IN SILICO GENOMICS: THE NEW PARADigm OF VIRUS SIMULATION

-AND-

PATHOGENIC PRIMING: WHAT DOES THE SCIENCE ACTUALLY SHOW?

Andrew Kaufman, MD
SARS-CoV-2
Does NOT EXIST!!

FACT No. 1

Computer generated, 3D Biomedical Art
by artists Alissa Eckert, and Dan Higgins
To Illustrate ultrastructural morphology
alleged to be exhibited by coronaviruses.
FACT No. 2

Covid-19 Does NOT EXIST!!

Computer generated, 3D Biomedical Art by artists Alissa Eckert, and Dan Higgins To Illustrate ultrastructural morphology alleged to be exhibited by coronaviruses.
EXTRAORDINARY CLAIMS REQUIRE EXTRAORDINARY EVIDENCE.

- CARL SAGAN
The Extraordinary Claim:

A microscopic particle, which has never been seen, emerged out of nowhere, spread around the world, and caused the worst disease in over 100 years!
WHAT CAN BE ASSERTED WITHOUT EVIDENCE...

CAN BE DISMISSED WITHOUT EVIDENCE.

HITCHENS’ RAZOR
Logical Fallacies

Informal

An **informal fallacy** refers to an argument whose proposed conclusion is not supported by the premises. This creates an unpersuasive or unsatisfying conclusion.

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<tr>
<th>Ad Hominem*</th>
<th>Ambiguity*</th>
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<td>Argumentum ad Populum</td>
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<td>Fallacy of Quoting Out of Context</td>
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<tr>
<td>False Cause &amp; False Attribution*</td>
<td>False Dilemma*</td>
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Logical Fallacies

Ad Hominem

The ad hominem attack is a logical fallacy associated with trying to undermine the opponent’s arguments by personal attacks, through attacking their character or skill level, etc. The ad hominem attack uses an accepted fact about a person to undermine their credibility despite the lack of causal connection between the two parts of the argument.

Example of Ad Hominem

- Bill claims that this was an accident, but we know Bill to be a liar, so we can’t take his word for it.

  Even though Bill may be a liar, his character does not automatically make anything he says untrue.

- Susan is an avid hunter, therefore she cannot possibly support gun control.

  Being a hunter is used as a negative characteristic to make a conclusion which could very well be untrue. Susan could support a variety of gun control legislation.
It can be proven that most claimed research findings are false.

Essay

Why Most Published Research Findings Are False

John P. A. Ioannidis

Summary

There is increasing concern that most current published research findings are false. The probability that a research claim is true may depend on study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationships among the relationships probed in each scientific field. In this framework, a research finding is less likely to be true when the studies conducted in a field are smaller; when effect sizes are smaller; when there is a greater number and lesser preselection of tested relationships; where there is factors that influence this problem and some corollaries thereof.

Modeling the Framework for False Positive Findings

Several methodologists have pointed out [9–11] that the high rate of nonreplication (lack of confirmation) of research discoveries is a consequence of the convenient, yet ill-founded strategy of claiming conclusive research findings solely on the basis of a single study assessed by formal statistical significance, typically for a $p$-value less than 0.05. Research is not most appropriately represented is characteristic of the field and can vary a lot depending on whether the field targets highly likely relationships or searches for only one or a few true relationships among thousands and millions of hypotheses that may be postulated. Let us also consider, for computational simplicity, circumscribed fields where either there is only one true relationship (among many that can be hypothesized) or the power is similar to find any of the several existing true relationships. The pre-study probability of a relationship being true is $R/(R + 1)$. The probability of a study finding a true relationship
THE ‘IN SILICO’ GENOME
AND
THE NEW PARADIGM OF GENETIC VIROLOGY
WHAT IS DNA?

DNA is the genetic material in our bodies. It is said that our DNA code makes us human. The code is in the sequence of 4 bases.
WHAT IS DNA?

