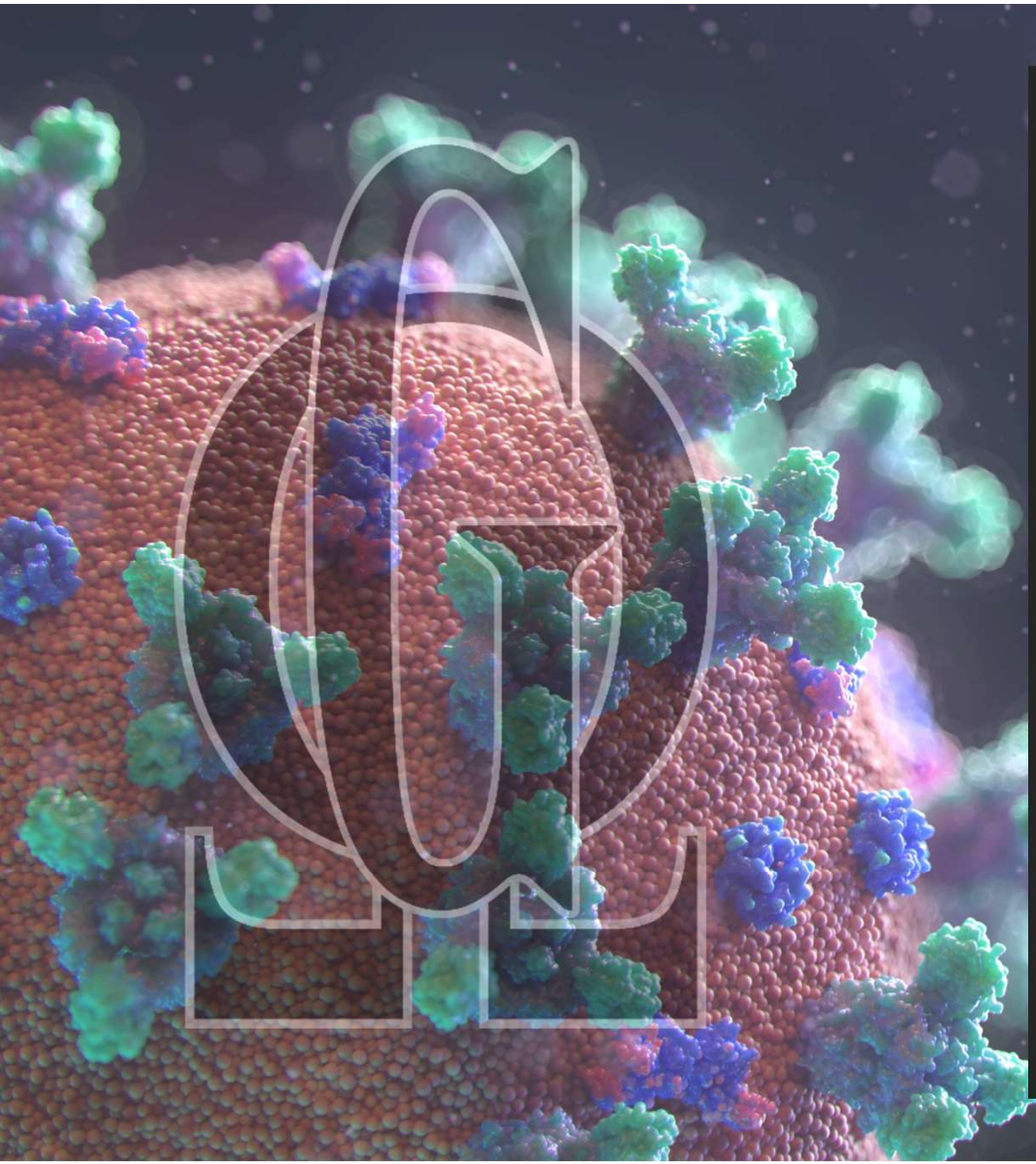
Key Words: Culex—Flaviviridae—Mosquito(es)—Transmission—West Nile.

Introduction

A NUMBER OF FLAVIVIRUS REVERSE GENETIC SYSTEMS have been developed and utilized for the study of basic viral genetics, *in vivo* interactions, and the advancement of vaccine technology (Rice et al. 1989, Lai et al. 1991, Sumiyoshi et al. 1995). Genes of interest (GOI) have been cloned into these recombinant genomes in proximity to the genomic 3′-end under the control of exogenous translation control elements, such as internal ribosomal entry sequences (Scholle et al. 2004, Pierson et al. 2005, Rossi et al. 2005). Further random mutagenesis studies have identified regions of the flavivirus genome that tolerate small GOI insertions. As a result, small epitopes derived from T cells, Rift Valley fever virus, Lassa fever virus, and Influenza A virus have been expressed via direct insertion into the flaviviral open reading frame (ORF) (Rice 1990, Tao et al. 2005, Bredenbeek et al. 2006, Pugachev et al. 2008). However, previous attempts to generate a full-length West Nile virus (WNV) construct with a fluorescent reporter gene using similar manipulations have been confounded by genetic instability and the rapid emergence of mutants with deleted GOI sequences (Pierson et al. 2005). In a recent study, Shustov et al. (2007) reported the development of a pseudoinfectious bipartite genome yellow fever virus 17D reverse genetic system that stably expressed GOI via insertion of a 5′ expression cassette into the genome-encoded polyprotein.

WNV is a mosquito-borne flavivirus that can infect the central nervous system of vertebrate hosts with potential for subsequent development of encephalitis, meningitis, meningoencephalitis, and poliomyelitis-like disease (www.cdc.gov/ncidod/dvbid/westnile). WNV is principally maintained in nature through transmission by ornithophilic *Culex* mosquitoes with amplification in susceptible avian hosts (Apperson et al. 2004). However, WNV has been recovered from numerous mosquito and tick species (Whitman and Aitken 1960, Karabatsos 1985, Abbassy et al. 1994, Anderson et al. 2003), and a number of nonconventional modes of infection have been identified (Granwehr et al. 2004, Higgs et al. 2005, McGee et al. 2007, Reisen et al. 2007). Like all of the members of the genus *flavivirus*, WNV possesses a positive-sense, single-stranded RNA genome containing a single ORF, which encodes three structural gene proteins (capsid,

Department of Pathology and Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas.



Unglossed cross-posted a post from PANDA Uncut

Brian Mowrey Feb 1 · Unglossed

Hudson articulates nearly perfectly why the idea of a functional "novel" virus is so outlandish: namely, the "job" of a virus is too complex and interconnected. He also goes further than I would in how to apply that premise to the evidence regarding SARS-CoV-2.

I have become convinced that the virus must be either 1) functional at injuring people*, implying successful biowarfare, good job Baric 2) somehow being directly deployed in high dose/purity.

Thus, I endorse Hudson's argument over JJ Couey's (which leans on an idea that no RNA viruses can sustain genetic fidelity, which isn't true) for why we should keep at least a grain of skepticism regarding viral spread vs vector delivery.

*Examples in my comment https://pandauncut.substack.com/p/the-bioweapons-bore/comment/12420689

The bioweapons bore

Are they wildly imaginative hyper-embroidered fairy tales?

