Natural and Vaccine-Induced Immunity to COVID-19 in Rhesus Macaques

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Two Critical Questions for COVID-19 Vaccine Development

• Is there natural protective immunity? Will individuals who recover from COVID-19 be protected against re-exposure?

• Is there vaccine-induced immunity? What are the immune correlates of protection?
Animal Models for COVID-19

• **Small animal models**
  - ACE2 transgenic mice
  - Ferrets
  - Hamsters

• **Large animal models**
  - Rhesus macaques
  - Cynomolgus macaques
SARS-CoV-2 infection protects against rechallenge in rhesus macaques

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DNA vaccine protection against SARS-CoV-2 in rhesus macaques

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SARS-CoV-2 Infection Study in Rhesus Macaques

• Animals: Indian-origin rhesus macaques (N=13)
  • 6-12 years old, SPF, mixed male/female

• Dose titration (N=9; 1 ml IN + 1 ml IT)
  • Group 1: $1.1 \times 10^6$ PFU (N=3)
  • Group 2: $1.1 \times 10^5$ PFU (N=3)
  • Group 3: $1.1 \times 10^4$ PFU (N=3)

• Necropsy for histopathology on day 2 and day 4 (N=4)
Viral RNA in BAL Following Challenge

Days Following Challenge

Group 1

Group 2

Group 3
Viral RNA in Nasal Swabs Following Challenge

Days Following Challenge
Subgenomic mRNA in Nasal Swabs Following Challenge

Days Following Challenge

Group 1

Group 2

Group 3
Viral RNA in Plasma Following Challenge

Days Following Challenge
S-Specific ELISA Responses Following Challenge

Days Following Challenge

Group 1

Group 2

Group 3

ELISA Titer

Days Following Challenge
Pseudovirus Neutralizing Antibody Responses Following Challenge

Days Following Challenge

Group 1

Group 2

Group 3
Live Virus Neutralizing Antibody Responses Following Challenge

Group 1

Group 2

Group 3

Days Following Challenge

David Martinez, Ralph Baric
Antibody Subclasses and Function Following Challenge
ELISPOT Responses Following Challenge

Days Following Challenge

Group 1

Group 2

Group 3
ICS Responses Following Challenge

Days Following Challenge

Group 1

% IFN+ / CD4+ CD3+ T Cells

% IFN+ / CD8+ CD3+ T Cells

Group 2

% IFN+ / CD4+ CD3+ T Cells

% IFN+ / CD8+ CD3+ T Cells

Group 3

% IFN+ / CD4+ CD3+ T Cells

% IFN+ / CD8+ CD3+ T Cells
Tissue Viral RNA Following Necropsy

Day 2

Day 4

Log RNA Copies / g

Nares / Pharynx, Trachea, Lung, Trach LN, Distal LN, Spleen, Tonsill, GI, Liver, Kidney
Extensive Lung Inflammation by IHC

SARS CoV2 RNA | MPO | CD4-CD68-CD163 | CD8 | MX1
---|---|---|---|---
A | B | C | D | E

SARS CoV2

Uninfected

F | G | H | I | J

Jake Estes
Extensive Lung Inflammation by IHC

A

Lung Alveoli PMN Infiltration (PMNs/mm²)

P = 0.0296

B

% Area Total Lung Mx1+

P = 0.0286
SARS-CoV-2 Re-Challenge Study

• Do rhesus macaques that have recovered from SARS-CoV-2 infection have immunity against re-challenge?

