Human B Cell Responses to Influenza Virus Vaccination
Influenza Virus

- Enveloped, single-stranded, negative-sense RNA virus with segmented genome.
- 3 types: A, B and C.
Influenza Virus Hemagglutinin (HA)

- Mediates virus binding to host cells
- Main target for neutralizing antibody responses
- 18 subtypes
Influenza Virus Hemagglutinin (HA) Subtypes

Group-1

Group-2

- H1
- H2
- H3
- H4
- H5
- H6
- H7
- H8
- H9
- H10
- H11
- H12
- H13
- H14
- H15
- H16
Influenza vaccine

- Two types of currently licensed vaccines:
  - **Inactivated** virus
  - Live attenuated virus

Trivalent \( \left\{ \begin{array}{c} \text{Influenza A/H3} \\ \text{Influenza A/H1} \\ \text{Influenza B} \end{array} \right\} \)
Rapid influenza specific plasmablast (ASC) responses after vaccination

Cloning Ig genes from influenza-specific ASC

Rapid method for generating human mAbs

Recombinant monoclonal antibodies

B Cell Responses: Division of Labor

Naïve or Resting Memory B Cell → Activated B Cells → Memory B Cells

Plasmablasts or ASC
Defining activated B Cells (ABC) after influenza vaccination

Gated on CD3\textsuperscript{neg}, CD19\textsuperscript{pos}

Day 0

Day 7

CD71

IgD

0.519

2.01

CD38

CD71\textsuperscript{hi}

IgD\textsuperscript{neg}

CD20

54.8

43

\textbf{- CD71} (TFRC, Transferrin Receptor, TfR) is a cell surface iron transport receptor that is upregulated in proliferating cells (Ki67 is intracellular)
Only ASC spontaneously secrete antibodies

- Day 7 after vaccination PBMC

*Gated on CD3^{neg}, CD19^{pos} IgD^{neg} CD71^{hi}
ABC represent a distinct lineage from ASC

We compared the gene expression profiles of naïve B cells, resting memory B cells, activated B cells and plasmablasts
Sorting of different B cell subsets at day 7 post-influenza vaccination
ABCs are distinct from ASCs and resting memory B cells
Using HA to label Ag-specific ASC and ABC after influenza vaccination

- HA is from the 2009 pandemic H1N1 virus

Gated on CD3/14/16\textsuperscript{neg}, CD19\textsuperscript{pos}, IgD\textsuperscript{neg}

Day 0

HA

CD20

ASC

Day 7

ABC

0.113

2.09

0.631

3\textsuperscript{-fold} dilution

61

0

67

0

H1 HA

H1 HA

IgG

IgG

HA+ ASC

HA+ ABC

HA+ ASC

HA+ ABC

HA+ ABC

(after 5-day in vitro culture)
Kinetics of the ABC response after influenza vaccination

*Gated on CD3/14/16$^{\text{neg}}$, CD19$^{\text{pos}}$, IgD$^{\text{neg}}$
Summary

- Antigen-specific activated B cells (ABCs) and ASCs emerge in blood at day 7 post-influenza vaccination

- ABCs are functionally and transcriptionally distinct from resting memory B cells and ASCs

- Can identify antigen-specific B cells induced by influenza vaccination and track them at later time points as they join the memory B cell pool
Questions we can address:

1. Are the same B cell clones present in the activated B cell and plasmablast compartments?

2. Are there any differences in the level of somatic hypermutation (SHM) in B cell clones present in the activated B cell and plasmablast compartments?

3. Does TIV immunization increase the levels of SHM in the responding B cells?
Study design

Days after TIV immunization

0 7 14 21 28 90

Sort
Resting MBCs
HA+ ABCs
IgG+ ASCs
HA+ ABCs
Resting MBCs
Sort
Resting MBCs
Sort
Resting MBCs

RNA
Deep sequencing of the BCR
Questions we can address:

1. Are the same B cell clones present in the activated B cell and plasmablast compartments?

2. Are there any differences in the level of somatic hypermutation (SHM) in B cell clones present in the activated B cell and plasmablast compartments?

3. Does TIV immunization increase the levels of SHM in the responding B cells?
Same B cell clones can be detected in the ABC and ASC compartments

![Bar graph showing percentage of HA+ ABC lineages detected in IgG+ ASCs for donors 4, 6, and 8.](image)
Similar clonal expansion within the ABC and ASC compartments

Donor 4

\[ R^2 = 0.56 \]
\[ p < 0.0001 \]

Donor 6

\[ R^2 = 0.79 \]
\[ p < 0.0001 \]

Donor 8

\[ R^2 = 0.96 \]
\[ p < 0.0001 \]
Questions we can address:

1. Are the same B cell clones present in the activated B cell and plasmablast compartments?

2. Are there any differences in the level of somatic hypermutation (SHM) in B cell clones present in the activated B cell and plasmablast compartments?

3. Does TIV immunization increase the levels of SHM in the responding B cells?
Similar levels of mutations within the ABC and ASC compartments
Questions we can address:

1. Are the same B cell clones present in the activated B cell and plasmablast compartments?

2. Are there any differences in the level of somatic hypermutation (SHM) in B cell clones present in the activated B cell and plasmablast compartments?