The Central Dogma of Biology Sates:

DNA into RNA into Proteins

Each of the three letters code for an amino acid (the building blocks of proteins)
WHAT IS THE GENOME?

The genome is the entire genetic code of an organism.

In humans, there are 23 strands of DNA called chromosomes.

The entire sequence of all the chromosomes, end to end, is the ‘Human Genome’.
How To Sequence A Genome:

Actual Human (Non-Theoretical)
Is this how they sequence the genomes of viruses?

NO SIR!
How to sequence
An ‘in silco’ Genome

In silico (adjective)
literally ‘in silicon’, i.e. ‘in the computer’; referring to analysis or experimentation carried out in a computer environment, rather than in the laboratory. It is the mimicking or modeling of biological processes within computer hardware and software.

In other words:
IMAGINARY
“NEXT GENERATION” SEQUENCING

- This purification step is NOT DONE with SARS-CoV-2
- The source of the RNA is UNKNOWN
- Amplifies EVERYTHING in the sample
- Alignment
- Discovery of a “Theoretical Virus”
A new coronavirus associated with human respiratory disease in China

Emerging infectious diseases, such as severe acute respiratory syndrome (SARS) and Zika virus disease, present a major threat to public health. Despite intense research efforts, how, when and where new diseases appear are still a source of considerable uncertainty. A severe respiratory disease was recently reported in Wuhan, Hubei province, China. As of 23 January 2020, at least 1,975 cases had been reported since the first patient was hospitalized on 12 December 2019. Epidemiological investigations have suggested that the outbreak was associated with a seafood market in Wuhan. Here we study a single patient who was a worker at the market and who was admitted
METHODS

1. Lung fluid taken from only ONE “suspected case”
2. RNA is extracted and includes all sources of RNA (human, microbial)
3. RNA is analyzed for concentration and length
4. RNA library is constructed
5. Next Generation Sequencing. Only short strands used! (150 bases)
6. Many sequences are “trimmed”
7. Total sequences (reads) = 56,565,928
How do they get a genome from 56 ½ million little pieces?
A PUZZLING ENIGMA

THE IMPOSSIBLE TASK OF ASSEMBLING A NON-EXISTANT VIRUS

THE CORONA CHRONICLES
Sequencing a viral genome is like reading a book.

The genome of the book is simply the entire sequence of letters.

But, what if you aren’t sure that the book even exists?
her viral symptoms struck at

struck at him vehemently

struck at nine o’clock just as we arrived
I was at home at that hour and writing in kindergarten and first grade.

The teachers of our country have to be taught to start teaching reading and writing in a foolish facetious tone that the perfection of mechanical appliances must ultimately supersede limbs.

For a moment he was seized by a kind of hysteria. He began writing in my study, and although my French windows face towards Ottershaw and the blind was up (for I loved in those days to look up at the night sky), I saw nothing of it.
at that hour, in the teachers of our foolish facetious tone that the perfection superseded limbs seized by a kind of hysteria. He began writing although my French windows faced towards victimization and the blind in those days look up at the night sky. I saw nothing of puffed mechanical appliances? The body of mind doesn’t like afternoon papers ultimately clashing against big headlines of taxing rhapsodic. A wave, loudly set a treehouse on fire! What with the future yet to come, smirkingly prodigious profile lies the guttural reward a head. Whiskey needs a shower in the morning dew of wondrous awe where the world racked by a trees utter nonsensical love. The other side sat down once more and passionate serendipity slips on a caring mother yet still not coherent where our
ASSEMBLY OF CONTIGS

Megahit | Trinity
---|---
384,096 Contigs | 1,329,960 Contigs
Range 200 – 30,474 | Range 201 – 11,760

- Longest Contig (30,474) Chosen!
- 89.1% Similar to bat SARS-like CV isolate sequence
- Claimed whole genome 29,903 bases long
  - What happened to the other 571 bases?
  - Assembled from 123,613 reads
ASSEMBLY OF CONTIGS