• 9 animals in original infection study plus 3 naïve positive control animals re-challenged with SARS-CoV-2 on day 35

• Same dose as in original infection study (1 ml IN + 1 ml IT)
  • Group 1: $1.1 \times 10^6$ PFU (N=3)
  • Group 2: $1.1 \times 10^5$ PFU (N=3)
  • Group 3: $1.1 \times 10^4$ PFU (N=3)
Viral RNA in BAL Following Re-Challenge

Days Following Re-Challenge

Group 1

Group 2

Group 3

Naive
Viral RNA in Nasal Swabs Following Re-Challenge

Days Following Re-Challenge

Group 1

Group 2

Group 3

Naive
Subgenomic mRNA in Nasal Swabs Following Re-Challenge

Days Following Re-Challenge
Days Following Challenge or Re-Challenge

Primary Challenge

Re-Challenge

Log sgmRNA Copies / Swab

Days Following Challenge or Re-Challenge

P=0.0003

Peak Log sgmRNA Copies / Swab

Primary Re-challenge
Anamnestic Immune Responses Following Re-Challenge

Days Following Re-Challenge

- Log ELISA Titer: $P=0.0034$
- Log Pseudovirus NAb Titer: $P=0.0003$
- Log Virus NAb Titer: $P=0.0003$
- Log SFC / $10^6$ PBMC: $P=0.1837$
SARS-CoV-2 Re-Challenge Study

- Rhesus macaques infected with SARS-CoV-2 show high amounts of virus in the upper and lower respiratory tract and pathologic features of viral pneumonia.

- Recapitulates key features of SARS-CoV-2 infection in humans, but not a model of severe COVID-19 disease.

- SARS-CoV-2 infection induces robust humoral and cellular immunity and dramatically protects against re-challenge, demonstrating natural protective immunity.

- Protection probably not sterilizing but instead likely mediated by rapid immunologic control.
SARS-CoV-2 DNA Vaccine Study

• Goal is to assess immunogenicity and protective efficacy of prototype vaccines against SARS-CoV-2 in rhesus macaques and to define immune correlates of protection

• To accomplish this, we evaluated prototype DNA vaccines expressing 6 variants of the SARS-CoV-2 Spike protein

• Aim is to advance our understanding of vaccine immunity and is not a test of a vaccine product in clinical development
Design of Prototype DNA Vaccines
Expression from Prototype DNA Vaccines

Lysate

<table>
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<tr>
<th>S</th>
<th>S.dCT</th>
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Supernatant

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Lysate

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Study Design

• 35 rhesus macaques, ages 6-12, mixed gender

• DNA vaccines (N=25)
  • N=4-5 for each vaccine (S, S.dCT, S.dTM, RBD, S1, S.dTM.PP)

• Sham controls (N=10)

• Dose: 5 mg, IM
• Schedule: week 0, 3
• Challenge: week 6, 1.2x10^8 vp (1.1x10^4 pfu) SARS-CoV-2
ELISA Responses

Week 0

Week 5
Pseudovirus NAb Responses
Live Virus NAb Responses

Week 0

Week 5

Virus NAb Titer

Sham  S  S.dCT  S.dTM  Sf  RBD  S.dTM.PP

Sham  S  S.dCT  S.dTM  Sf  RBD  S.dTM.PP

David Martinez, Ralph Baric
Pseudovirus and Live Virus NAb Titer Correlations

Log Virus NAb Titer

Log Pseudovirus NAb Titer

P<0.0001
R=0.8052
Comparison of NAb Titers in Vaccinated NHPs, Convalescent NHPs, Convalescent Humans
ELISPOT Responses

Week 0

Week 5

SFC / 10^6 PBMC

Sham  S  S_dCT  S_dTM  S_1  RBD  S_dTM_PP

Sham  S  S_dCT  S_dTM  S_1  RBD  S_dTM_PP
IFN+ CD4+ and CD8+ T Cell Responses
IL-4+ CD4+ and CD8+ T Cell Responses
Viral Loads (sgmRNA) Following Challenge

**BAL**

- **Sham**
- N=10

**Nasal Swab**

- **Sham**
- N=10

Days Following Challenge

Log sgmRNA Copies

0 2 4 6 8 10 12 14
Days Following Challenge

Log sgmRNA Copies / Swab

Nasal Swab

S

S.dCT

S.dTM

N=4

N=4

N=3

S1

RBD

S.dTM.PP

N=4

N=4

N=5

Days Following Challenge
Viral Loads (sgmRNA) Following Challenge

**BAL Nasal Swab**

- **P=0.03**

**Nasal Swab**

- **P=0.01**
Pseudovirus NAb Titers Inversely Correlate with Peak sgmRNA Titers in BAL and Nasal Swabs