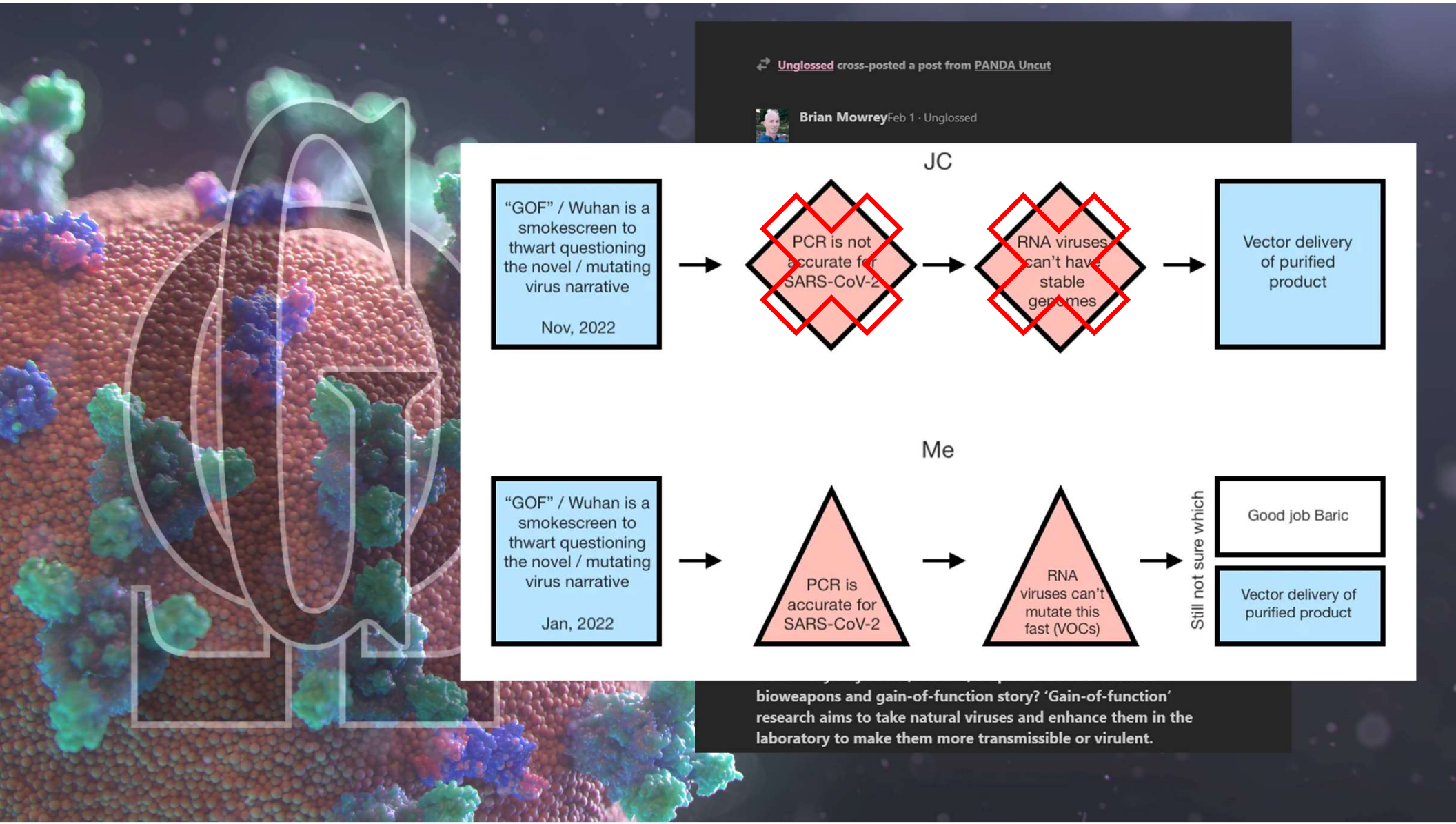
NICK HUDSON

FEB 1

CROSS-POST

Adapted from Nick Hudson's Twitter thread

How many of you are, like me, sceptical about the entire bioweapons and gain-of-function story? 'Gain-of-function' research aims to take natural viruses and enhance them in the laboratory to make them more transmissible or virulent.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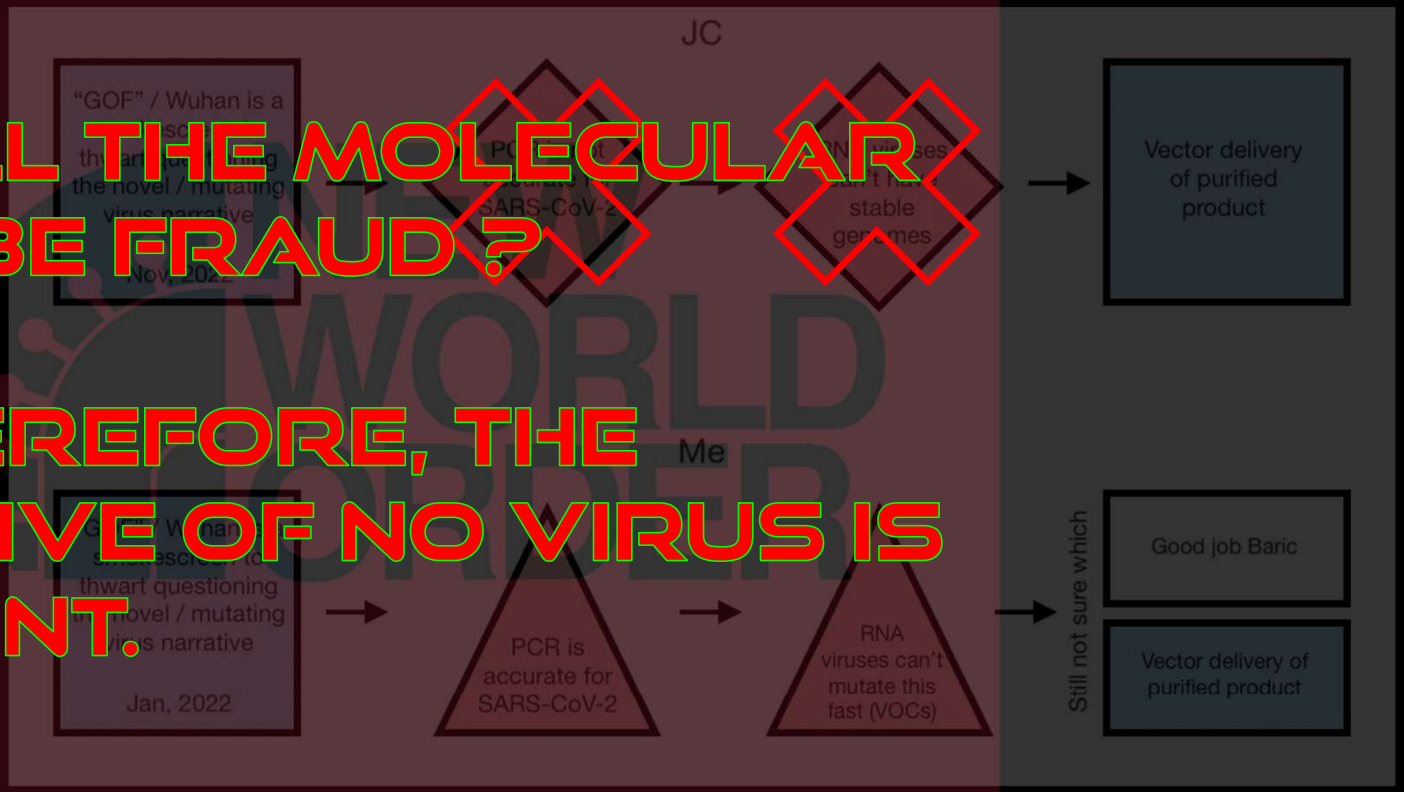
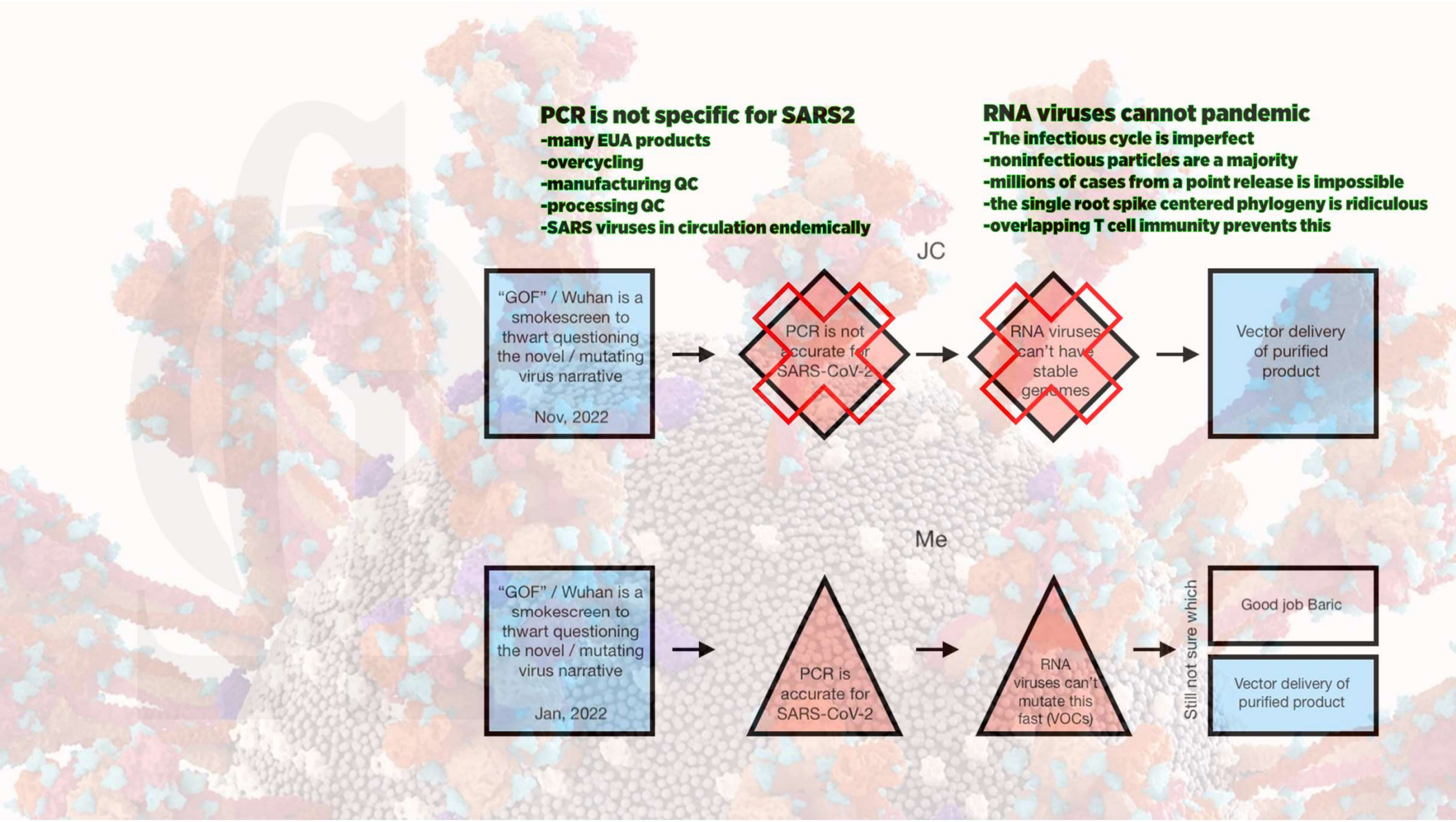
Brian MowreyFeb 1 · Unglossed
JC
"GOF" / Wuhan is a smokescreen to thwart questioning the novel / mutating virus narrative
Nov, 2022
PCR is not accurate for SARS-CoV-2
RNA viruses can't have stable genomes
Vector delivery of purified product
Me
"GOF" / Wuhan is a smokescreen to thwart questioning the novel / mutating virus narrative
Jan, 2022
PCR is accurate for SARS-CoV-2
RNA viruses can't mutate this fast (VOCs)
Still not sure which
Good job Baric
Vector delivery of purified product
bioweapons and gain-of-function story? 'Gain-of-function' research aims to take natural viruses and enhance them in the laboratory to make them more transmissible or virulent.

Q: CAN ALL THE MOLECULAR BIOLOGY BE FRAUD?

A: NO. THEREFORE, THE ALTERNATIVE OF NO VIRUS IS INSUFFICIENT.




PCR is not specific for SARS2
-many EUA products
-overcycling
-manufacturing QC
-processing QC
-SARS viruses in circulation endemically
RNA viruses cannot pandemic
-The infectious cycle is imperfect
-noninfectious particles are a majority
-millions of cases from a point release is impossible
-the single root spike centered phylogeny is ridiculous
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Gain of Function is a Mythology

- -thwarts questioning novel virus narrative
- -hides the widespread synthetic virus use
- -discourages speculation
- -provides a recyclable justification for policy
- -coerces citizens into compliance

PCR is not specific for SARS2

- -many EUA products
- -overcycling
- -manufacturing QC
- -processing QC
- -SARS viruses in circulation endemically

RNA viruses cannot pandemic

- -The infectious cycle is imperfect
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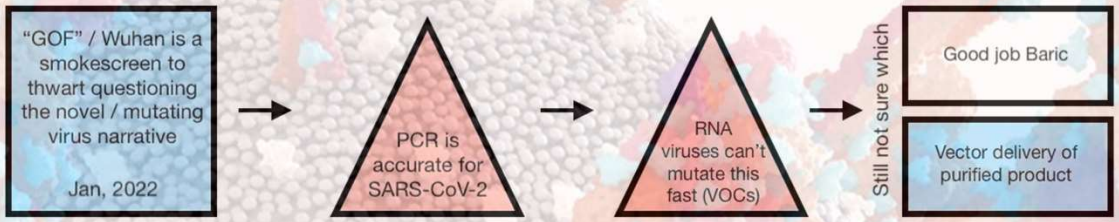
→ Vector delivery of purified product



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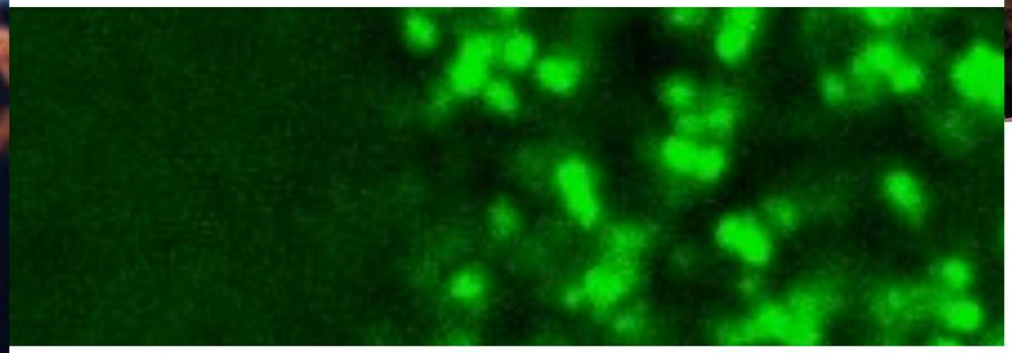


Research Focus

* miRNA sorting into exosomes
* Exosomes in (auto)immunity
* Exosome biogenesis

Curriculum Vitae

Michiel Pegtel obtained a master's degree in biology from Groningen University while trained by Prof J.J. Letterio in immunology/oncology at the National Cancer Institute (NCI/NIH). Michiel obtained his PhD degree from Tufts University in Boston in viral oncology supervised by Prof. D. Thorley-Lawson and in collaboration with Prof. T. Golub (MIT/Harvard). Next he moved to the Netherlands Cancer Institute (Amsterdam, The Netherlands) for post-doctoral training in cell polarity and cancer metastasis in the laboratory of Dr. J. Collard. Currently, Michiel is assistant professor at the VU university medical center (Amsterdam) leading investigations how and why cancerous cells release genetic material via small vesicles called exosomes. The aim of the laboratory is to translate this knowledge into practical methods for non-invasive cancer diagnosis.

EXOSOME RESEARCH GROUP

HOME RESEARCH TEAM AND STAFF MEDIA PUBLICATIONS VACANCIES CONTACT

Welcome to our website! Here we briefly introduce the projects we are currently running. We work in multi-disciplinary teams on various topics with exosome biology as underlying focus. Our translational studies include the role of exosomes in cancer biology, metastasis, mesenchymal stem cell biology, oncogenic herpesviruses, microRNAs, innate immunity and auto-immune diseases. We use innovative mouse models, advanced live-imaging techniques, spectral flow cytometry, next generation sequencing and proteomics to unravel the underpinnings of exosomes in health and disease with the aim to translate this knowledge into clinical applications.

Latest NEWS

29-05-2021
We are proud that **Maarten P Bebelman** won one of the ISEV 2022 award for outstanding oral presentation.

17-12-2021
Caitrin Crudden was awarded a Veni grant in collaboration with Prof. Dr. Martine Smit

01-12-2021
Rubina Baglio, **Caitrin Crudden** and **Cristina Gómez** (last two as head applicant) projects were funded from the Stitching CCA!

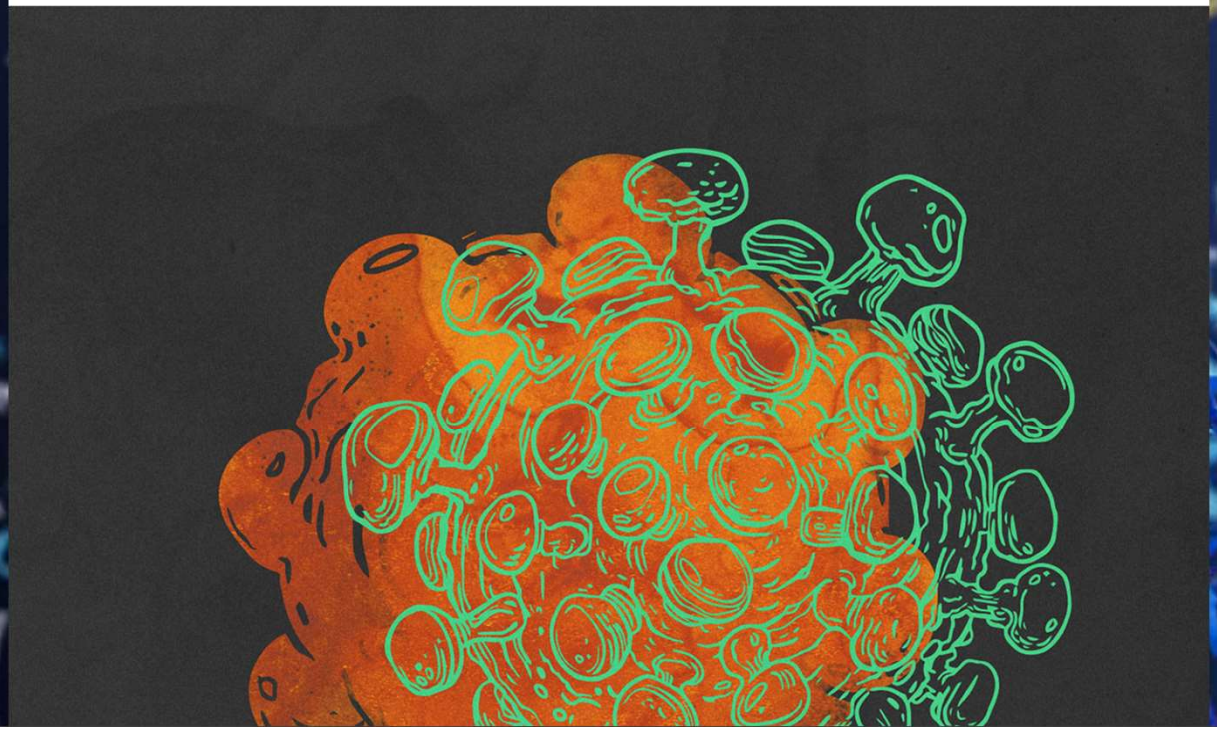
https://www.quantamagazine.org/cells-talk-in-a-language-that-looks-like-viruses-20180502/

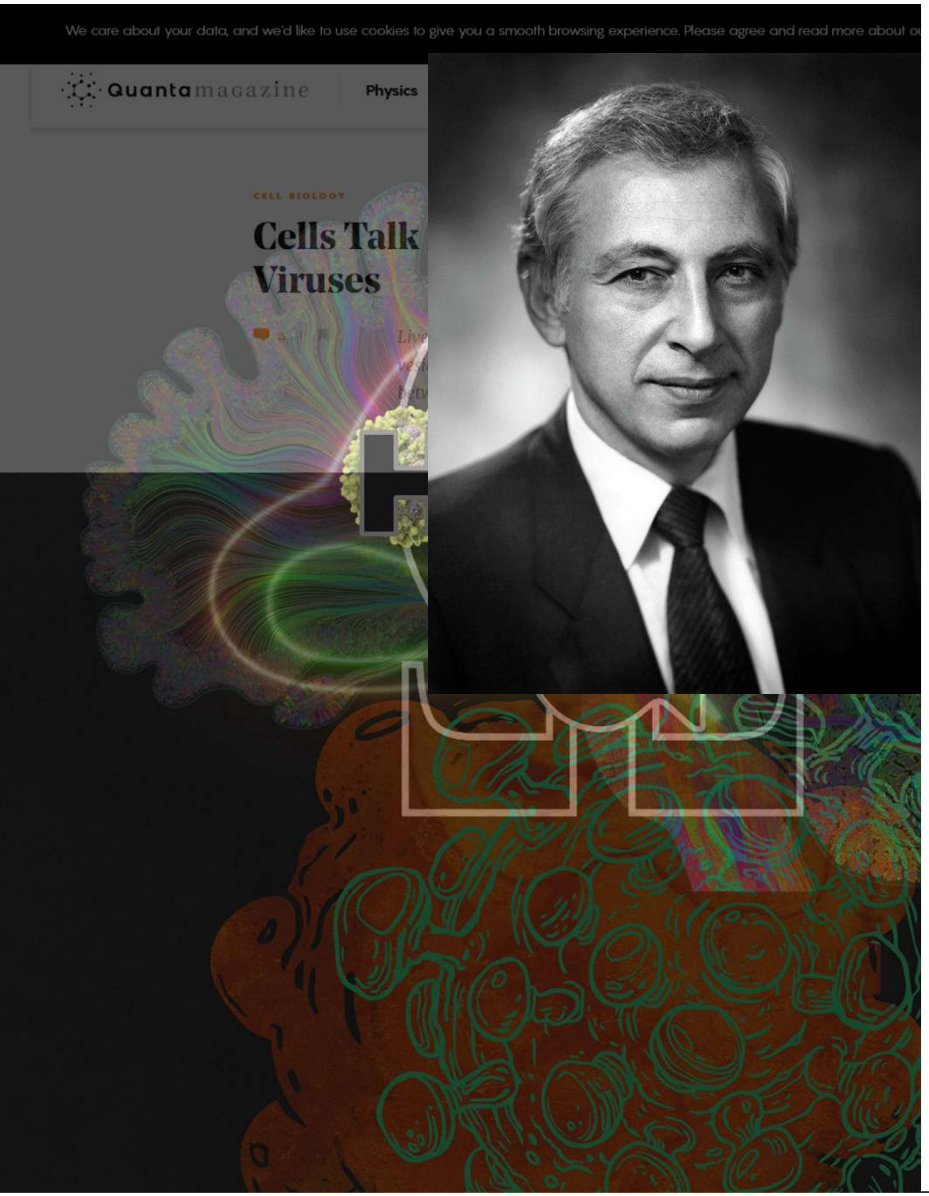
CELL BIOLOGY

Cells Talk in a Language That Looks Like Viruses

3

Live viruses may seem completely different from the message-carrying vesicles that cells release. But a vast population of particles intermediate between the two hints at their deep evolutionary connection.