• How did they know which reads were correct?
• How do they know this genome is real?
• How do they know the sequences’ origin?
• How much error is there in this process?
• Can the results be replicated?
COMPARE AND CONTRAST

Real Genome

Comes from:
Intact, isolated, and characterized organism

Can be reproduced without error

Represents:
ACTUAL code, an ACTUAL organism

in silico Genome

Comes from:
A mixture of unknown sources

Cannot reproduce without error, false mutations

Represents:
THEORETICAL code, a THEORETICAL organism
VARIANTS
WHAT ARE THEY?
SARS-CoV-2 Variant Classifications and Definitions

Updated Aug. 3, 2021

Key Points

- Genetic variants of SARS-CoV-2 have been emerging and circulating around the world throughout the COVID-19 pandemic.
- Viral mutations and variants in the United States are routinely monitored through sequence-based surveillance, laboratory studies, and epidemiological investigations.
- A US government SARS-CoV-2 Interagency Group (SIG) developed a Variant Classification scheme that defines three classes of SARS-CoV-2 variants:
  - Variant of Interest
  - Variant of Concern
  - Variant of High Consequence
Pseudo-Viruses

• “... we created VSV-based SARS-78 CoV-2 pseudoviruses that contain each of the individual mutations as well as one with all 8 mutations of the B.1.1.7 variant (UKΔ8) and another with all 9 mutations of the B.1.351 variant (SAΔ9). A total of 18 mutant pseudoviruses were made as previously described (20,21), and each was found to have a robust titer (Extended Data Fig. 1) adequate for neutralization studies.”
Delta Variant

• No known attempt to isolate!!! Does not exist in reality!
• Discovered only by in silico genome sequencing
• Clinical properties only studied by
  – Monoclonal antibodies
  – Pseudoviruses
  – Computer modeling
• There is no clinical test authorized, approved, or available for purchase for any variant!!!
Variants are simply the inability to reproduce or validate the original results.
WHAT IS A mRNA VACCINE?
Method for placing ‘in silico’ virus gene into a ready-made platform...

- Alleged SARS-CoV-2 Virus
- Spike Protein
- mNRA packaged in lipid nanoparticles
- mNRA is made with instructions to make viral proteins
- mNRA released into cell
- mNRA used to make viral proteins
- Host Cell
- Vaccine delivered as injection
Pandemic Virus Recipes

• First Generation
  – Proof of new virus: “virus isolation”
  – Vaccine: toxic tissue culture filtrate
  – FDA approval: ≥ 10 years

• Next (covid) Generation
  – Proof of new virus: ‘in silico’ genome
  – Vaccine: “viral gene” in delivery vehicle (pre-made)
  – FDA EUA: < 1 year
A genome is the full code of an organism
An *in silico* genome is a theoretical construct
The new paradigm of virus pandemic creation:
- Mimic the symptoms of another disease
- Perform tissue culture “isolation” (soon obsolete)
- Create an ‘*in silico*’ genome
- PCR test based on above
- Manufacture gene therapy “vaccine”
PATHOGENIC PRIMING, ANTIBODY DEPENDENT ENHANCEMENT

What does the science show?
FACT No. 1

SARS-CoV-2

Does NOT

EXIST!!

Computer generated, 3D Biomedical Art by artists Alissa Eckert, and Dan Higgins To Illustrate ultrastructural morphology alleged to be exhibited by coronaviruses.
• “One potential hurdle for antibody-based vaccines and therapeutics is the risk of exacerbating COVID-19 severity via antibody-dependent enhancement (ADE).”