3. Does TIV immunization increase the levels of SHM in the responding B cells?
Minimal changes in SHM levels of HA-specific B cells responding to TIV immunization

[Graph showing minimal changes in SHM levels for different donors over time]
Expansion of HA+ B cells following TIV vaccination

Donor 4

Donor 6
Tracking the evolution of individual HA+ clonal lineages

Donor 4
Tracking the evolution of individual HA+ clonal lineages

Donor 6
Conclusions

- Activated B cells are a phenotypically, functionally and transcriptionally distinct B cell subset that can be detected as early as 7 days after influenza vaccination

- Influenza vaccination-induced B cell clonal lineages are shared between the ABC and the ASC compartments

- ABC clonal lineages persist in the memory B cell pool

- Immunization with TIV does not induce a significant increase in the levels of SHM in responding B cells. Important implications for vaccination strategies.
Acknowledgement

Emory Vaccine Center
  Ali Ellebedy
  Carl Davis
  Rama Akondy
  Haydn Kissick

Stanford University
  Scott Boyd
  Katherine Jackson

St. Jude Children’s Research Hospital
  Paul Thomas
  Richard Webby
  Christine Oshansky

Clinical
  Aneesh Mehta
  Edmund Waller
  Rivka Elbein
  Nicole Battle
  Shine Thomas

Mount Sinai School of Medicine
  Peter Palese
  Adolfo Garcia-Sastre
  Florian Krammer

Bioinformatics
  Helder Nakaya

Sorting facility
  Sommer Durham
  Bob Karaffe
The Zika Virus

It's a mysterious illness with devastating effects. Is the next public health crisis in your backyard?

Plus:
The controversial plan to genetically manipulate mosquitoes out of existence.
Cross-reactivity of dengue-specific antibodies against Zika virus
Immune responses against Dengue infection

Collaboration with Siriraj Hospital, Bangkok


Kwissa et al. Dengue virus infection induces expansion of a CD14(+)CD16(+) monocyte population that stimulates plasmablast differentiation. *Cell Host Microbe* 2014.

Zika Virus

Key issues in understanding Zika virus pathogenesis and immunopathology:

• Cross-reactivity of pre-existing immunity against other Flaviviruses (Dengue, YFV, WNV, JEV)

• Does this pre-existing immunity protect or cause antibody dependent enhancement of disease?
## Percentage homology of Zika virus amino acid identity with other flaviviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zika (PR)</td>
<td>--</td>
</tr>
<tr>
<td>Den 1</td>
<td>57</td>
</tr>
<tr>
<td>Den 2</td>
<td>52</td>
</tr>
<tr>
<td>Den 3</td>
<td>57</td>
</tr>
<tr>
<td>Den 4</td>
<td>55</td>
</tr>
<tr>
<td>WNV</td>
<td>54</td>
</tr>
<tr>
<td>JEV</td>
<td>54</td>
</tr>
<tr>
<td>YF-17D</td>
<td>41</td>
</tr>
</tbody>
</table>

Adapted from Yvonne (Bonnie) Maldonado, MD, UCSF
Serum samples from Dengue infected patients bind Zika virus
Serum samples from Dengue infected patients can neutralize Zika virus
Summary of Zika virus neutralization by dengue sera

FRNT50 titer

- Dengue acute
- Dengue convalescent
- Naive control
Is the “cross-reactivity” of dengue sera to Zika virus due to truly cross-reactive antibodies or is it due to the presence of dengue-specific and Zika-specific antibodies in the sera of these individuals?

• To address this question we analyzed human monoclonal antibodies derived from PCR confirmed dengue infected patients. These monoclonal antibodies were isolated from plasmablasts showing that these B cells were responding to dengue infection
Dengue-specific monoclonal antibodies react with Zika virus

A

Binding to ZIKV lysate

B

Binding to ZIKV whole virus

C

Neutralization of ZIKV

D

Neutralization of DENV2

DENV-reactive human monoclonal antibodies
Summary

• Over 50% (26/47) of the dengue specific antibodies isolated during acute dengue infection cross-react to Zika virus in a binding assay

• 7 of these 26 mAbs showed neutralizing activity against Zika virus

• Neutralizing titers ranged from weak to very potent

What about antibody mediated enhancement (ADE) of Zika infection?
Human dengue-specific monoclonal antibodies isolated following dengue infection can enhance Zika virus infection in vitro.
In vivo models of Zika virus infection

• Preliminary data from animal models suggests that Zika virus readily infects both mice and primates

• Good model systems to test:
  • Protective immunity vs. enhancement of Zika infection
Acknowledgement

**Emory Vaccine Center**
Jens Wrammert  
Lalita Priyamvada  
Siddharta Bhaumik

Mehul Suthar  
Kendra Quicke  
William Hudson

**Emory Dept. of Pediatrics**
Anita McElroy

**Emory VTEU**
Mark Mulligan/Paul Spearman  
and their teams

**Siriraj Hospital, Bangkok, Thailand**
Kulkanya Chokephaibulkit  
and her team
Kovit Pattanapanyasat  
and his team

**University of Chicago**
Dr. Patrick Wilson and  
his team