Carrie Arnold

Contributing Writer

May 2, 2018

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biology | cell biology | viruses | All topics →

For cells, communication is a matter of life and death. The ability to tell other members of your species — or other parts of the body — that food supplies are running low or that an invading pathogen is near can be the difference between survival and extinction. Scientists have known for decades that cells can secrete chemicals into their surroundings, releasing a free-floating message for all to read. More recently, however, scientists discovered that cells could package their molecular information in what are known as extracellular vesicles. Like notes passed by children in class, the information packaged in an extracellular vesicle is folded and delivered to the recipient.

The past five years have seen an explosion of research into extracellular vesicles. As scientists uncovered the secrets about how the vesicles are made, how they package their information and how they're released, it became clear that there are powerful similarities between vesicles and viruses.

A small group of researchers, led by Leonid Margolis, a Russian-born virologist at the National Institute of Child Health and Human Development (NICHD), and Robert Gallo, the HIV pioneer at the University of Maryland School of Medicine, has proposed that this similarity is more than mere coincidence. It's not just that viruses appear to hijack the cellular pathways used to make extracellular vesicles for their own production — or that cells have also taken on some viral components to use in their vesicles. Extracellular vesicles and viruses, Margolis argues, are part of a continuum of membranous particles produced by cells. Between these two extremes are lipid-lined sacs filled with a variety of genetic material and proteins — some from hosts, some from viruses — that cells can use to send messages to one another.

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CELL BIOLOGY

Cells Talk in a Language That Looks Lik Viruses

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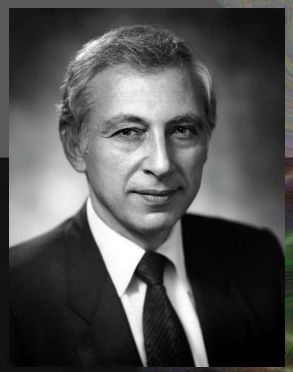


The Virus-Vesicle Connection

Viruses have similarities to the membranous vesicles that cells release for communication. Diverse particles that lack enough viral functionality to infect cells occupy a middle ground between those entities.

TWO CATEGORIES OF VESICLES:

A **Exosomes** are assembled within a cell before being released through its membrane.

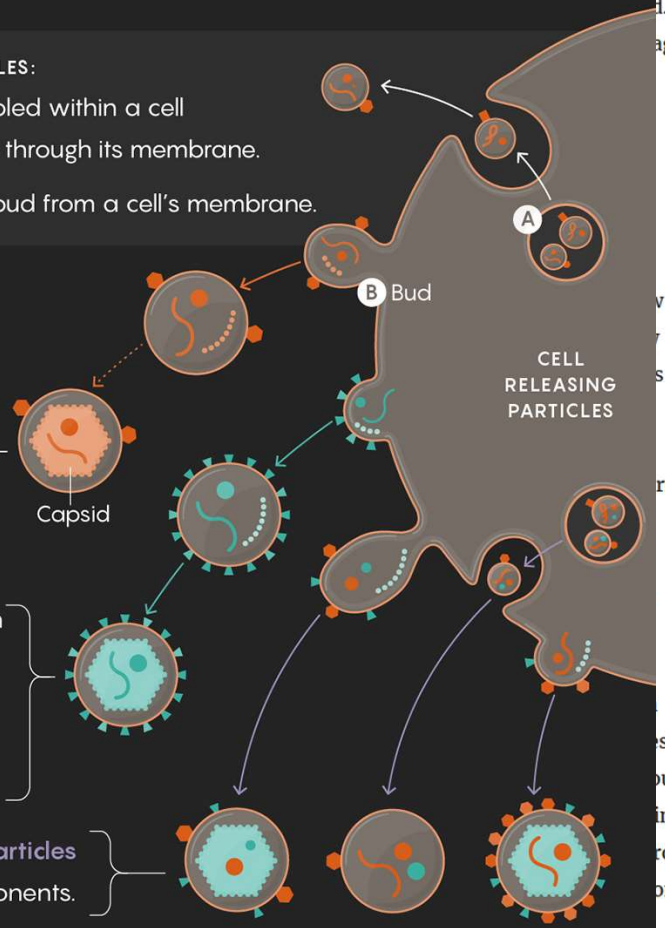
B **Extracellular vesicles** bud from a cell's membrane.

Shades of Difference:

Vesicles carry various RNAs and proteins, including some that resemble viral proteins and can form capsid structures.

Infectious viruses contain viral proteins and genes needed for replication, often wrapped in a cellular membrane.

Noninfectious viruslike particles lack essential viral components.

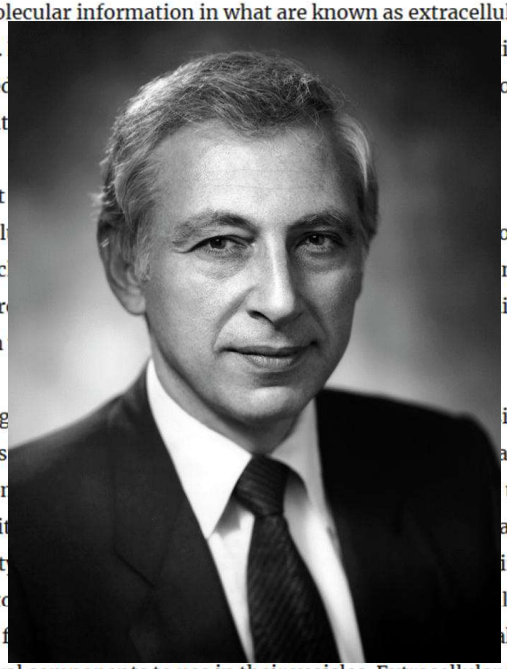


Carrie Arnold

Quanta Magazine

SIMONS FOUNDATION

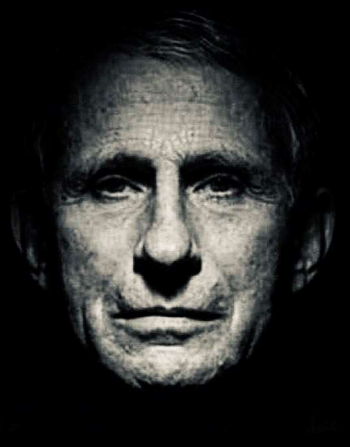
For cells, communication is a matter of life and death. The ability to tell other members of your species — or other parts of the body — that food supplies are running low or that an invading pathogen is near can be the difference between survival and extinction. Scientists have known for decades that cells can secrete chemicals into their surroundings, releasing a free-floating message for all to read. More recently, however, scientists discovered that cells could package their molecular information in what are known as extracellular vesicles. ion packaged o the recipient

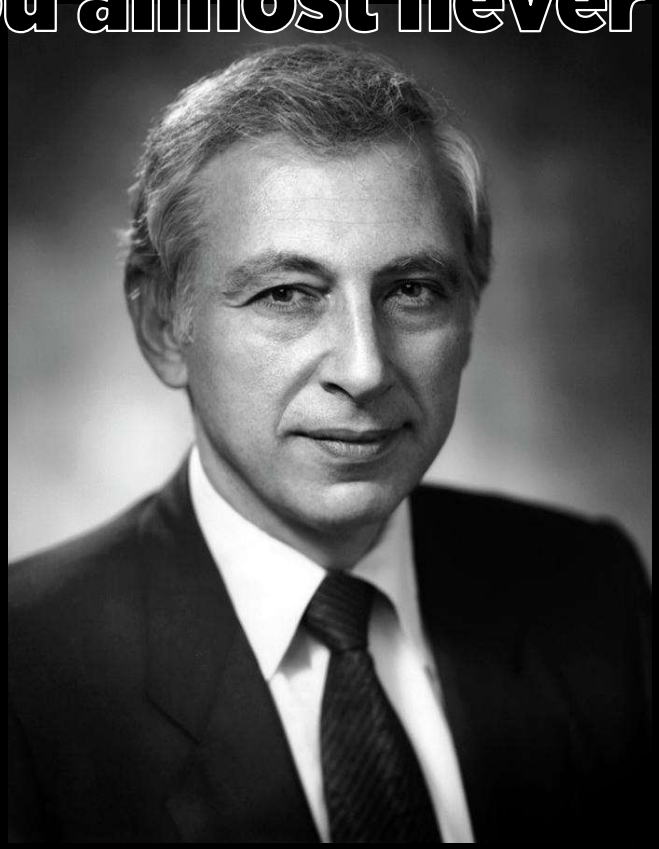


The past out how extracell the vesic nd how they're r ilarities between

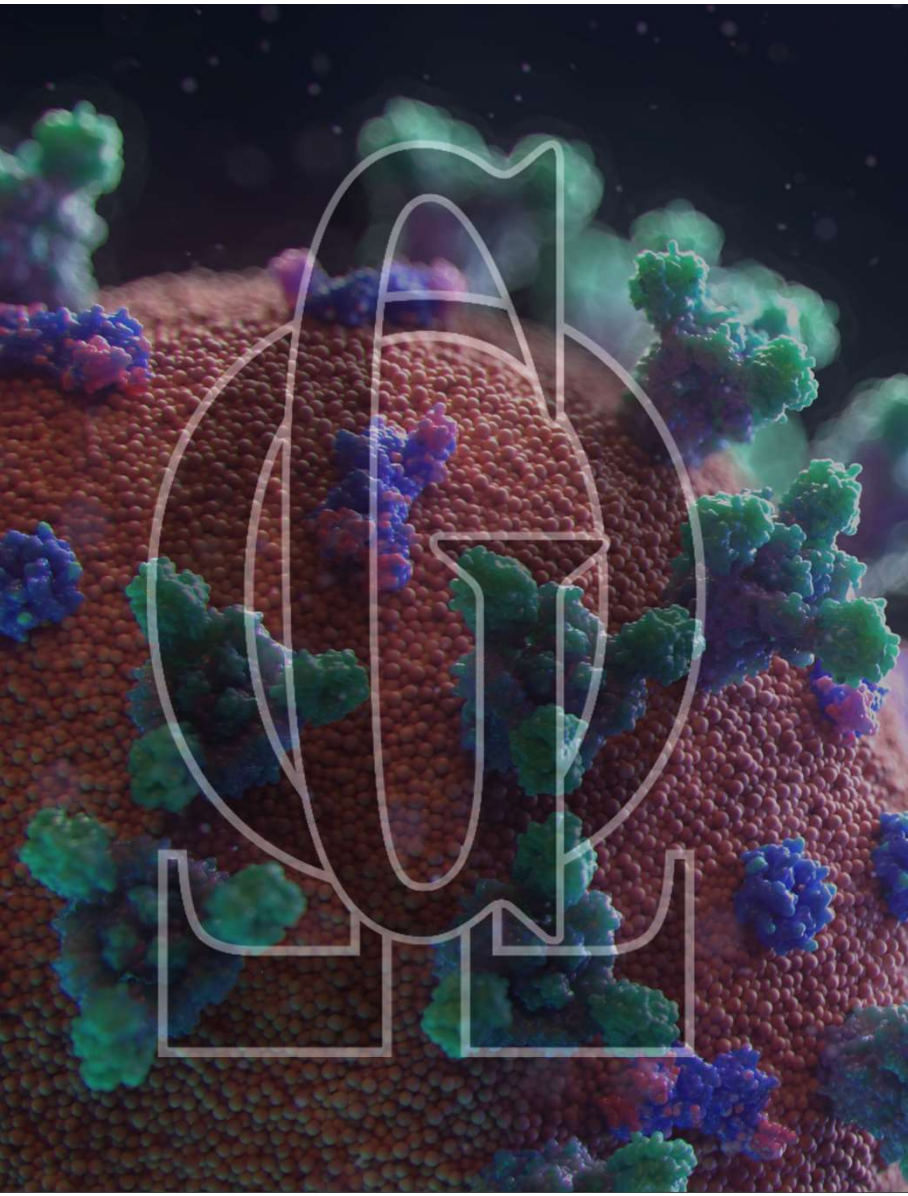
A small g ian-born virologis an Developr the Universit at this similarit iruses appear t lular vesicles f aken on some viral components to use in their vesicles. Extracellular vesicles and viruses, Margolis argues, are part of a continuum of membranous particles produced by cells. Between these two extremes are lipid-lined sacs filled with a variety of genetic material and proteins — some from hosts, some from viruses — that cells can use to send messages to one another.

Once you give a charlatan power over you, you almost never get it back.





Gigaohm Biological
High Resistance
Low Noise
Information Brief
8 February 2023



2020 Origin of the virus: Likely a lab leak
2023 Origin of the virus: Scooby Doo (Lab Leak Drama)

2020 Spike Protein: Kevin and others say it's toxic; not so sure
2023 Spike Protein: Might have been relevant in the clone release, very relevant for transfections

2020 Situation Estimate: Seems like a cover-up
Early Treatment: Are they suppressing to increase effect?
NPI Stance: Masks cannot work for a CoV

2023 Situation Estimate: Ongoing Scooby Doo (Lab Leak Drama)
Early Treatment: Protocol changes killed majority
NPI Stance: Sovereign rights violation

2020 Position on Transfection: Cannot work as expected
2023 Position on Transfection: Did not work; likely harmed many

2020 Position on Childhood Vaccination Schedule USA: Fantastic
2023 Position on CVS-USA: Holy crap, what was I thinking?

Antibody Position: *THE* #1 main bamboozle
PCR Testing: Works, but not specific as claimed
Virus versus stupid: Nearly all scripted stupid
Swarm Position : Part of the biology that says CoVs can't pandemic
Clone Position: The methodology
GOF Position: A mythology that hides all of synthetic biology

2020 Origin of the virus: Zoonosis, most likely a market
2023 Origin of the virus: Zoonosis, most likely market, but maybe lab.

