Viruses

dnagase [redacted] Mon, Aug 22, 2022 at 4:58 PM

[redacted]

"cmssyc@gmail.com" <cmssyc@gmail.com>

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8674568/

I know this may be beyond most unless you did microbiology or biochemistry at mcgill but here it is fyi.

From figure 3 if you don't want to read the whole paper.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8674568/

"Primary cardiac PCs (n=6 patients) and the Caco-2-ACE2 cell line were either mock-infected or inoculated with SARS-CoV-2 isolated early in the pandemic (REMRQ001) or the α (B.1.1.7) or δ (B.1.617.2) variants, all at an MOI = 10, and incubated for 24 h before immunostaining for viral and PC markers. (A) Immunofluorescence images show SARS-CoV-2 nucleocapsid protein (N, magenta) and double-stranded RNA (dsRNA, orange) indicative of virus replication. Nuclei are stained with DAPI (blue). PDGFRβ stains PCs (green). For PCs, we show example images from two patients. Because of the high magnification used (20×), the images shown for patient #2 are not representative of the real % of infection, but we aimed to provide examples of infected PCs in each experimental group. (B) Quantification of the percentage of PCs positive for dsRNA in the three experimental groups. The bar graph reports individual values and means ± SEM. (C) Analysis of ACE2 and CD147 protein levels in cardiac PCs using Western blotting. (D) Table showing the row data of the PC infection and summarising the ..."

Original and variants isolated, tested on cardiac cells, found to grow more copies.
Growing more copies means living thing and Not a Toxin.

Sent with Proton Mail secure email.

------- Original Message -------
On Wednesday, August 17th, 2022 at 2:53 PM, [redacted]

Viruses

Christine Massey <cmssyc@gmail.com> Mon, Aug 22, 2022 at 5:54 PM

[redacted]

Hi Daniel,

That study was published 2 years after the fake virus was said to have been discovered.

The authors did not claim to have demonstrated the existence of a virus. They began with the false premise that it was already known to exist.

They used pre-existing fake "isolates" of the nonexistent SARS-CoV-2, and pretended to show that "SARS-COV-2 spike protein" disrupts human cardiac pericytes function.

From the Methods section

> *Stocks of SARS-CoV-2 viral isolates, SARS-CoV-2/human/Liverpool/REMRQ0001/2020 (REMRQ0001, wildtype strain, GenBank: MW041156.1, isolated as previously described [30]), hCoV-19/England/204690005/2020 (lineage B.1.1.7 – α variant; GISAID ID: EPI_ISL_693401, kindly provided by Professor Wendy Barclay, Imperial College, London and Professor Maria Zambon, Public Health England), and hCoV-19/England/SHEF-10E8F3B/2021 (lineage B.1.617.2 – δ variant; GISAID ID: EPI_ISL_1731019; kindly provided by Professor Wendy Barclay, Imperial College, London and Dr Thushan de Silva, Sheffield Teaching Hospitals, University of Sheffield) were produced by inoculation of VeroE6/TMPRSS2 cells [31] and titred as previously described [32,33]*

If you check the supplementary methods in the paper cited as #30, you will find;

> *SARS-76 CoV-2 isolation and infection*
> *77 **A clinical specimen in viral transport medium**, confirmed SARS-CoV-2 positive by qRT-PCR*
> *78 (kindly proved by Dr Lance Turtle, University of Liverpool), was adjusted to 2 ml with OptiMEM*
> *79 (Gibco™, ThermoFisher), **filtered through a 0.2 µm filter and used to infect Vero E6 cells.** After*
> *80 1 h the inoculum was diluted 1:3 (vol/vol) with **MEM supplemented with 2% FCS** and incubated*
> *81 at 37 °C in a 5% CO2 incubator for 5 days. The **culture supernatant was passaged twice more on***
> ***82 Vero E6 cells until cytopathic effect was observed and then once on Caco-2 cells** to produce the*
> *83 stock used in the experiments. The intracellular viral genome sequence and the titre of virus in the*
> *84 supernatant were determined as previously described (20) and the virus termed SARS-CoV-*
> *85 2/human/Liverpool/REMRQ0001/2020.*

So they assumed the clinical specimen contained the fake virus based on a completely meaningless PCR test, stored the clinical sample in transport medium (the CDC's SOP for transport medium contains fetal calf serum and toxic drugs, so the specimen was quite possibly already contaminated with cow material), filtered the specimen, added it to monkey kidney (Vero) cells and more calf serum (and possibly more toxic drugs because these studies almost invariably use antibiotics and antifungals). The cells are being malnourished (with only 2% fcs) and likely poisoned. When the cells start to break down they insist that an imaginary virus is to blame.

