Antibody Dependent Cellular Cytotoxicity in HIV protection

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Antibody Dependent Cellular Cytotoxicity

1. Antibody-dependent cellular cytotoxicity (ADCC) is one mechanism used by the immune system to destroy cells infected with intracellular pathogens.

2. Acquired immune responses establish the specificity of ADCC by generating antibodies (Abs) capable of binding viral antigens (Ags) on the surface of virus-infected cells.

3. The innate immune system provides peripherally located Fcγ receptor-expressing cells, including natural killer (NK) cells, monocytes/macrophages, and subsets of γδ T cells, which constitute the effectors of ADCC.
Antibody Dependent Cellular Cytoxicity (ADCC)/ Antibody Dependent Cellular Viral Inhibition (ADCVI)

HIV-1-infected Cells: CD4+ T; Monocytes; DC

Effector Cells: FcγR-bearing cells: NK; Monocytes/Macrophages; γδ T cells

Ab-Fab

Ab-Fc

γ-R

Difference in Antigen recognition

≠

Neutralizing Ab = prevention of virus entry

ADCC

ADCVI

Cytokine/Chemokines = Cell activation

Viral inhibition

= GranzymeB: Cytotoxicity
Mechanisms of HIV-1 Entry & Role of Antibodies

Lumen
Stratified Squamous Epithelium

HIV-1

Columnar Epithelium

HIV-1-infected cells

Mucus Layer

CD4 T cells

Langerhan/dendritic cells

HIV-1-CD4, CCR5, α4β7 interactions

Macrophage FcR-mediated phagocytosis of virions and ADCC of virus infected CD4 T cells.
NK cell FcR-mediated ADCC

Neutralizing Activity

Ab Fc-Fc Receptor Mediated Activities

CD4 T cells

Virus-infected cells
Background

- Neutralizing antibodies (passive and active immunization) can prevent HIV-1 or SIV infection through intravenous or vaginal routes of challenge in nonhuman primates (Mascola et al, Barouch et al).

- ADCC response correlates with protection after active and passive immunization in NHP (Gómez-Román et al., Hessler et al., Alpert et al).

- ADCC responses inversely correlate with rates of HIV-1 disease progression (Baum et al., Lambotte et al.).

- ADCC responses prevent mother-to-children transmission via breast milk (Overbaugh et al.).

- ADCC responses detected in the RV144 study in subjects with low IgA responses to HIV correlated with low risk of infection (Haynes et al.).
Topics of this presentation

- Chronological appearance and breadth of ADCC responses during HIV-1 infection.

- Impact of ADCC response on controlling virus replication during HIV-1 infection.

- Specificity of RV144 vaccine induced ADCC responses compared to those induced by natural infection.

- Competition between IgA and IgG responses induced by RV144 vaccine.
How early can we detect ADCC after acute HIV-1 Infection?

How does ADCC Ab responses correlate to Ab responses to Env?
Neutralizing and ADCC Ab against autologous Transmitted/Founder HIV-1 were detectable earlier than heterologous Ab responses. ADCC Ab parallel anti-gp120 responses more than anti-gp41.

Pollara J., Ferrari G., unpublished
Conclusions

- The appearance of ADCC-mediating Ab responses is asynchronous with that of Ab with different functions (i.e. ADCC Ab = months, NAb = years).
- This is consistent with the fact that Abs that mediate ADCC recognize HIV-infected cells instead of free virions do not necessarily share the same breadth and specificities of neutralizing Abs.
Are the early ADCC responses broadly reactive?
Cross-clade ADCC following acute subtype B (n=3) and C (n=3) HIV-1 infection against subtype AE-infected target cells.

Cross-clade ADCC against Clade A/E HIV-infected target cells is detectable within 2 months of transmission for 3/3 Clade B infected and 1/3 Clade C infected individuals, and within 1.5 years for all.

Pollara J., Ferrari G., unpublished
Are ADCC responses correlating with control of virus replication?
Relationship between ADCC responses and control of virus replication.