2020 Spike Protein: Huh?
2023 Spike Protein: Great choice for the mRNA target

2020 Situation Estimate: Natural Crisis (I told you so)
Early Treatment: Nothing available except Remdesivir
NPI Stance: Work when used right

2023 Situation Estimate: Success of System; Will Never End
Early Treatment: Pax and Mol
NPI Stance: Still work when used

2020 Position on Transfection: Must be better
2023 Position on Transfection: Saved Millions

2020 Position on Childhood Vaccination Schedule USA: Fantastic
2023 Position on CVS-USA: Fantastic; missing COVID mRNA

Antibody Position: *THE* correlate of immunity
PCR Testing: Works grrrrreat
Virus versus stupid: Virus
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Done, doing, must be done, could be bad

Robert F. Kennedy, Jr.

2020 Origin of the virus: Zoonosis, most likely a market
2023 Origin of the virus: GOF lab leak?

2020 Spike Protein: Huh?
2023 Spike Protein: Toxic?

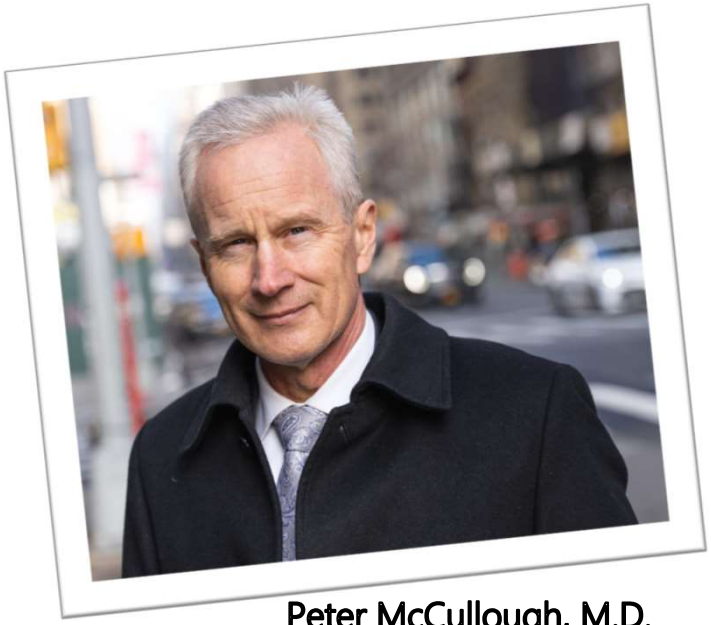
2020 Situation Estimate: Natural Crisis, I hope
Early Treatment: Doctors' choice
NPI Stance: Noticed the threat to sovereign rights as citizens

2023 Situation Estimate: Clusterfunk
Early Treatment: HCQ, IVM, and whatever else any doctor wants
NPI Stance: Sovereign rights of citizens were violated

2020 Position on Transfection: Aware this was an up and coming idea
2023 Position on Transfection: YIKES

2020 Position on Childhood Vaccination Schedule USA: YIKES
2023 Position on CVS-USA: YIKES

Antibody Position: *Knows this is a scam for all vaccines*
PCR Testing: Remembers AIDS
Virus versus stupid: Virus + Stupid
Swarm Position : Huh?
Clone Position: CD versus mixed tapes (kinda gets it)
GOF Position: Done, doing, must be done, could be this



Peter McCullough, M.D.

2020 Origin of the virus: Zoonosis, most likely a market
2023 Origin of the virus: GOF lab leak?

2020 Spike Protein: Huh?
2023 Spike Protein: Toxic?

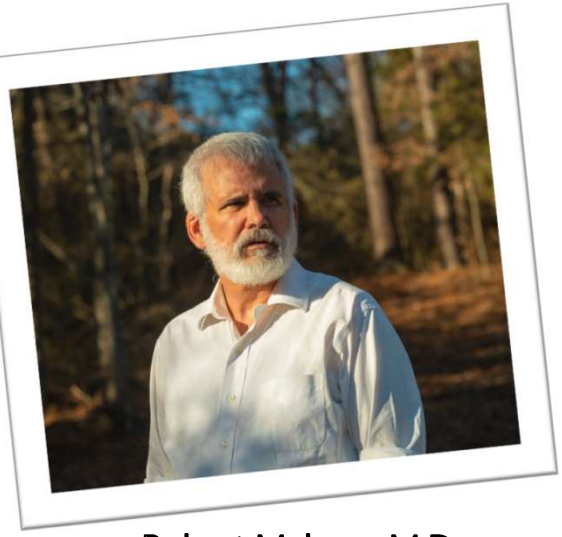
2020 Situation Estimate: Natural Crisis, I hope
Early Treatment: I am a doctor; whatever TF I want. HCQ works.
NPI Stance: Noticed the threat to sovereign rights as citizens

2023 Situation Estimate: Clusterfunk
Early Treatment: HCQ, IVM, and whatever else any doctor wants
NPI Stance: Sovereign rights of citizens were violated

2020 Position on Transfection: UNK
2023 Position on Transfection: YIKES

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Kinda*
PCR Testing: Was on first published objection
Virus versus stupid: Virus + Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Done, doing, must be done, could be this



Robert Malone, M.D.

2020 Origin of the virus: Zoonosis, most likely a market
2023 Origin of the virus: GOF lab leak

2020 Spike Protein: Huh?
2023 Spike Protein: Wrong target

2020 Situation Estimate: Natural Crisis, I hope
Early Treatment: I spun my DTRA team up after I got a call from Mike
NPI Stance: Followed all recommendations; transfected early

2023 Situation Estimate: Clusterfunk
Early Treatment: Wishy washy
NPI Stance: Sovereign rights of citizens were violated; 5gen war

2020 Position on Transfection: Wasn't on his resume AFAIK
2023 Position on Transfection: Claims he invented the tech. It isn't perfect but probably saved some more than zero

2020 Position on Childhood Vaccination Schedule USA: Fine
2023 Position on CVS-USA: Fine

Antibody Position: *Not the only thing, but worth talking about*
PCR Testing: Has no comment
Virus versus stupid: Virus >> Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Done, doing, must be done, resulted in this

Pierre Kory, M.D.

2020 Origin of the virus: Zoonosis, most likely a market
2023 Origin of the virus: GOF lab leak?

2020 Spike Protein: Huh?
2023 Spike Protein: Toxic?

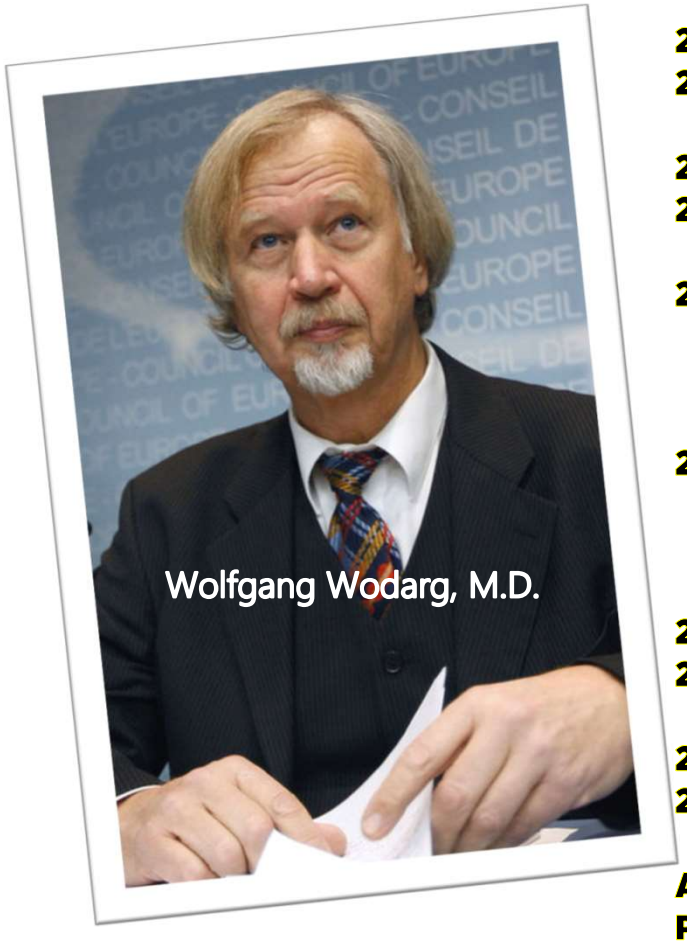
2020 Situation Estimate: Natural Crisis, I just want to treat patients. Went to NYC early
Early Treatment: Fought for it. Strong IVM advocate.
NPI Stance: UNK

2023 Situation Estimate: Clusterfunk
Early Treatment: HCQ, IVM, and whatever else any doctor wants
NPI Stance: Sovereign rights of citizens were violated

2020 Position on Transfection: UNK
2023 Position on Transfection: YIKES

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Kinda*
PCR Testing: UNK
Virus versus stupid: Virus + Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Done, doing, must be done, could be this



2020 Origin of the virus: Called it likely nonsense from day 1
2023 Origin of the virus: There are hundreds of unknown respiratory viruses

2020 Spike Protein: Huh?
2023 Spike Protein: Huh?

2020 Situation Estimate: Orchestrated WHO pandemic scheme
Early Treatment: Said we needed to do nothing different
NPI Stance: Lockdowns would not stop a pandemic

2023 Situation Estimate: Clusterfunk
Early Treatment: Should never have been forbidden
NPI Stance: Sovereign rights of citizens were violated

2020 Position on Transfection: UNK
2023 Position on Transfection: YIKES

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Didn't really buy it*
PCR Testing: UNK
Virus versus stupid: Probably just stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: UNK

2020 Origin of the virus: UNK
2023 Origin of the virus: UNK

2020 Spike Protein: UNK
2023 Spike Protein: UNK

2020 Situation Estimate: UNK
Early Treatment: UNK
NPI Stance: UNK

2023 Situation Estimate: Clusterfunk
Early Treatment: UNK
NPI Stance: UNK

2020 Position on Transfection: UNK
2023 Position on Transfection: YIKES; quality control issues

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *UNK*
PCR Testing: UNK
Virus versus stupid: Virus + Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: UNK




Andrew Kaufman, M.D.

2020 Origin of the virus: Plandemic
2023 Origin of the virus: Plandemic

2020 Spike Protein: UNK
2023 Spike Protein: UNK

2020 Situation Estimate: Plandemic
Early Treatment: UNK
NPI Stance: Violation of rights

2023 Situation Estimate: Plandemic
Early Treatment: UNK
NPI Stance: Violation of rights

2020 Position on Transfection: UNK
2023 Position on Transfection: Wishy washy?

2020 Position on Childhood Vaccination Schedule USA: UNK?
2023 Position on CVS-USA: UNK?

Antibody Position: *Do we even have an immune system?*
PCR Testing: Doesn't detect anything
Virus versus stupid: Just Planned Stupid
Swarm Position : There isn't one
Clone Position: Huh?
GOF Position: Fake

2020 Origin of the virus: Zoonosis or bushmeat like others, then lab leak
2023 Origin of the virus: Lab Leak


2020 Spike Protein: Altered
2023 Spike Protein: Toxic?

2020 Situation Estimate: Worldwide Emergency
Early Treatment: There are no treatments for a novel virus
NPI Stance: Bandanas work



2023 Situation Estimate: Clusterfunk
Early Treatment: HCQ a bit, IVM much more
NPI Stance: Doesn't really say much, obviously

2020 Position on Transfection: Thought it would work. Hoped?
2023 Position on Transfection: Harms were done, but might also work

2020 Position on Childhood Vaccination Schedule USA: Accepted as stood
2023 Position on CVS-USA: Unclear; Definitely not openly questioning

Antibody Position: *Has become aware of this myth*
PCR Testing: Unclear
Virus versus stupid: Virus or Viruses + Stupid
Swarm Position : Says he doesn't get it, even though evolution is swarms
Clone Position: Huh? I worked on telomeres!
GOF Position: Did it, doing it, this is from it

2020 Origin of the virus: Zoonosis?
2023 Origin of the virus: Lab Leak?

2020 Spike Protein: UNK
2023 Spike Protein: Toxic?

2020 Situation Estimate: Worldwide Emergency
Early Treatment: Gonna get me an EUA by calling Robert
NPI Stance: UNK

2023 Situation Estimate: Clusterfunk
Early Treatment: Unclear focus
NPI Stance: Doesn't really say much

2020 Position on Transfection: Thought it would work. Hoped?
2023 Position on Transfection: Thinks it was a mistake?

2020 Position on Childhood Vaccination Schedule USA: Accepted as stood
2023 Position on CVS-USA: Unclear; Definitely not openly questioning

Antibody Position: *Unclear*
PCR Testing: Unclear
Virus versus stupid: Virus > Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Did it, doing it, this is probably it

2020 Origin of the virus: Plandemic?
2023 Origin of the virus: Plandemic

2020 Spike Protein: Huh?
2023 Spike Protein: Huh?

2020 Situation Estimate: Plandemic
Early Treatment: Doctors' choice, duh?
NPI Stance: Sovereign rights violated by lockdown, etc.

2023 Situation Estimate: Plandemic
Early Treatment: Change in protocols killed majority
NPI Stance: Sovereign rights violated

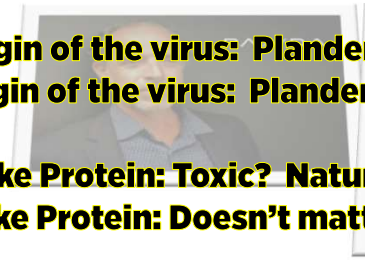
2020 Position on Transfection: UNK
2023 Position on Transfection: Thinks it was mistake

2020 Position on Childhood Vaccination Schedule USA: Accepted as stood
2023 Position on CVS-USA: Questions everything now

Antibody Position: *See the myth*
PCR Testing: False positives and background virus swarm
Virus versus stupid: Almost all Stupid
Swarm Position : Understands this idea doesn't help the narrative
Clone Position: Understands this idea is being ignored
GOF Position: Estimates this is a mythology

Michael Yeadon, Ph.D.

2020 Origin of the virus: Plandemic? Variants are BS
2023 Origin of the virus: Plandemic



2020 Spike Protein: Toxic? Natural Immunity Paramount
2023 Spike Protein: Doesn't matter anymore except in the POISON

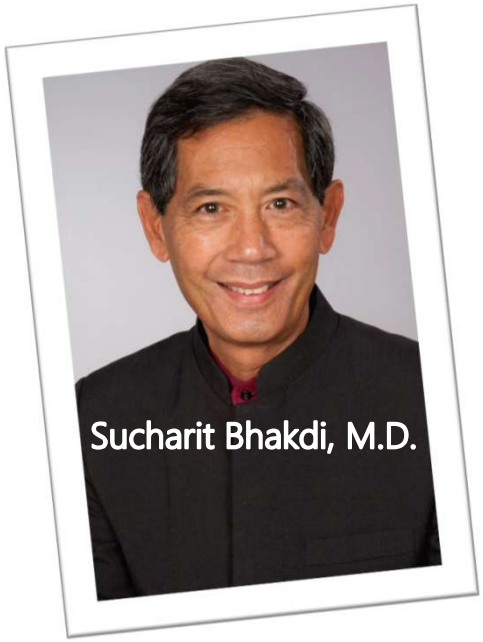
2020 Situation Estimate: Plandemic
Early Treatment: Doctors' choice, duh?
NPI Stance: Sovereign rights violated by lockdown, etc.