The monkey/cow/human/bacteria/fungi mixture (cell culture) is passed off as the latest "virus isolate".

No "virus" was found in any clinical sample, as always. No "virus" was isolated/purified and available for use as the independent variable in a controlled experiment. No science has taken place, whatsoever. Just wild idiotic, irresponsible assumptions, which is virology in a nutshell.

If you look up the studies from which the other fake isolates came, you will surely find the same basic recipe being used to create the other fake "isolates".

We need you to grasp this Daniel, we need your voice on the side of reason. I beg you to study the methods in "virus isolation" papers, and you will see for yourself that virology is pure idiocy and delusion, we have all been lied to all our lives. It is not a science.

Christine

[Quoted text hidden]

Viruses

dnagase [redacted] Tue, Aug 23, 2022 at 6:42 PM

[redacted]

again, if you don't comprehend the paper don't make the claim there's no such thing as viruses.

the one logical fallacy that Tavistock mass psychology agents often use is a non-sequitr

i.e. The model T had a lot of deficiencies therefore there is no such thing as a car.

of course the first scientists who pioneered microbiology made mistakes and wrong assumptions. This paper is where microbiology is today.
Western blot proofs of non cellular proteins found in infected cells,
Sequencing proofs of non-cellular segments of DNA only found in infected cells.

If you don't know how a sequence differs from pcr then making the claim that viruses have never been proven to exist is ignorant.

[redacted]

------- Original Message -------
[Quoted text hidden]

Viruses

Christine Massey <cmssyc@gmail.com> Tue, Aug 23, 2022 at 8:46 PM

[redacted]

Daniel, you sent me an email about "viruses" and a study with nothing remotely approaching a "virus".

You seem to have forgotten what "virus" means. Let me help you:

A replication-competent intracellular obligate parasite that transmits between hosts and causes disease via natural modes of exposure.

You sent a study involving monkey/cow/human/bacteria/fungi soups and "***Production*** *and purification of the* ***recombinant*** *SARS-CoV-2 S protein".*

You seem to have forgotten scientific method too. It requires an independent variable with which to do a controlled experiment.

You have zero science so you resort to insults and accusations. That's pathetic.

Show me a paper where the alleged virus, or even the alleged spike protein was found in any bodily fluid/tissue of anyone on Earth. Not indirect tests that have no gold standard, but the actual alleged thing found in "hosts". If you can't even do that, then there is nothing to discuss

Viruses

dnagase [redacted]
Reply-To: [redacted]
To: Christine Massey <cmssyc@gmail.com>

Wed, Aug 24, 2022 at 1:37 AM

[redacted]

Please, if you don't comprehend the paper just say so.
Stop with the accusations and made up definitions.
The thing these scientists did was good science.
Excellent in my opinion, because they showed that spike proteins were bad for heart pericytes, but whole covid 19, both the original and 2 subsequent variants were not.
These researchers did a really good job.

Viruses

Christine Massey <cmssyc@gmail.com> Wed, Aug 24, 2022 at 7:44 AM

[redacted]

Oh but I do understand, Daniel. You're relying on a wildly unnatural lab-dish study using man-made monkey/cow/human/bacteria/fungi soups, man-made protein, engineered cell lines and indirect tests that have no gold standard as evidence of alleged real-life particles and in vivo effects. This isn't science. There is zero validity here.

Mike Stone already shredded this and other "spike protein" studies. I suggest you study Mike's entire website if you really buy into this study you've cited.