Lambotte, Ferrari; Submitted
Study in HIV-1 Infection: Conclusions

1. Anti-gp120 Ab responses seem to correlate better than anti-gp41 Ab with ADCC detected during HIV-1 infection.

2. Cross-clade ADCC responses are detectable as early as 30 days after estimated HIV-1-infection compared to 2.5 years for broadly neutralizing Ab.

3. ADCC responses correlate with control of virus replication; interestingly, this is more significant in HLA-57 negative individuals.
What ADCC epitope specificities are elicited by vaccine strategy compared to natural infection?
RV144 Vaccination and Follow-up Schedule

6-month vaccination schedule

- ALVAC®-HIV (vCP1521) priming at week 0, 4, 12, 24
- AIDSVAX® B/E gp120 boosting at week 12, 24

3 years of follow-up (every 6 mo.)

16,402 Volunteers
Vaccine:Placebo = 1:1
RV144 Vaccine Efficacy Trial in Thailand

31.2% Estimated Vaccine Efficacy
(Rerks-Ngarm, NEJM 361: 2209, 2009)

Immune Correlates Study Completed
Two Correlates of Infection Risk Found
(Haynes, NEJM 366: 1275, 2012)

1. IgG antibodies that bind to a V1V2 recombinant fusion protein correlated \textit{inversely} with infection rate. (Higher V1V2, lower infection rate)

2. Env binding plasma (monomeric) IgA correlated \textit{directly} with infection rate. (Higher IgA to Env, \textit{higher} infection rate).

**Interaction Secondary Analyses:**
High IgA and high ADCC: No Protection
Low IgA and high ADCC: Protection
We approach the problem of characterizing the Ab responses elicited by the RV144 vaccine regimen by generating mAbs from the vaccine recipients.
Vaccine-induced ADCC monoclonal Abs

1. C1-IgG – (19) CH40, CH49, CH51-CH55, CH57-CH59, CH77-CH78, CH80, CH81, CH89-CH91

1. V1V2-IgG - (2) CH58, CH59

2. V3-IgG - (2) CH21, CH23

3. Unknown Conformational-IgG - (1) CH20
New RV144 V2 Human MAbs

CH58: ELRDKKKQKVHALFYKLDIVPIED

CH59: ELRDKKKQKVHALFYKLDIVPIED

HG107: ELRDKKKQKVHALFYKLDIVPIED

HG120: ELRDKKKQKVHALFYKLDIVPIED

Liao, Bonsignori, Haynes; Immunity 2013
RV144 Virus Genetic Analysis (Rolland and Kim, Nature Sept 10, 2012)

1. Compared breakthrough viruses in vaccinees vs. placebo recipients

2. Found increased vaccine efficacy (48%) when K169 in V2 (virus matched vaccine—site of immune pressure)
Do mutations in the V2 169 aa residue affect ADCC mediated by these three anti-V2 RV144 mAbs?
Recognition of gp120 variants by anti-V2 mAb

AE.703357 gp120 represents a transmitted/founder isolate from a breakthrough RV144 vaccinees.

Amino acid residues representing escape mutants were replaced with those present in the vaccine immunogen.

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Are RV144 anti-V2 mAb recognizing the same region as the anti-V2 mAb generated from chronically infected subjects?
Epitopes in the gp120 trimer association domain (TAD)
RV144 CH58 mAb epitope
PG9/16 and CH01-04 epitopes

Asn 156 glycan

Asn 160 glycan
Anti-V2 Responses Elicited by Natural HIV1-Infection versus AIDS RV144 Vaccine

- Anti-V2 responses were more prevalent in the RV144 clinical trial compared to natural infection:
  - anti-V2 responses elicited in 97% of the Thai vaccine recipients
  - Only detectable in 50% of the HIV-1 CRF01_AE-infected Thai individuals (Karasavvas N, AIDS Res Hum Retroviruses 28: 1444; 2012).