• “No definitive role for ADE in human coronavirus diseases has been established.”
“ADE has been observed in SARS, MERS and other human respiratory virus infections including RSV and measles, which suggests a real risk of ADE for SARS-CoV-2 vaccines and antibody-based interventions. However, clinical data has not yet fully established a role for ADE in human COVID-19 pathology.”
“Autopsies of Chinese citizens who have died from COVID-19 following SARS-CoV-19 infection show evidence of interstitial changes, suggesting the development of pulmonary fibrosis [1]. This suggests, at least partly, an autoimmunology basis of the pathogenesis of COVID-19.”

Causes of Pulmonary Fibrosis

Occupational and environmental factors
• Silica dust, asbestos, metal dusts, coal dust, grain dust, bird/animal droppings

Radiation treatments

Medications
• Chemotx, anti-arrythmics, antibiotics, immunosuppressive antibodies

Medical conditions
• Connective tissue disorders, rheumatoid arthritis, sarcoidosis, scleroderma, lupus, pneumonia

https://www.mayoclinic.org/diseases-conditions/pulmonary-fibrosis/symptoms-causes/syc-20353690
THE STUDIES…

Is there evidence for ADE?
RESPIRATORY SYNCYTIAL VIRUS DISEASE IN INFANTS
DESPITE PRIOR ADMINISTRATION OF ANTIGENIC
INACTIVATED VACCINE$^1, 2$

HYUN WHA KIM, JOSE G. CANCHOLA$^3$, CARL D. BRANDT, GLORIA PYLES,
ROBERT M. CHANOCK, KEITH JENSEN, AND ROBERT H. PARROTT$^4$

(Received for publication August 8, 1968)

Kim, H. W., J. G. Canchola, C. D. Brandt, G. Pyles, R. M. Chanock, K. Jensen
and R. H. Parrott (Children's Hosp. of D.C., Wash., D.C. 20009). Respiratory
syncytial virus disease in infants despite prior administration of antigenic in-
activated vaccine. Amer. J. Epid., 1969, 89: 422–434.—In response to three
injections of alum precipitated, 100X concentrated, formalin inactivated RS
vaccine (lot 100), 43% of infant vaccinees displayed a 4-fold or greater rise in
serum neutralizing antibody and 91% displayed a 4-fold or greater rise in
serum CF antibody. When RS virus became prevalent in the community, the
rate of RS virus infection in infants who received this vaccine was not remarkably
different from that in control infants who received parainfluenza vaccines. How-
ever, 80% of RS vaccinees required hospitalization at the time of RS infection
54 infants received RSV vaccine, 3 injections

- Toxic cell culture derived, monkey and human embryonic kidney cells
- Formaldehyde added
- Concentrated 4X and precipitated with aluminum (alum)
- Antibiotics added: polymixin B, streptomycin, neomycin
- Benzethonium chloride preservative (seizures, tumors, CNS)

Control: parainfluenza vaccine

- No antibiotics or preservatives added

Followed to observe incidence RSV infection
## Table 5

*RS virus infection and serious illness in comparable groups of infants receiving one or more injections of inactivated RS and parainfluenza vaccines*

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>No. infants</td>
<td>Age (mo.)</td>
<td>No. infants</td>
<td>Age (mo.)</td>
</tr>
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<td>RS lot 100</td>
<td>At risk*</td>
<td>20</td>
<td>5.1</td>
<td>25†</td>
</tr>
<tr>
<td></td>
<td>RS infection†</td>
<td>5</td>
<td>16</td>
<td></td>
</tr>
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<td></td>
<td>Hospitalized</td>
<td>4</td>
<td>12</td>
<td></td>
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<tr>
<td>Para 1 lot 23</td>
<td>At risk*</td>
<td>20</td>
<td>5.0</td>
<td>17†</td>
</tr>
<tr>
<td></td>
<td>RS infection†</td>
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<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospitalized</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trivalent parainfluenza lot 6279</td>
<td>At risk*</td>
<td>20</td>
<td>8.4</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>RS infection†</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospitalized</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Total parainfluenza</td>
<td>At risk*</td>
<td>20</td>
<td>5.0</td>
<td>37</td>
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<tr>
<td></td>
<td>RS infection†</td>
<td>2</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospitalized</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*No prior natural infection.
† As indicated by 4-fold or greater CF antibody rise only; reinfection counted only once.
‡ One infant was not available to follow up.
§ Mean age at peak of RS prevalence.
|| Per cent of infants at risk who sustained RS infection.
¶ Per cent of infected individuals who were hospitalized.
Conclusions: Valid?