The Elephant and The Spike -- Mike Stone
https://viroliegy.com/2022/07/20/the-elephant-and-the-spike/

> This was another study used by Hammond to try and claim that the spike protein alone is pathogenic. He believed that the study showed a plausible way blood clots may occur due to "SARS-COV-2" and/or injection from the mRNA vaccine. However, once again, this study did not use purified and isolated particles but instead experimented with lab-created recombinant S proteins made from insect cells. The primary cell cultures used for the study were grown in dedicated medium supplemented with human recombinant growth factors and 2% fetal calf serum which hardly sounds like something the cells would encounter within a living organism. These cells were passaged between 4 to 7 times which can have detrimental effects on the culture as the passage number increases. The cell line cultures consisted of human gut epithelial cell line, Caco2, expressing hACE2 as well as African green monkey kidney cell line VeroE6 engineered to overexpress the human ACE2 and TMPRSS2. All cells were cultured in Dulbecco's modified Eagle's medium plus GlutaMAX supplemented with 10% FBS, 1% sodium pyruvate, and 0.1 mM non-essential amino acids. The human lung epithelial cell line Calu3 (ATCC HTB-55) was cultured in Eagle's minimum essential medium plus GlutaMAX with 10% FBS, 0.1 mM non-essential amino acids, and 1% sodium pyruvate. In other words, there is absolutely nothing natural about the materials nor the chemical additives that they were kept in and experimented with.
>
> The researchers stated that their study provided novel (as in fictional) *proof-of-concept* evidence for S protein capacity to cause molecular and functional changes in human vascular PCs. However, they admitted that their small sample size was inadequate and that further

investigation in a larger population of patients was warranted to determine the cause for the inter-individual variability in PC infection. They also could not exclude that different scenarios may happen *in vivo*, i.e. within a living organism, as compared to that seen *in vitro*, i.e. inside a petri dish in a lab, thus essentially admitting that their results can not be applied to what occurs within a human body. Interestingly, the researchers also admitted that low amounts of the S protein *could be detected in pre-pandemic control sera*. They stated that this could be explained by the sequence homology between some regions of the S protein and other human proteins/peptides. In other words, the S protein contains similar sequences to normal human proteins/peptides and thus the tests that the researchers were using may have been picking up nothing more than normal human proteins/peptides rather than the theoretical S protein. Sadly, the immunogen sequence for the ELISA kit they used was locked away behind proprietary information (as is always the case), and therefore they could not determine if it recognised the S protein residues that have homology with unrelated peptides. Thus, the results from this study truly were worthless:

The SARS-CoV-2 Spike protein disrupts human cardiac pericytes function through CD147 receptor-mediated signalling: a potential non-infective mechanism of COVID-19 microvascular disease

“Exposure to the recombinant S protein alone elicited signalling and functional alterations, including: (1) increased migration, (2) reduced ability to support endothelial cell (EC) network formation on Matrigel, (3) secretion of pro-inflammatory molecules typically involved in the *cytokine storm*, and (4) production of pro-apoptotic factors causing EC death.”

Primary cell cultures

“Cardiac PCs were immunosorted as CD31neg/CD34pos cells from human myocardial samples, and expanded in a dedicated medium supplemented with human recombinant growth factors and 2% v/v foetal calf serum (FCS) (ECGM2 complete kit, C-22111, PromoCell) as previously described [11,28]. Briefly, samples were finely minced using scissors and scalpel until nearly homogenous and digested with Liberase (Roche) for up to 1 h at 37 C, with gentle rotation. The digest was passed through 70-, 40-, and 30-μm strainers. Finally, the cells were recovered and sorted using anti-CD31 and -CD34 microbeads (Miltenyi) to deplete the population of CD31pos ECs and select CD31neg/CD34pos cells, which distinguish a population of perivascular cells *in situ* [11,28]. After expansion to passage 3, the purity of the cell population was verified using immunocytochemistry (ICC) or flow cytometry [11,28].

Human coronary artery ECs (CAECs) were purchased from PromoCell and expanded in the same medium used for PCs. All cells used in the present study

tested negative for mycoplasma contamination (assessed using the PCR Mycoplasma Test Kit I/C, PromoCell, cat# PK-CA91-1096). **Cells were used between passages 4 and 7.**

Cell line cultures

The human gut epithelial cell line, Caco2, expressing hACE2 (Caco-2-ACE2) was a kind gift from Dr Yohei Yamauchi, University of Bristol. **The African green monkey kidney cell line VeroE6 engineered to overexpress the human ACE2 and TMPRSS2** (VeroE6/ACE2/TMPRSS2) [29] was a kind gift from Dr Suzannah Rihn, MRC-University of Glasgow Centre for Virus Research. **All cells were cultured in Dulbecco's modified Eagle's medium plus GlutaMAX (DMEM, Gibco, Thermo Fisher, cat# 10567014) supplemented with 10% v/v FBS (Gibco, Thermo Fisher, A3840001), 1% v/v sodium pyruvate, and 0.1 mM non-essential amino acids. The human lung epithelial cell line Calu3 (ATCC HTB-55) was cultured in Eagle's minimum essential medium plus GlutaMAX (MEM, Gibco, Thermo Fisher, cat# 41090036) with 10% v/v FBS, 0.1 mM non-essential amino acids, and 1% v/v sodium pyruvate."**