- Anti-V2 mAbs isolated from RV144 vaccine recipients have different specificities from those isolated from HIV-1 infected individuals:
  - Vaccine-induced CH58 and CH59 mAbs recognized a linear V2 epitope amino acid residues 163-181 (Liao H-X, Immunity 38: 176; 2013)
  - Binding of CH58 and CH59 is not affected by the presence of glycans (Liao H-X, Immunity 38: 176; 2013).
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Interaction Secondary Analyses:
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Model of Monomeric Plasma IgA gp120 Antibodies blocking IgG ADCC (via NK)
How can the hypothesis that specific HIV IgA responses block functional HIV IgG responses be tested?
• Determine relative levels of Env specific IgA vs. IgG in plasma.

• Determine whether there are differences in affinity of Env specific IgA and IgG monoclonal antibodies from RV144.

• Determine whether RV144 IgA mAbs can inhibit ADCC (via NK cells) by IgG mAbs of similar specificity.
Does the ratio of Env Specific IgA/IgG provide information about relative risk of infection?
### Odds Ratio for Env IgA binding alone or relative to Env IgG (IgA/IgG ratio).

<table>
<thead>
<tr>
<th>Envelope Protein/Peptide</th>
<th>Odds Ratio</th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>Vaccine Strain Clade AE.A244gp120&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.28</td>
<td>0.72</td>
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<tr>
<td>Env Panel IgA Primary Score&lt;sup&gt;1,3&lt;/sup&gt;</td>
<td>1.39</td>
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<tr>
<td>CRF01 AE.C1 Peptide</td>
<td>1.69</td>
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<tr>
<td>Clade A.1ConEnv gp140</td>
<td>1.57</td>
<td>0.87</td>
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<tr>
<td>Con6 gp120</td>
<td>1.01</td>
<td>0.90</td>
</tr>
<tr>
<td>Non-HIV&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.01</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**Significant Increased Risk of Infection for IgA/IgG Ratio or for IgA**

**No correlation with HIV-1 infection risk**

Tomaras G., Ferrari G., PNAS 2013
Isolation of IgA Env Binding Mabs and ADCC Mediating Mabs from RV144 vaccinees

1. 2 IgA HIV-1 binding mAbs from RV144
   – CH38- IgA2 and CH29- IgA2

2. IgG ADCC mAbs from RV144
   – CH54, CH57-IgG (same donor as CH38), and CH81-IgG (same donor as CH29)
   – Specific for the C1 region of gp120.
RV144 Monoclonal antibodies of IgA and IgG origin Bind with Different Affinity to HIV-1 Env

Binding affinity to A244Δ11 gp120

CH38 IgA
- $k_a = 11.0 e^4 \text{ M}^{-1}\text{s}^{-1}$
- $k_d = 5.83 e^{-4} \text{ s}^{-1}$
- $K_d = 4.8 \text{ nM}$

CH38 IgG
- $k_a = 4.08 e^4 \text{ M}^{-1}\text{s}^{-1}$
- $k_d = 5.83 e^{-4} \text{ s}^{-1}$
- $K_d = 14.3 \text{ nM}$

CH57 IgG
- $k_a = 7.32 e^4 \text{ M}^{-1}\text{s}^{-1}$
- $k_d = 4.28 e^{-2} \text{ s}^{-1}$
- $K_d = 585 \text{ nM}$
CH38 IgA Inhibits ADCC of CM235 Infected Target Cells by RV144 C1 Region IgG mAbs

Tomaras G., Ferrari G., PNAS 2013
Conclusions

1. Infected RV144 Vaccinees are enriched for higher HIV-1 Env IgA/IgG plasma ratios for some Env specificities.

2. CH38 IgA mAb has ~ 2 logs higher avidity for HIV-1 Env than CH57 IgG mAb (same Env specificity from same vaccinee).

3. Purified RV144 Plasma IgA from some vaccinees are C1 region specific- similar to ADCC Ab generated from RV144.

4. CH38 IgA mAb can block IgG mAb mediated ADCC (via NK cells).
Mapping conformational epitopes: Fab blocking

- mAb Fab fragments of known specificities used to block ADCC activity of isolated mAbs

- Fabs used:
  - A32: C1 epitope that represents a major target of ADCC Abs in natural infection
  - 17b: CD4i coreceptor binding site
  - 19B: V3 loop
ADCC Fab blocking assay

HIV_{CM235}^-infected Target Cells (Clade A/E)

ADCC activities of 19/20 conformation-dependent mAbs were inhibited by A32 Fab.