2023 Situation Estimate: Plandemic
Early Treatment: Change in protocols killed majority
NPI Stance: Sovereign rights violated

2020 Position on Transfection: As designed, it's a poison
2023 Position on Transfection: Thinks it is a depopulation technique

2020 Position on Childhood Vaccination Schedule USA: Accepted as stood
2023 Position on CVS-USA: Questions everything now

Antibody Position: *Sees the myth*
PCR Testing: Was on first paper calling it a fraud
Virus versus stupid: Just stupid
Swarm Position : UNK
Clone Position: UNK
GOF Position: UNK



2020 Origin of the virus: Plandemic?
2023 Origin of the virus: Plandemic

2020 Spike Protein: Huh? Don't need a vaccine for this virus
2023 Spike Protein: Transfection of spike causes endothelial damage, etc.

2020 Situation Estimate: Plandemic
Early Treatment: Doctors' choice, duh?
NPI Stance: Sovereign rights violated by lockdown, etc.

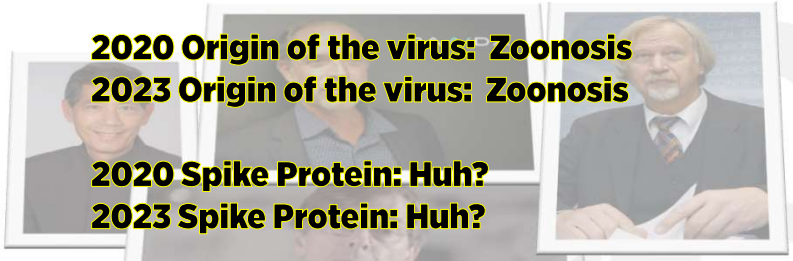
2023 Situation Estimate: Plandemic
Early Treatment: Change in protocols killed majority
NPI Stance: Sovereign rights violated

2020 Position on Transfection: UNK
2023 Position on Transfection: Terrible methodology; not immunization

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Sees the myth; discovered complement system*
PCR Testing: Was sure variants were mythology
Virus versus stupid: Just stupid
Swarm Position : UNK
Clone Position: UNK
GOF Position: UNK

Sam Harris, Ph.D.



2020 Origin of the virus: Zoonosis
2023 Origin of the virus: Zoonosis

2020 Spike Protein: Huh?
2023 Spike Protein: Huh?

2020 Situation Estimate: Crisis of Public Health and Leadership
Early Treatment: There are no early treatments for a NOVEL virus
NPI Stance: They worked when done right

2023 Situation Estimate: Crisis of belief in Science and misinformation
Early Treatment: Whatever they say, who am I?
NPI Stance: Probably need more of this in the future

2020 Position on Transfection: UNK
2023 Position on Transfection: Saved millions, hurt almost zero

2020 Position on Childhood Vaccination Schedule USA: Fine and Great
2023 Position on CVS-USA: Even better now probably

Antibody Position: *Doesn't understand basic immunology*
PCR Testing: They have done their job
Virus versus stupid: Virus + Anti-Science Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Have done, necessary, should continue?

Vinay Prasad, M.D.

2020 Origin of the virus: Zoonosis
2023 Origin of the virus: Zoonosis

2020 Spike Protein: Huh?
2023 Spike Protein: Huh?

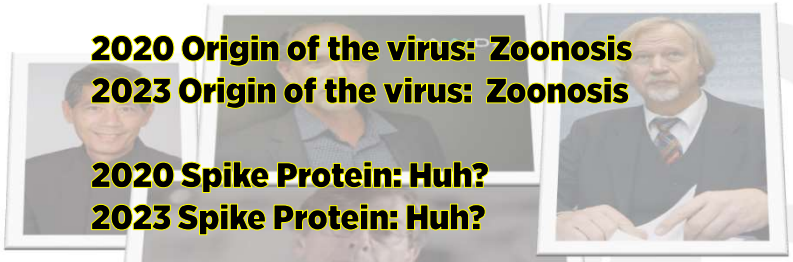
2020 Situation Estimate: Natural Crisis
Early Treatment: There are no early treatments for a NOVEL virus
NPI Stance: They worked when done right

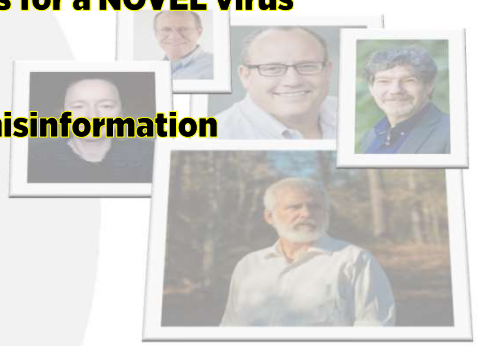
2023 Situation Estimate: Crisis of belief in Science and misinformation
Early Treatment: Unclear
NPI Stance: Realizes masks didn't work

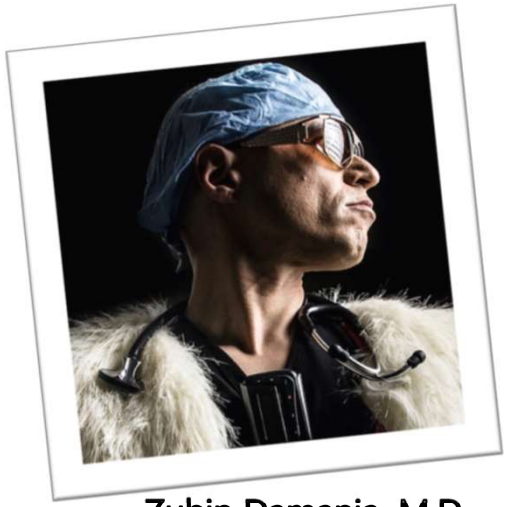
2020 Position on Transfection: UNK
2023 Position on Transfection: Unclear

2020 Position on Childhood Vaccination Schedule USA: Fine and Great
2023 Position on CVS-USA: Fine and Great?

Antibody Position: *Hasn't expressed any objection?*
PCR Testing: They have done their job
Virus versus stupid: Virus >> Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Have done, necessary, should continue?







Zubin Damania, M.D.

2020 Origin of the virus: Zoonosis
2023 Origin of the virus: Zoonosis

2020 Spike Protein: Huh?
2023 Spike Protein: Huh?

2020 Situation Estimate: Natural Crisis
Early Treatment: There are no early treatments for a NOVEL virus
NPI Stance: They worked when done right

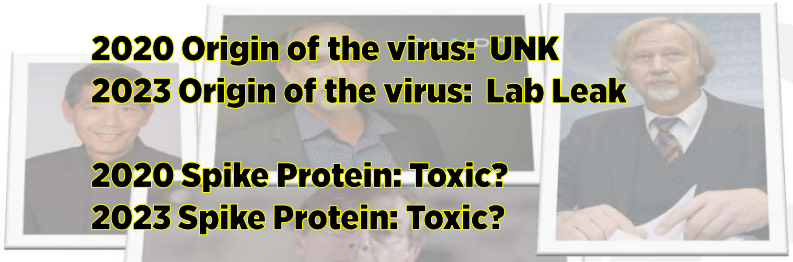
2023 Situation Estimate: Crisis of belief in Science and misinformation
Early Treatment: Unclear
NPI Stance: Unclear

2020 Position on Transfection: UNK
2023 Position on Transfection: Saved some old folks

2020 Position on Childhood Vaccination Schedule USA: Fine and Great
2023 Position on CVS-USA: Fine and Great

Antibody Position: *Hasn't expressed any objection?*
PCR Testing: They have done their job
Virus versus stupid: Virus >> Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Have done, necessary, should continue

Jessica Rose, Ph.D.



2020 Origin of the virus: UNK
2023 Origin of the virus: Lab Leak

2020 Spike Protein: Toxic?
2023 Spike Protein: Toxic?

2020 Situation Estimate: Natural Crisis?
Early Treatment: UNK
NPI Stance: UNK

2023 Situation Estimate: Plandemic
Early Treatment: Unclear
NPI Stance: Unclear

2020 Position on Transfection: UNK
2023 Position on Transfection: Bad idea for 1000 reasons

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Has expressed awareness of T cells, natural immunity*
PCR Testing: Unclear
Virus versus stupid: Virus + Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Have done, is likely the cause of pandemic

Kevin McCairn, Ph.D.

2020 Origin of the virus: Lab Leak
2023 Origin of the virus: Lab Leak or Intentional

2020 Spike Protein: Toxic
2023 Spike Protein: Toxic

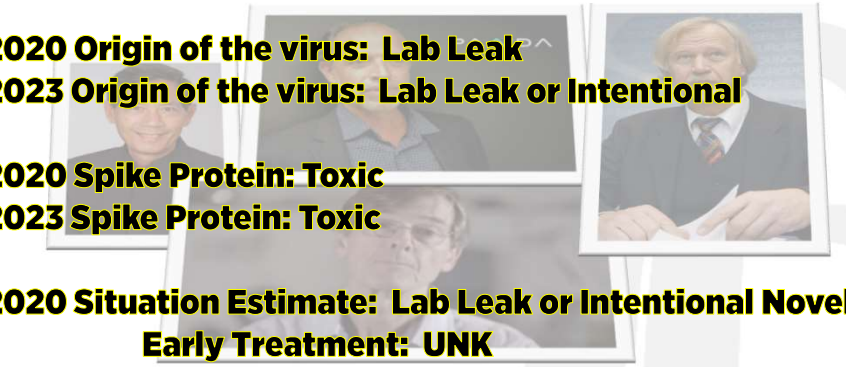
2020 Situation Estimate: Lab Leak or Intentional Novel Virus
Early Treatment: UNK
NPI Stance: Lockdowns and masks work

2023 Situation Estimate: 5th generation warfare
Early Treatment: Unclear
NPI Stance: Unclear

2020 Position on Transfection: UNK
2023 Position on Transfection: Bioweapon

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Has expressed awareness of T cells, natural immunity*
PCR Testing: Seems to work?
Virus versus stupid: Virus >> Stupid
Swarm Position : Doesn't matter
Clone Position: GOF
GOF Position: Have done, is likely the cause of intentional EcoHealth pandemic



Charles Rixey MA, MBA

2020 Origin of the virus: UNK
2023 Origin of the virus: Lab Leak or Intentional

2020 Spike Protein: UNK
2023 Spike Protein: Toxic designer protein

2020 Situation Estimate: Lab Leak or Intentional Novel Virus
Early Treatment: Opposed the control of early treatments?
NPI Stance: Lockdowns and masks work?

2023 Situation Estimate: 5th generation warfare
Early Treatment: Unclear
NPI Stance: Unclear

2020 Position on Transfection: UNK
2023 Position on Transfection: Bioweapon

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Has expressed awareness of T cells, natural immunity*
PCR Testing: Seems to work?
Virus versus stupid: Virus >> Stupid
Swarm Position : Doesn't matter
Clone Position: GOF
GOF Position: Have done, is likely the cause of intentional EcoHealth pandemic

Andrew Huff, Ph.D.

2020 Origin of the virus: UNK
2023 Origin of the virus: Lab Leak or Intentional

2020 Spike Protein: UNK
2023 Spike Protein: Toxic designer protein

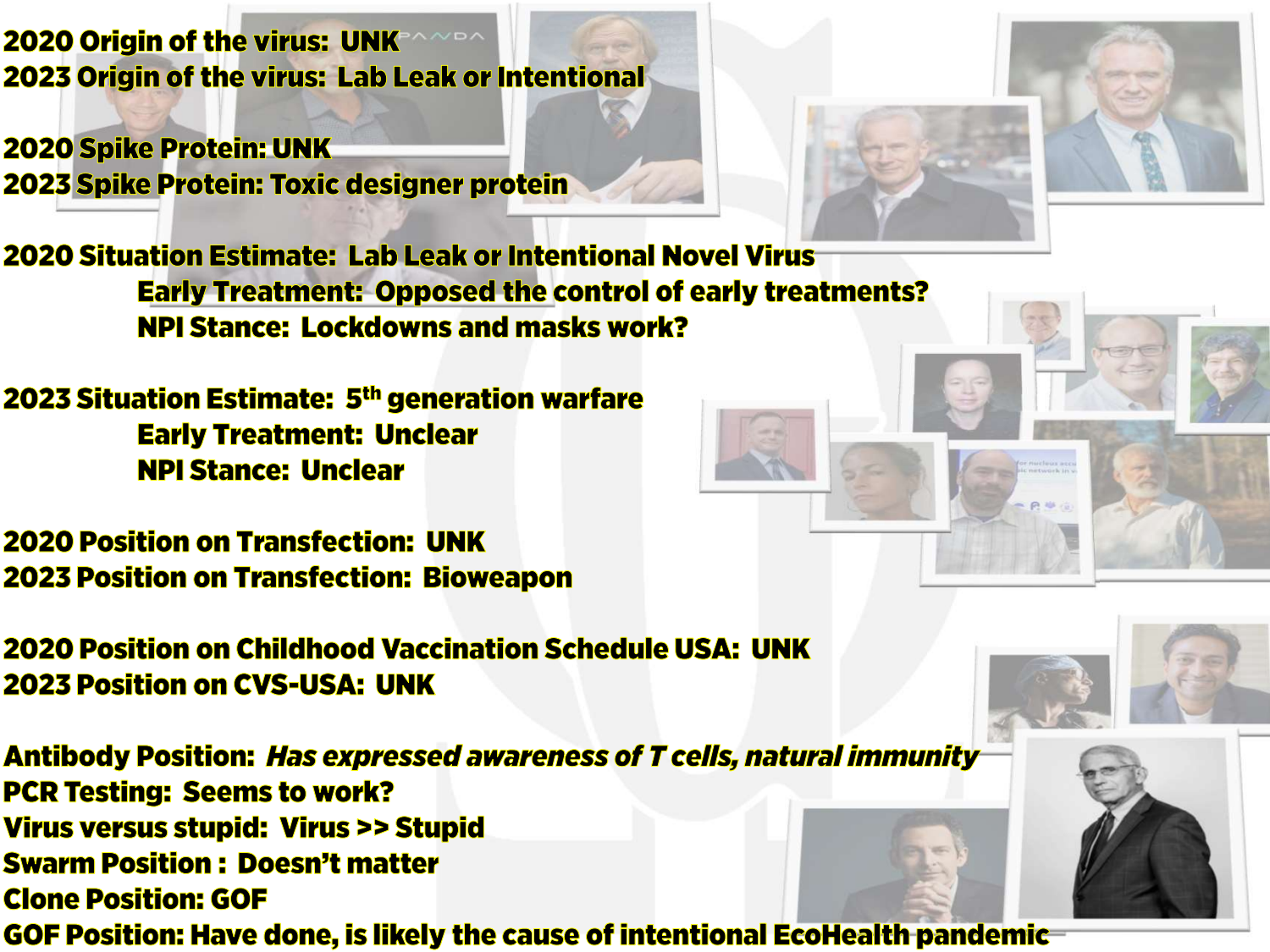
2020 Situation Estimate: Lab Leak or Intentional Novel Virus
Early Treatment: Opposed the control of early treatments?
NPI Stance: Lockdowns and masks work?