- Vaccine did NOT prevent RSV infection
- No increased susceptibility to RSV
- Increased clinical severity of RSV
  - “We lack a definite explanation for this phenomenon…”
  - Antibodies may play a role

Validity
- Small study size
- Not randomized
- Improper control intervention
- Did not consider direct toxicity of vaccine, antibiotics, preservatives
11 children with atypical acute illness with rash in small town

All the children were exposed to "measles"

4 years earlier they were subjects in measles vaccine study

"Little is currently known about the etiology of such reactions, or the basic immunologic mechanisms involved."

Validity

- Small sample
- Case control design, not RCT
- 4-year delay suggests not related to vaccine
- Small geographic region suggests common exposure to toxin

Atypical exanthem following exposure to natural measles: Eleven cases in children previously inoculated with killed vaccine

A severe illness characterized by high fever, tachypnea, myalgia, prostration, and an atypical exanthem occurred after exposure to measles in children previously immunized with killed measles vaccine. Laboratory and epidemiological data were suggestive of recent measles infection. Other previously immunized children had local reactions at the site of injection of live measles vaccine. The host response to subsequent challenge with live measles virus is apparently sometimes altered by prior vaccination with inactivated measles vaccine, but the exact mechanisms remain obscure.

Philip R. Nader, M.D.,* Marshall S. Horwitz, M.D., and John Rousseau, M.D.

ATLANTA, GA., AND RIVERTON, WYO.
Naturally or artificially “infected” immunized cats with 2 strains of FIPV

No clinical information

Antibody studies in cell culture only

Found “ADE” in one strain only – what does it mean?

What happened to the cats?

How were they infected?

What vaccines did they receive?

Antibody-Dependent Enhancement of Feline Infectious Peritonitis Virus Infection in Feline Alveolar Macrophages and Human Monocyte Cell Line U937 by Serum of Cats Experimentally or Naturally Infected with Feline Coronavirus

Tsutomu HOHDATSU, Mika YAMADA, Ritsuko TOMINAGA, Kaori MAKINO, Kouji KIDA and Hiroyuki KOYAMA

Department of Veterinary Infectious Diseases, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034, Japan

(Received 3 July 1997/Accepted 28 August 1997)

Abstract. Infection of the type II feline infectious peritonitis virus (FIPV) strain 79–1146 to primary feline alveolar macrophages and human monocyte cell line U937 was enhanced by the sera of cats experimentally infected with the 79–1146 strain, but not those of cats infected with KU-2 or UCD-1 strain of type I FIPV. The experiments using sera of cats with feline infectious peritonitis (FIP) and of cats naturally infected with feline coronavirus (FCoV) revealed that infection of the FIPV 79–1146 strain to the U937 cells was enhanced only by the sera of cats infected with type II FIPV or feline enteric coronavirus. The samples positive for antibody-dependent enhancement (ADE) activity had high neutralizing antibody titers against the FIPV 79–1146 strain and the samples negative for ADE activity had low neutralizing antibody titers. These findings support the previous results where a monoclonal antibody with neutralizing activity had high ADE activity, suggesting that there was a close relationship between the neutralization and enhancement sites. And then it is also suggested that ADE of infection is likely to be induced by re-infection with the same serotype of virus in type II FIPV infection. Furthermore, U937 cells are considered useful and can be substituted for the feline macrophages for determining ADE of FIPV-infection.