Measurement of S protein in patients' sera

"The presence of S protein in COVID-19 patients' serum was evaluated using the COVID-19 Spike Protein ELISA Kit from Abcam (ab274342), according to manufacturer's instructions. Pre-pandemic sera were employed as controls. All test sera were diluted 1:2. The S protein concentration was expressed as nanogram per millilitre serum. **The antibody supplied in the kit recognised the S2 domain."**

Production and purification of the recombinant SARS-CoV-2 S protein

"SARS-CoV-2 S protein was expressed in insect cells and purified as described previously [33,35]. Briefly, the S construct encoded amino acids 1–1213 (extracellular domain – ECD) fused with a thrombin cleavage site, followed by a T4-foldon trimerisation domain and a hexahistidine (HIS) affinity purification tag at the C-terminus. The polybasic furin cleavage site was mutated (RRAR to A) to increase the stability of the protein for *in vitro* studies [33,35]. S protein was expressed in Hi5 cells using the MultiBac system [36]. **Secreted S protein was harvested 3 days after infection by centrifuging the cell culture at 1000×*g* for 10 min followed by another centrifugation of supernatant at 5000×*g* for 30 min. S protein-containing medium was incubated with HisPur Ni-NTA Superflow Agarose (Thermo Fisher Scientific) for 1 h at 4°C.** Resin bound with S protein was separated from unbound proteins and medium using a gravity flow column, followed by 30 column volume wash with wash buffer (65 mM NaH2PO4, 300 mM NaCl, 20 mM imidazole, pH 7.5). Finally, the protein was eluted with a step-gradient of elution buffer (65 mM NaH2PO4, 300 mM NaCl, 235 mM imidazole, pH 7.5). Eluted fractions were analysed by reducing SDS/PAGE. **Fractions containing the S protein were pooled and concentrated using 50-kDa MWCO Amicon centrifugal filter units (EMD Millipore).** During

concentration, proteins were buffer-exchanged in PBS, pH 7.5. Concentrated protein was aliquoted, flash-frozen in liquid nitrogen, and stored at −80°C until use. In all the *in vitro* experiments of the manuscript, we will refer to the S-ECD protein simply as S protein.

Recombinant Spike S1 (#10522-CV) and S2 (#10584-CV) were purchased from R&D, resuspended in PBS according to manufacturer's instructions, aliquoted and stored at −80°C until use. **Similarly to the S-ECD, the S1 and S2 proteins were produced in insect cells."**

Discussion

"Our study provides novel *proof-of-concept* evidence for S protein capacity to cause molecular and functional changes in human vascular PCs, either dependently or independently of the CD147 receptor (summarised in Figure 11)."

"Here, we report that two-third of patients tested did not have their PCs infected by SARS-CoV-2, while the rate of infection was below 8% in the remaining subjects, **suggesting a very low permissiveness of these cells to the coronavirus, at least *in vitro*."**

"Further investigation in a larger population of patients is warranted to determine the cause for the inter-individual variability in PC infection. **Moreover, we cannot exclude different scenarios may happen *in vivo*."**

"In our study, **low amounts of the S protein could be detected in pre-pandemic control sera. This could be explained by the sequence homology between some regions of the S protein and other human proteins/peptides.** A previous report identified pathogenic regions of SARS-CoV-1 S protein, which share sequence homology with Angrgm-52 (GenBank accession number AAL62340), a novel gene up-regulated in human mesangial cells stimulated by angiotensin II and bradykinin [53]. **Unfortunately, the immunogen sequence for this particular ELISA kit ab274342 is proprietary information, therefore we could not determine if it can recognise the S protein residues that have homology with unrelated peptides."**

Study limitations

"The study was conducted on isolated cells and therefore **the evidence must be confirmed *in vivo*.**

The amount of S protein used for *in vitro* studies was higher than the average S protein concentration detected in COVID-19 patients' serum. However, circulating S protein represents the spill-over from infected organs, where concentration may be higher due to retention at the receptor level. **Because we do not have access to post-mortem myocardial samples, we could not verify this hypothesis."**