2023 Situation Estimate: 5th generation warfare
Early Treatment: Unclear
NPI Stance: Unclear

2020 Position on Transfection: UNK
2023 Position on Transfection: Bioweapon

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Has expressed awareness of T cells, natural immunity*
PCR Testing: Seems to work?
Virus versus stupid: Virus >> Stupid
Swarm Position : Doesn't matter
Clone Position: GOF
GOF Position: Have done, is likely the cause of intentional EcoHealth pandemic



Ryan Cole, M.D.

2020 Origin of the virus: UNK
2023 Origin of the virus: Lab Leak or Intentional

2020 Spike Protein: UNK
2023 Spike Protein: Toxic designer protein

2020 Situation Estimate: UNK
Early Treatment: Opposed the control of early treatments
NPI Stance: Against lockdown and masks

2023 Situation Estimate: Clusterfunk
Early Treatment: Opposes control of doctors, period
NPI Stance: Don't work

2020 Position on Transfection: UNK
2023 Position on Transfection: Terrible idea

2020 Position on Childhood Vaccination Schedule USA: UNK
2023 Position on CVS-USA: UNK

Antibody Position: *Early advocate for natural previous immunity*
PCR Testing: Seems to work?
Virus versus stupid: Virus + Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Have done, possible source of pandemic

Geert Vandenbossche, Ph.D., D.V.M.

2020 Origin of the virus: UNK
2023 Origin of the virus: Natural or Lab leak?

2020 Spike Protein: UNK
2023 Spike Protein: Spike has non-specific T cell epitopes in it

2020 Situation Estimate: UNK
Early Treatment: UNK
NPI Stance: UNK

2023 Situation Estimate: Disaster of variant generation
Early Treatment: Wishy washy
NPI Stance: Unclear

2020 Position on Transfection: Aware of it as a methodology
2023 Position on Transfection: Works but not during a pandemic

2020 Position on Childhood Vaccination Schedule USA: Fine
2023 Position on CVS-USA: Fine

Antibody Position: *Antibodies are important for sure; no T cell memory here*
PCR Testing: Works
Virus versus stupid: Virus >> Stupid
Swarm Position : Huh?
Clone Position: Huh?
GOF Position: Have done, possible source of pandemic

Denis Rancourt, Ph.D.

2020 Origin of the virus: UNK
2023 Origin of the virus: Skeptical of there being a significant virus

2020 Spike Protein: UNK
2023 Spike Protein: UNK

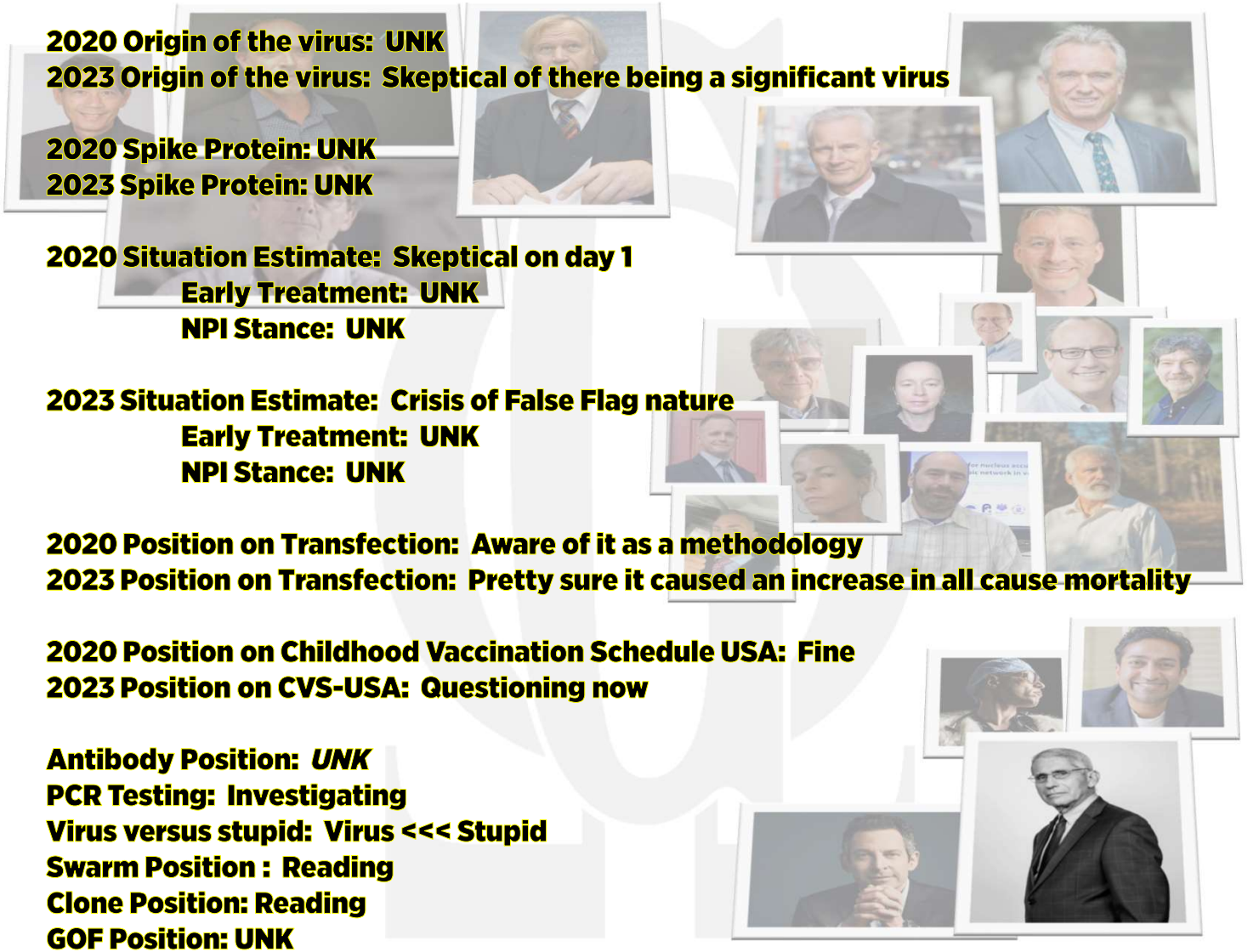
2020 Situation Estimate: Skeptical on day 1
Early Treatment: UNK
NPI Stance: UNK

2023 Situation Estimate: Crisis of False Flag nature
Early Treatment: UNK
NPI Stance: UNK

2020 Position on Transfection: Aware of it as a methodology
2023 Position on Transfection: Pretty sure it caused an increase in all cause mortality

2020 Position on Childhood Vaccination Schedule USA: Fine
2023 Position on CVS-USA: Questioning now

Antibody Position: *UNK*
PCR Testing: Investigating
Virus versus stupid: Virus <<< Stupid
Swarm Position : Reading
Clone Position: Reading
GOF Position: UNK



Vladimir Zelenko, M.D.

2020 Origin of the virus: UNK
2023 Origin of the virus: Passed in 2022

2020 Spike Protein: UNK
2023 Spike Protein: UNK

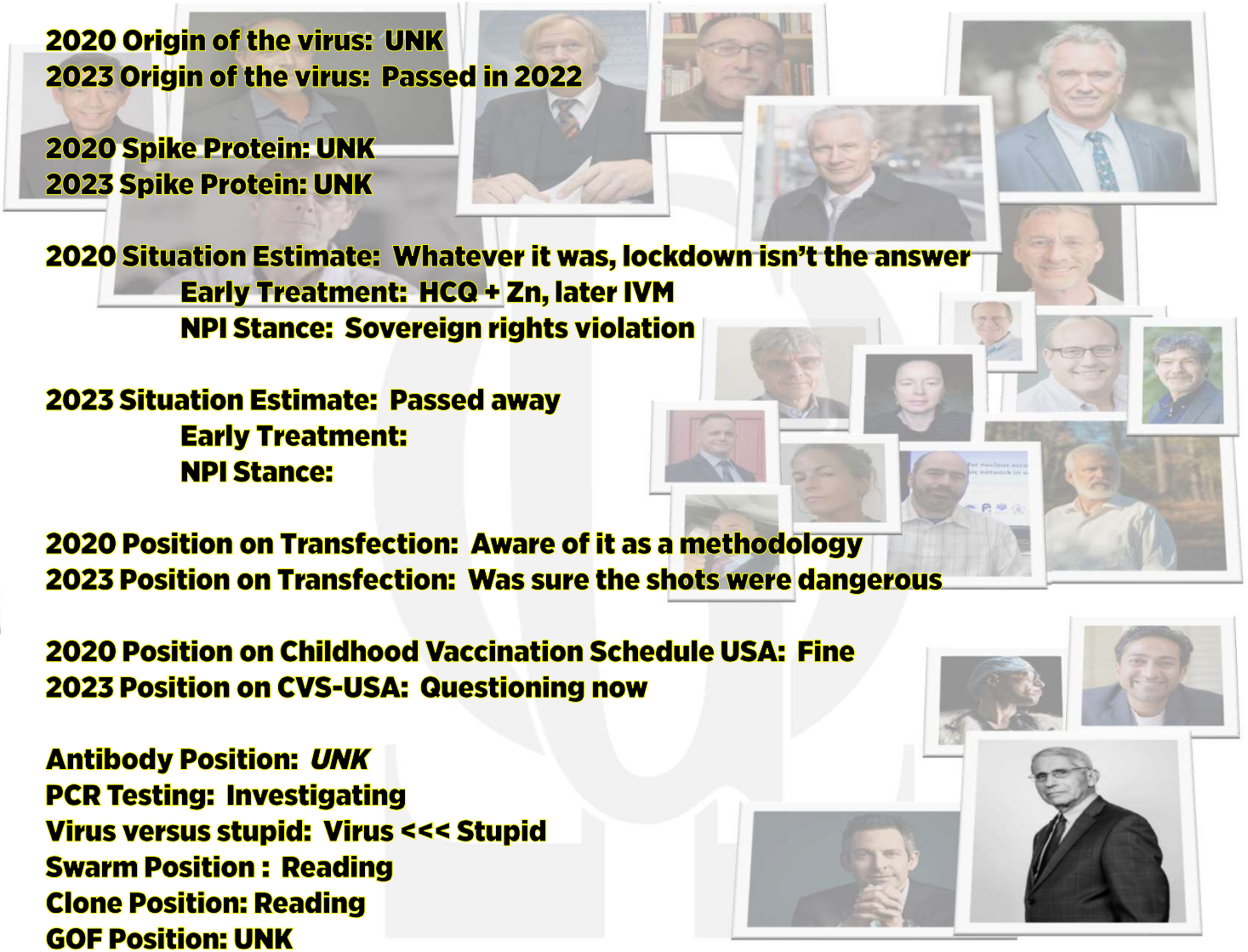
2020 Situation Estimate: Whatever it was, lockdown isn't the answer
Early Treatment: HCQ + Zn, later IVM
NPI Stance: Sovereign rights violation

2023 Situation Estimate: Passed away
Early Treatment:
NPI Stance:

2020 Position on Transfection: Aware of it as a methodology
2023 Position on Transfection: Was sure the shots were dangerous

2020 Position on Childhood Vaccination Schedule USA: Fine
2023 Position on CVS-USA: Questioning now

Antibody Position: *UNK*
PCR Testing: Investigating
Virus versus stupid: Virus <<< Stupid
Swarm Position : Reading
Clone Position: Reading
GOF Position: UNK





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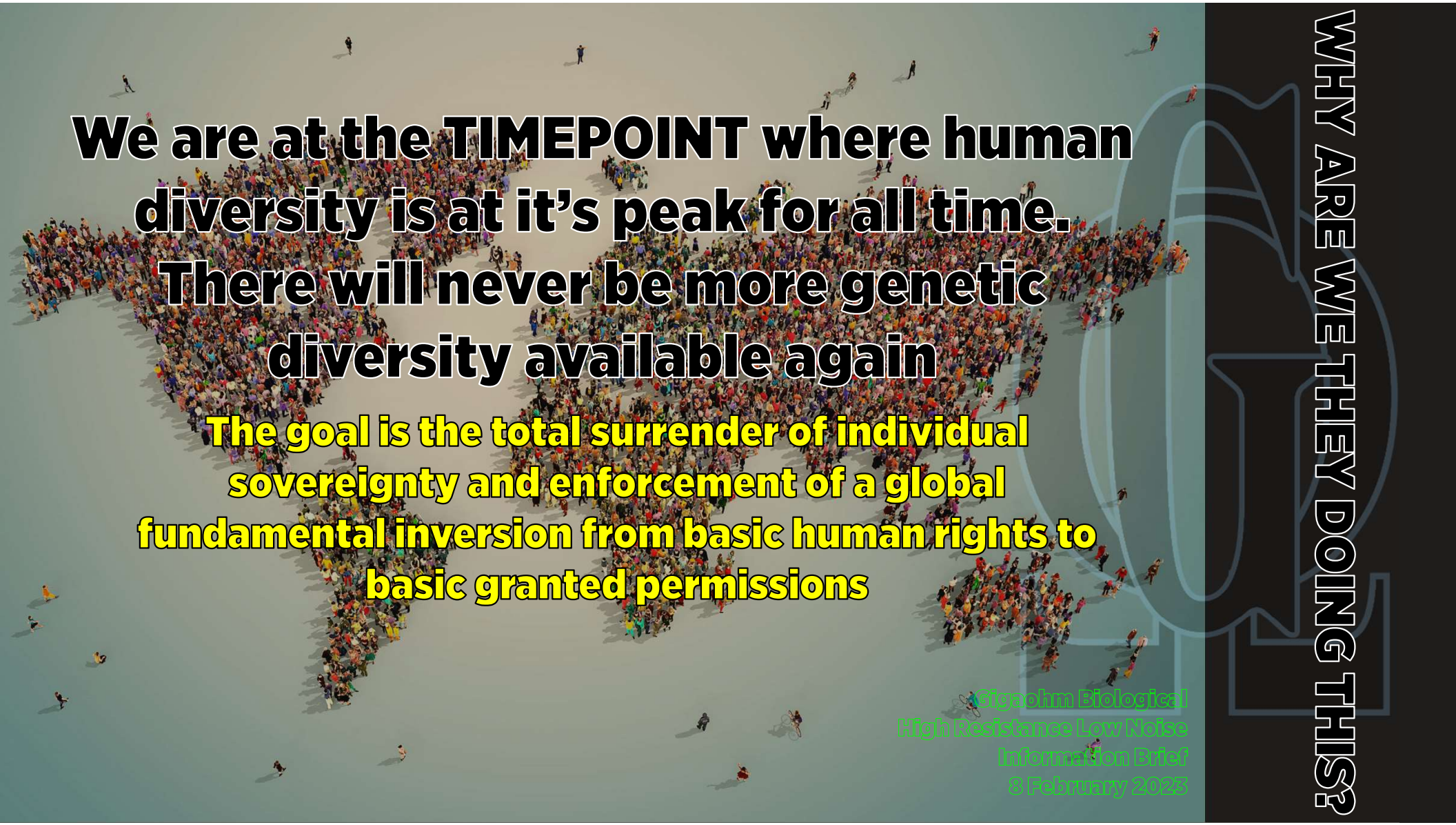
MYSTERY MACHINE


THEY ARE CHANGING
THE WAY WE THINK
for a REASON
PBS NEWS HOUR
Gigaohm Biological
High Resistance Low Noise
Information Brief
8 February 2023


We are at the TIMEPOINT where human diversity is at it's peak for all time. There will never be more genetic diversity available again
The goal is the total surrender of individual sovereignty and enforcement of a global fundamental inversion from basic human rights to basic granted permissions
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High Resistance Low Noise
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8 February 2023
WHY ARE WE THEY DOING THIS?