— Key words: antibody-dependent enhancement of infection, feline infectious peritonitis virus, feline macrophage, U937 cell.
Thus, our data suggest that vaccination with rMVA expressing SARS-CoV S protein is associated with enhanced hepatitis."
Study Design: SARS

- Recombinant modified vaccinia virus Ankara (rMVA) expressing SARS-CoV S protein (rMVA-S) – similar to J&J
- 10-week-old castrated ferrets in solitary confinement, GMO food
- Control group 1: vaccine MVA (ferrets 1 to 3)
- Ferrets 4 to 6 ???
- Experimental group: rMVA-S (ferrets 7 to 9)
- Control group 2: PBS (ferrets 10 to 12)
- Day 0 and 14 by intraperitoneal and subcutaneous routes
- 14d later, ferrets challenged with SARS-CoV Tor2 isolate (intranasal)
What happened to the poor ferrets?

“…no clinical signs (e.g., elevated temperature and altered behavior including feeding) were observed up to 29 days post challenge…”

Transient elevation in AST, other LFTs normal

Animals sacrificed day 27 to 29

“…it is likely that the liver inflammation…in fact, may represent the recovering stage.”
All groups of ferrets showed clinical signs after challenge, indicating that ferrets became infected with SARS-CoV and that none of the vaccines tested blocked infection. All ferrets had increased temperatures after challenge, but significant differences between groups were not seen…

There was no apparent evidence of enhanced liver pathology…” (no intraperitoneal injections)

Gross pathology indicated frequent significant hemorrhage in lung and mediastinum (the area between the lungs containing the heart, trachea, esophagus, thymus and lymph nodes), specifically the thymus (Fig. 5), but other organs appeared largely normal.

Although all ferrets were sacrificed by day 6 post-challenge, our results did not suggest vaccine-induced immune enhancement of disease in any tissues.”
NO FERRETS DIED IN THE MAKING OF THIS PRESENTATION (they were killed by the scientists)
“Six- to eight-week-old, female Balb/c and C57BL/6 mice…were housed in cages covered with barrier filters…”

Given a variety of SARS vaccines and challenged with “SARS”

Sacrificed 2 days later, no clinical outcomes reported (none sick)

“…all animals exhibited pathologic changes after challenge…”

Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the SARS Virus

Chien-Te Tseng1,2, Elena Strana1, Naoko Iwata-Yoshikawa1,2, Patrick C. Newman1, Tania Garron1, Robert L. Atmar1,3, Clarence J. Peters1,2, Robert B. Couch1,4,5

1 Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, Texas, United States of America; 2 Center for Biodiversity and Emerging Disease, The University of Texas Medical Branch, Galveston, Texas, United States of America; 3 Department of Medicine, Baylor College of Medicine, Houston, Texas, United States of America; 4 Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas, United States of America

Abstract

Background: Severe acute respiratory syndrome (SARS) emerged in China in 2002 and spread to other countries before being brought under control. Because of a concern for reemergence or a deliberate release of the SARS coronavirus, vaccine development was initiated. Evaluations of an inactivated whole virus vaccine in ferrets and other nonhuman primates and a virus-like particle vaccine in mice induced protection against infection but challenged animals exhibited an immunopathologic lung disease.

Design: Four candidate vaccines for humans with or without alum adjuvant were evaluated in a mouse model of SARS, a VLP vaccine, the vaccine given to ferrets and NHP, another whole virus vaccine and an IDNA-produced S protein, Balb/c or C57BL/6 mice were vaccinated IM on day 0 and 28 and sacrificed for serum antibody measurements or challenged with live virus on day 50. On day 50, challenged mice were sacrificed and lungs obtained for virus and histopathology.

Results: All vaccines induced serum neutralizing antibody with increasing dosages and/or alum significantly increasing responses. Significant reductions of SARS-CoV two days after challenge were seen for all vaccines and prior live SARS-CoV. All mice exhibited histopathologic changes in lungs two days after challenge including all vaccinated Balb/c and C57BL/6 mice given live virus, influenza vaccine, or PBS suggesting infection occurred in all. Histopathology seen in animals given one of the SARS-CoV vaccines was uniformly a Th2-type immunopathology with prominent eosinophil infiltration, confirmed with special eosinophil stains. The pathologic changes seen in all control groups lacked the eosinophil prominence.