**BAL**

\[ P < 0.0001 \]
\[ R = -0.6877 \]

**Nasal Swab**

\[ P = 0.0199 \]
\[ R = -0.4162 \]
Live Virus NAb Titers Inversely Correlate with Peak sgmRNA Titers in BAL and Nasal Swabs

**BAL**

P<0.0001
R=-0.7702

**Nasal Swab**

P=0.1006
R=-0.3360
NAAb Titers Represent the Principal Correlate of Protection But ADCD Responses Also Contribute
NAb and ADCD Responses Differentiate Animals with Complete Protection vs Partial Protection

P=0.0004

P=0.0001

P=0.0010

P=0.0005
Anamnestic NAb Responses Suggest Rapid Immunologic Control Rather than Sterilizing Immunity
Anamnestic ELISPOT Responses Suggest Rapid Immunologic Control Rather than Sterilizing Immunity
SARS-CoV-2 DNA Vaccine Study

- Prototype DNA vaccines expressing six S variants induced humoral and cellular immune responses in rhesus macaques; full-length S immunogen provided optimal protection

- 8 of 25 animals showed no virus in BAL or nasal swabs following SARS-CoV-2 challenge; remainder showed reduced viral loads

- Vaccine-elicited NAb titers (by both pseudovirus and live virus neutralization assays) correlated with protective efficacy

- Protection probably not sterilizing but instead likely to be mediated by rapid immunologic control
Conclusions

• These studies demonstrate natural protective immunity and vaccine-induced immunity to SARS-CoV-2 in macaques

• Our data suggest that NAb titers may be a useful biomarker and correlate of protection for vaccines

• These are proof-of-concept studies in animals and any conclusions for humans must await rigorous clinical studies
**Acknowledgements (Re-Challenge Study)**

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*University of North Carolina*

| Michael Nekorchuk                           | Hanne Anderson | Ralph Baric    |
| Kathleen Busman-Sahay                      |                 |                 |
| Margaret Terry                             |                 |                 |
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**Acknowledgements (Vaccine Study)**

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Viral RNA Following Challenge

Days Following Challenge

Log Viral RNA Copies

BAL
Sham

Nasal Swab
Sham
Days Following Challenge

[Graphs showing Log Viral RNA Copies/ml for different samples: S, S.dCT, S.dTM, S1, RBD, S.dTM.PP]
Viral RNA Following Challenge

**BAL**

- **P=0.02**

**Nasal Swab**

- **P=0.04**

**Nasal Swab**

- **P=0.04**
ELISA Titers Inversely Weakly Correlate with Peak sgmRNA Titers in BAL and Nasal Swabs

- **BAL**
  - $P=0.0041$
  - $R=-0.4733$

- **Nasal Swab**
  - $P=0.2712$
  - $R=-0.2039$
ELISPOT Responses Do Not Correlate with Peak sgmRNA Titers in BAL and Nasal Swabs

For BAL:
- Log sgmRNA Copies/ml vs Log ELISPOT SFC / 10^6 PBMC
- P=0.9258
- R=0.0196

For Nasal Swab:
- Log sgmRNA Copies/Swab vs Log ELISPOT SFC / 10^6 PBMC
- P=0.6037
- R=-0.1025
CD4 ICS Responses Do Not Correlate with Peak sgmRNA Titers in BAL and Nasal Swabs

BAL

P=0.4829
R=-0.1383

Nasal Swab

P=0.8855
R=0.0303
CD8 ICS Responses Do Not Correlate with Peak sgmRNA Titers in BAL and Nasal Swabs
Anamnestic ELISA Responses Suggest Rapid Immunologic Control Rather than Sterilizing Immunity
Anamnestic NAb Responses Suggest Rapid Immunologic Control Rather than Sterilizing Immunity