They changed how we THINK

They changed how we THINK about the
Human Coronavirus Swarm

They changed how we THINK about
All Cause Mortality

They changed how we THINK about
our immune response to a respiratory virus

They changed how we THINK about
Immunization, vaccination, and immunity

THEY HAVE
CHANGED
THE WAY
WE THINK

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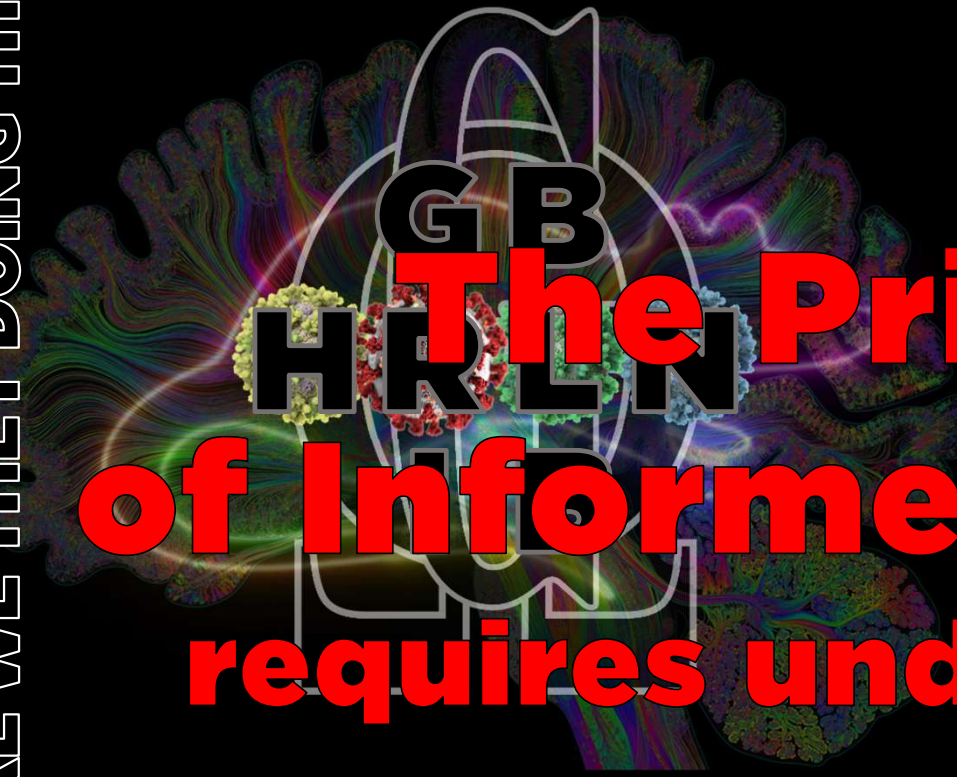
WHY ARE WE THEY DOING THIS?



The Principle of Informed Consent requires understanding.

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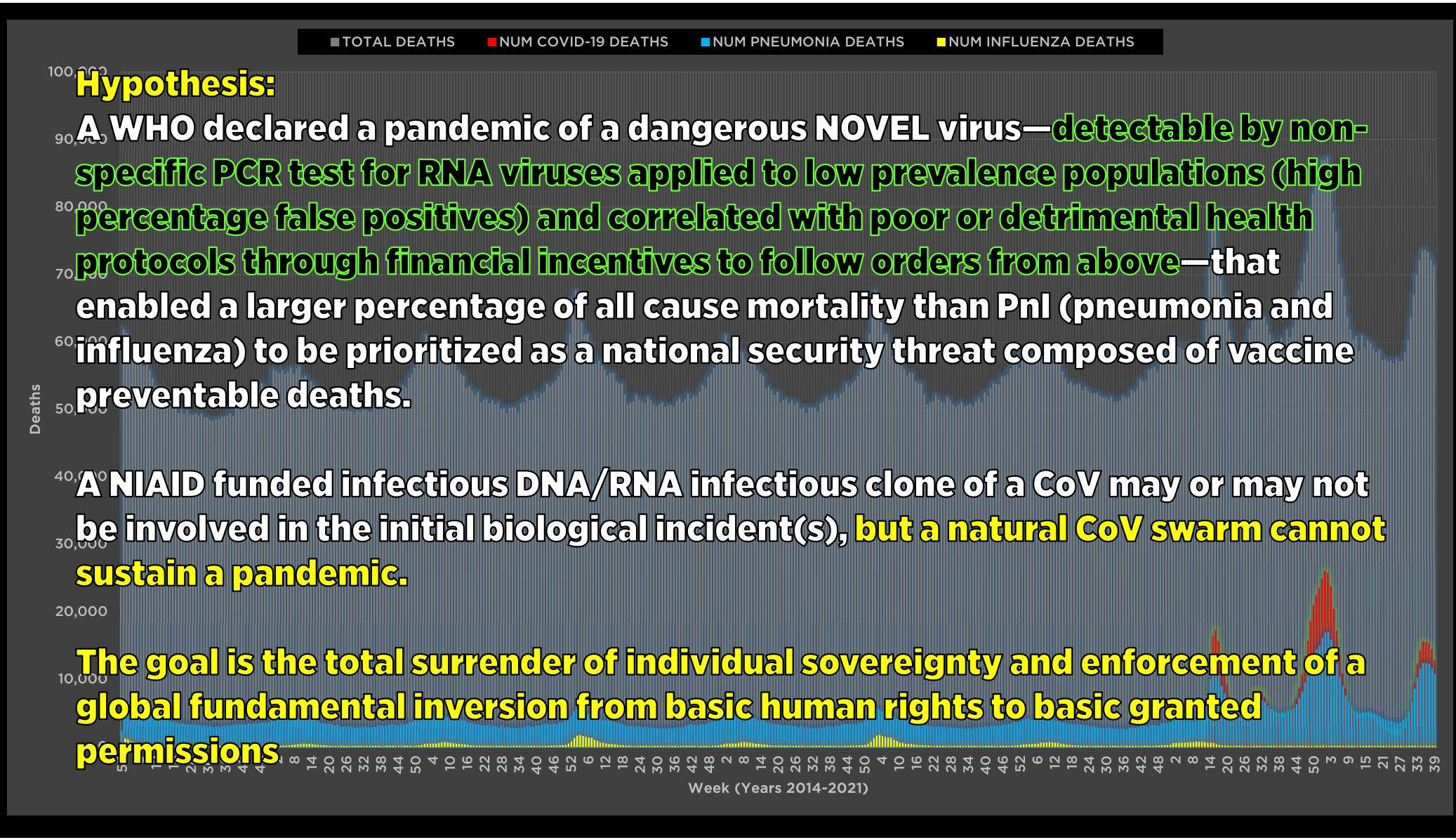
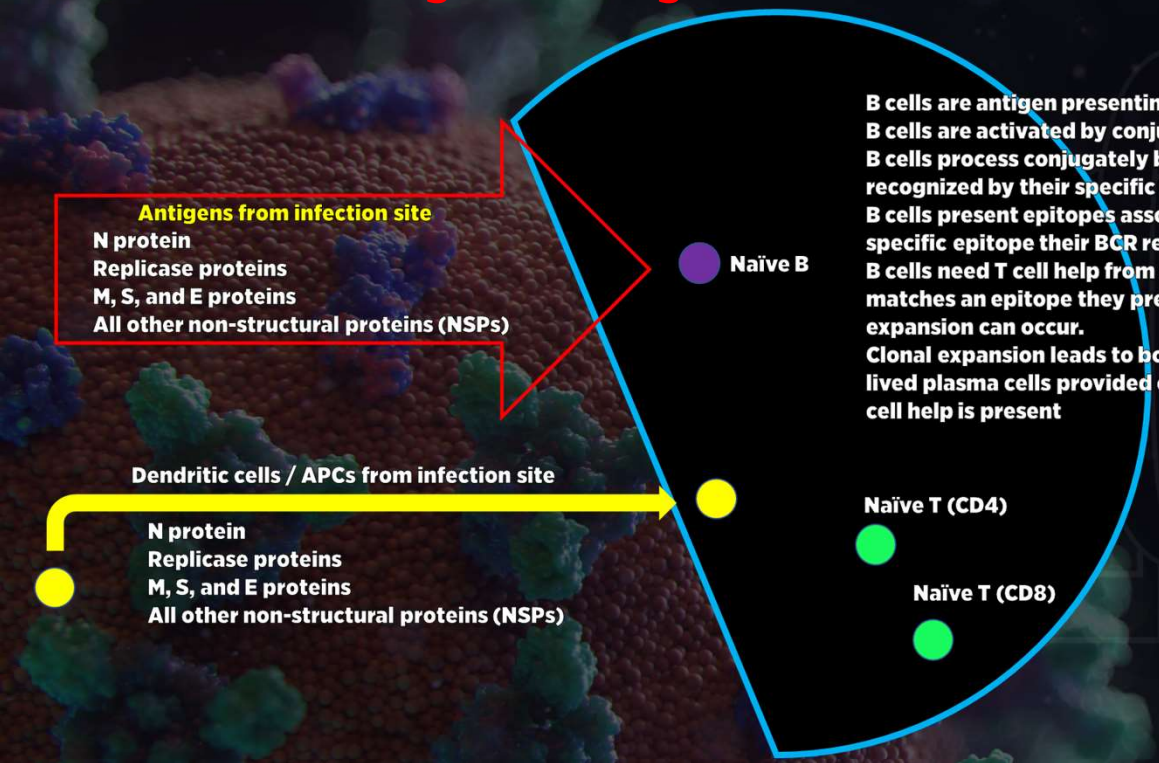
WHY ARE WE THEY DOING THIS?

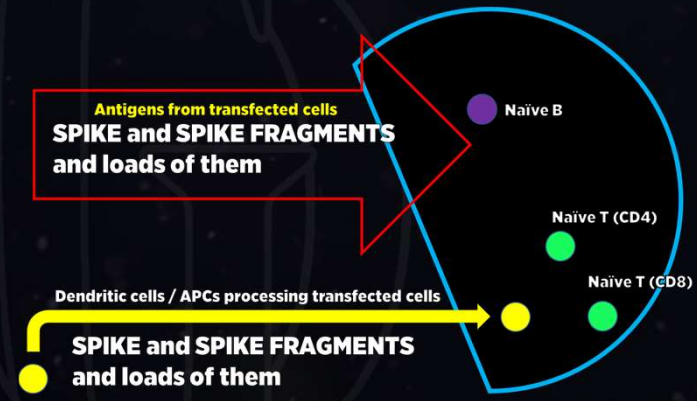

TOTAL DEATHS
NUM COVID-19 DEATHS
NUM PNEUMONIA DEATHS
NUM INFLUENZA DEATHS
Deaths
Week (Years 2014-2021)
Hypothesis:
A WHO declared a pandemic of a dangerous NOVEL virus—detectable by non-specific PCR test for RNA viruses applied to low prevalence populations (high percentage false positives) and correlated with poor or detrimental health protocols through financial incentives to follow orders from above—that enabled a larger percentage of all cause mortality than PnI (pneumonia and influenza) to be prioritized as a national security threat composed of vaccine preventable deaths.
A NIAID funded infectious DNA/RNA infectious clone of a CoV may or may not be involved in the initial biological incident(s), but a natural CoV swarm cannot sustain a pandemic.
The goal is the total surrender of individual sovereignty and enforcement of a global fundamental inversion from basic human rights to basic granted permissions

How are these two processes different?

Immune Linked Recognition during Infection



Immune Linked Recognition during Transfection



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https://www.researchgate.net/publication/362427136

Figure 1 shows the all-cause mortality by month (ACM/m) for the USA from January 1999 to December 2021.

So millions of doses didn't change all cause mortality?

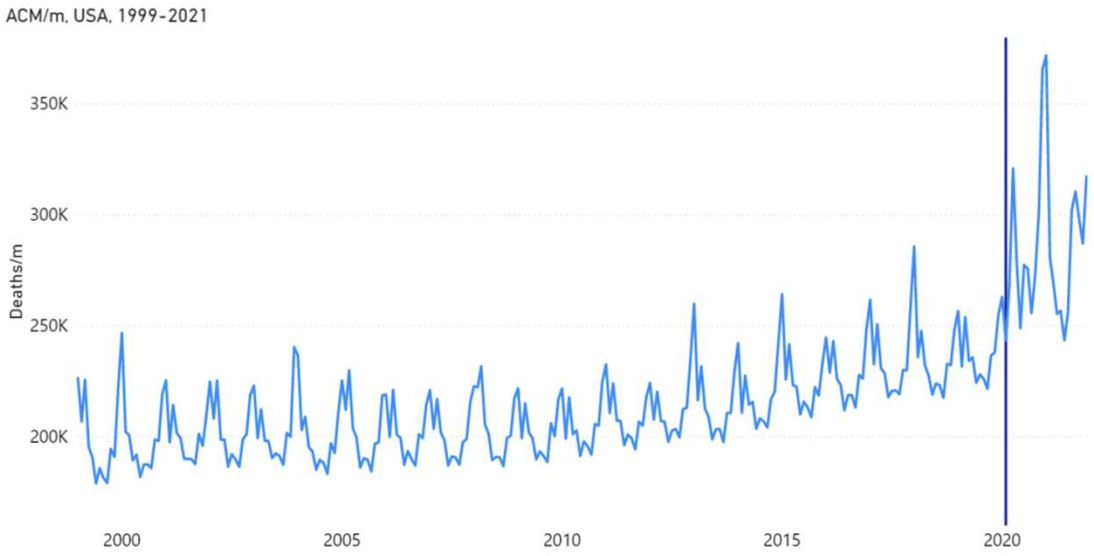

Figure 1. All-cause mortality by month in the USA from 1999 to 2021. Data are displayed from January 1999 to December 2021. The vertical dark-blue line indicates the month of February 2020, intended to point the beginning of the covid period. Data were retrieved from CDC (CDC, 2022a), as described in Table 1.