Conclusions: These SARS-CoV vaccines all induced antibody and protection against infection with SARS-CoV. However, challenge of mice given any of the vaccines led to occurrence of Th2-type immunopathology suggesting hypersensitivity to SARS-CoV components was induced. Caution in proceeding to application of a SARS-CoV vaccine in humans is indicated.


Editor: Stefan Riedstra, German Primate Center, Germany

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: rbcouch@bcm.edu
Lung Pathology

“Analyses: A. Mean lesion scores were different $p < .001$. DIV without alum greater than with alum $p = .001$, VLP without alum greater than with alum $p = .008$. Post-hoc comparisons: DIV lower than SV $p = .001$ and controls $p < .001$ but not VLP $p > .05$. SV lower than controls $p .048$.”
- Transgenic mice, groups of 6
- WIV – Whole Inactivated Virus vaccine
- Two IM injections, 3 weeks apart
- Groups
  - WIV alone
  - WIV plus aluminum hydrogel
  - WIV plus MF59 adjuvant - muramyl tripeptide linked to dipalmitoyl phosphatidyl ethanolamine
  - Control 1 – alum only
  - Control 2 – MF59 only
- Three weeks later challenged with MERS-CoV (intranasally)
- Sacrificed day 3 or 6
- No clinical outcomes!!

**Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus**

Anurodh Shankar Agrawal, Xinrong Tao, Abdullah Algaissi, Tania Garron, Krishna Narayanan, Bi-Hung Peng, Robert B. Couch, and Chien-Te K. Tseng

*Departments of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA; ‡Pathology, University of Texas Medical Branch, Galveston, TX, USA; ‡Internal Medicine, Division of Infectious Disease, University of Texas Medical Branch, Galveston, TX, USA; †Center for Biodefense and Emerging Infectious Disease, University of Texas Medical Branch, Galveston, TX, USA; †Department of Medical Laboratories Technology, College of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia

**ABSTRACT**

To determine if a hypersensitive-type lung pathology might occur when mice were given an inactivated MERS-CoV vaccine and challenged with infectious virus as was seen with SARS-CoV vaccines, we prepared and vaccinated mice with an inactivated MERS-CoV vaccine. Neutralizing antibody was induced by vaccine with and without adjuvant and lung virus was reduced in vaccinated mice after challenge. Lung mononuclear infiltrates occurred in all groups after virus challenge but with increased infiltrates that contained eosinophils and increases in the eosinophil promoting IL-5 and IL-13 cytokines only in the vaccine groups. Inactivated MERS-CoV vaccine appears to carry a hypersensitive-type lung pathology risk from MERS-CoV infection that is similar to that found with inactivated SARS-CoV vaccines from SARS-CoV vaccination.
“No gross pathology was noted on either day 3 or 6 (data not shown); however, histopathology was noted in all groups on both days.”

<table>
<thead>
<tr>
<th>Vaccination Groups</th>
<th>Day 3**</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum only</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MF59 only</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>WIV/Alum</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>WIV/MF59</td>
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</tr>
<tr>
<td>WIV only</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* Pathology severity scores (0-3): 0: no pathology, 1: mild, 2: moderate, and 3: severe.
** Day post challenge.
A Rumor of a Shadow of a Fingerprint

- No animals died from vaccination followed by challenge with toxic cell culture
- When clinical outcomes were observed, there was no difference between vaccinated and control groups that supported ADE/PP
- Results suggest vaccines and cell culture supernatants are sub-clinically to clinically toxic
- Human cohort studies suggest vaccines are toxic
- Antibody and cell culture experiments based upon false premise, should disregard