Poverty level
Household income
Serious mental illness
Obesity
and the excess mortality is a direct correlation across states

True with and without vaccines
Not correlated with age?!

This means that it cannot be from a CoV because there is an exponential relationship between age and CoV infection fatality

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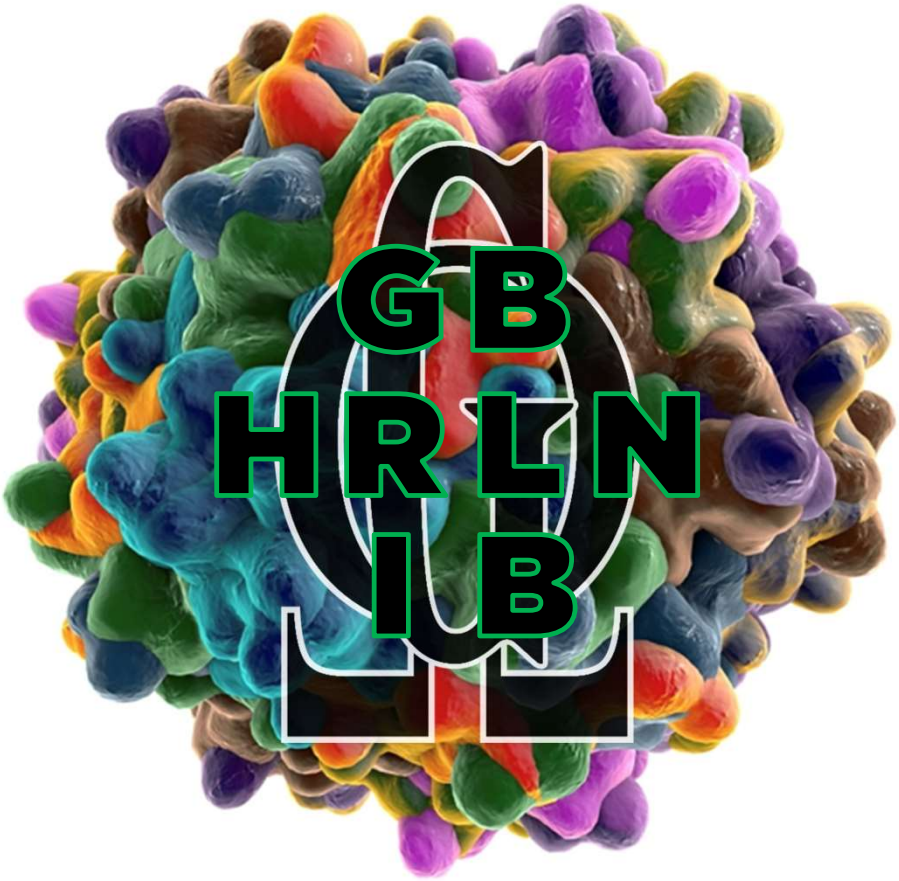
STOP
Stop all transfection in humans!

#1 DANGER: Elimination of Control Group by any means necessary



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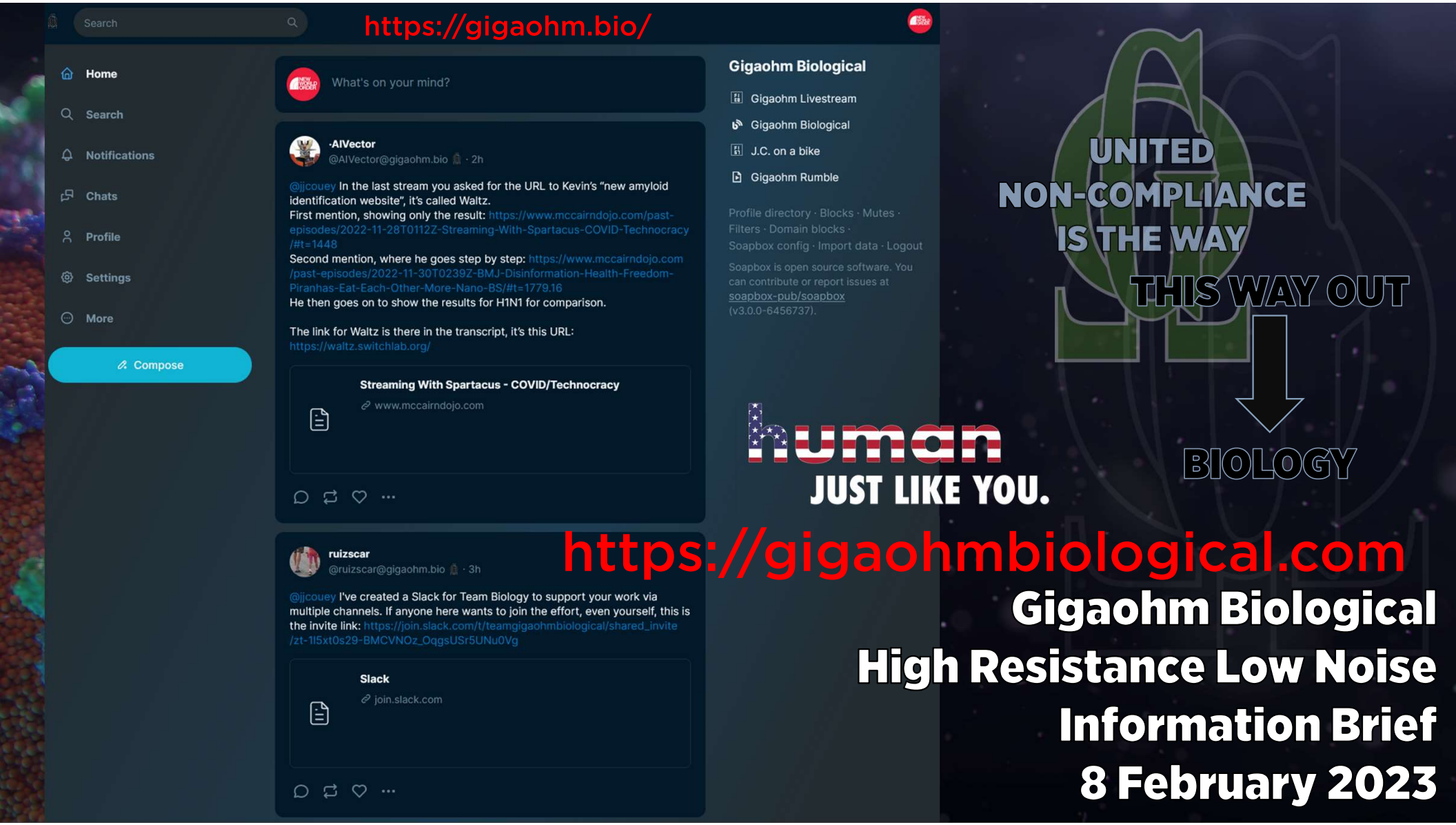
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What's on your mind?
·AIVector
@AIVector@gigaohm.bio · 2h
@jjcouey In the last stream you asked for the URL to Kevin's "new amyloid identification website", it's called Waltz.
First mention, showing only the result: https://www.mccairndojo.com/past-episodes/2022-11-28T0112Z-Streaming-With-Spartacus-COVID-Technocracy/#t=1448
Second mention, where he goes step by step: https://www.mccairndojo.com/past-episodes/2022-11-30T0239Z-BMJ-Disinformation-Health-Freedom-Piranhas-Eat-Each-Other-More-Nano-BS/#t=1779.16
He then goes on to show the results for H1N1 for comparison.
The link for Waltz is there in the transcript, it's this URL: https://waltz.switchlab.org/
Streaming With Spartacus - COVID/Technocracy
www.mccairndojo.com
ruizscar
@ruizscar@gigaohm.bio · 3h
@jjcouey I've created a Slack for Team Biology to support your work via multiple channels. If anyone here wants to join the effort, even yourself, this is the invite link: https://join.slack.com/t/teamgigaohmbiological/shared_invite/zt-1l5xt0s29-BMCVNOz_OqgsUSr5UNu0Vg
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Soapbox is open source software. You can contribute or report issues at soapbox-pub/soapbox (v3.0.0-6456737).
UNITED
NON-COMPLIANCE
IS THE WAY
THIS WAY OUT
BIOLOGY
human
JUST LIKE YOU.
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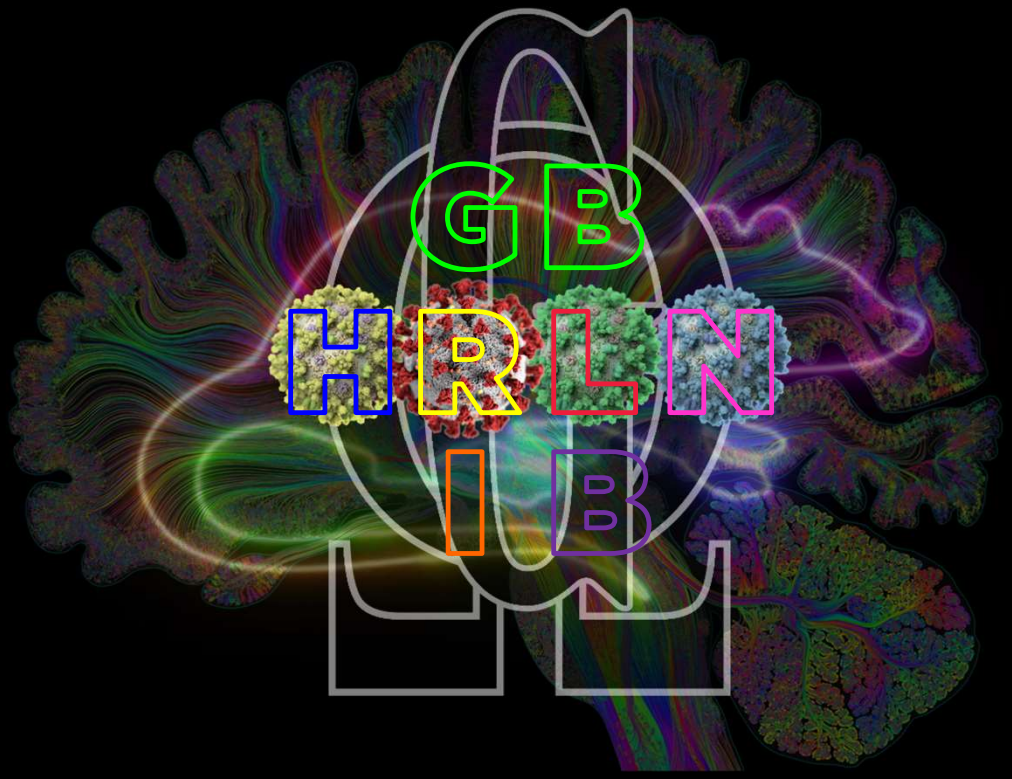
GB
HRLN
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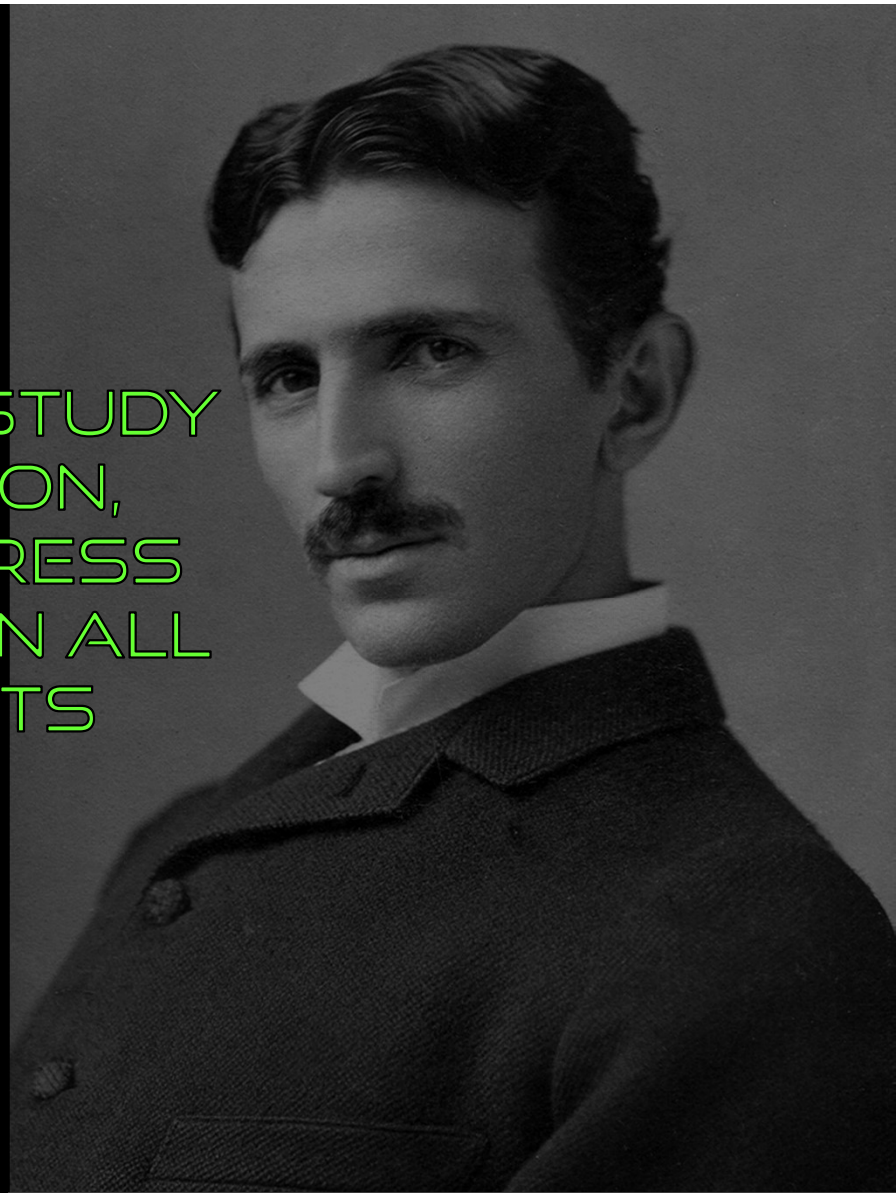
THE DAY SCIENCE BEGINS TO STUDY NON-PHYSICAL PHENOMENON, WE WILL MAKE MORE PROGRESS IN A DECADE THAN WE HAVE IN ALL PREVIOUS CENTURIES OF ITS EXISTENCE.

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