Bonsignori/Pollara//Moody/Munir/Liao/Haynes/Ferrari; Journal of Virology 2012
Each RV144 A32-blockable mAb displays a unique profile of recognition of gp120; and overall these RV144 mAbs differ from those isolated from infected individuals.
The anti-Env ADCC responses detectable during acute infection follow the appearance of anti-gp120 Ab responses.

What is the role of the early binding anti-gp41 responses? Can we exclude their role in ADCC responses?

The profile of epitope specificity of anti-V2 ADCC Abs elicited by natural HIV-1 infection differs from that elicited by RV144 vaccine and its impact on protection has to be determined.

RV144 IgA from vaccinees are C1 region specific and can block ADCC function mediated by IgG Ab responses. To balance vaccine induced IgA and IgG responses has to be considered in designing new vaccine strategies.
Acknowledgements

1. 16,402 Thai men and women who participated in the trial...

2. SCHARP team including
   Peter Gilbert, Mark Bollenbeck, Christine Cooper-Trenbeath
   Cheryl DeBoer, Allan DeCamp
   Youyi Fong, Erin Gabriel
   Raphael Gottardo. Linda Harris
   Tomer Hertz, Drienna Hollman
   Ying Huang, Yunda Huang, Holly Janes, Craig Magaret
   Zoe Moodie, Cindy Molitor
   Daryl Morris, Laura Saganic
   Alicia Sato, Steve Self
   Xuesong Yu

3. Thai RV144 Clinical Trials team including
   Supachai Rerks-Ngarm, Punee Pitisittithum

4. RV144 Immune Correlate Leadership team including
   Bart Haynes,
   Jerome Kim,
   Nelson Michael
   M. Julie McElrath
   Kelly Soderberg,
   Charla Andrews
Acknowledgments

ADCC lab
  Justin Pollara
  Joy Pickeral
  Kaylan Whitaker

Tony Moody and Lab

Mattia Bonsignori and Lab
  Daniel Kozink
  Kwan-Ki Hwang
  Chun-Yen (Jim) Tsao

Hua-Xin (Larry) Liao and Lab
  Xi (Stephen) Chen

Barton Haynes and Lab
  Robert Parks
  Krissey Lloyd
  Bradley Lockwood

Feng Gao and Lab

Munir Alam and Lab

David Montefiori and Lab

Georgia Tomaras and Lab

DHVI Flow Cytometry Core Facility
  Dawn Marshall
  John Whitesides

CHAVI and CHVI-ID Leadership
  Kelly Soderberg
  Thomas Denny
Collaborating Organizations

- GSID: Global Solutions for Infectious Diseases
- MOPH-TAVIK: Ministry of Public Health - Thailand Avalokitesvara Institute
- WRAIR: Walter Reed Army Institute of Research
- NIAID: National Institute of Allergy and Infectious Diseases
- sanofi pasteur: The vaccines division of sanofi-aventis Group
- MHRP: US Agency for HIV Research Program
- CAVD: Collaboration for AIDS Vaccine Discovery
- Bill & Melinda Gates Foundation
- Global HIV Vaccine Enterprise
- CHAVI: Center for HIV/AIDS Vaccine Immunology
- Vaccine Research Center: National Institute of Allergy and Infectious Diseases
- University of Washington
- HIV Vaccine Trials Network
- CFAR: Centers for AIDS Research
Acknowledgements

Supported by:

Bill and Melinda Gates Foundation
B Cell Lineage Envelope Immunogen Design from RV144 Antibodies - Haynes
Role of IgA in HIV-1 Protection - Haynes
CAVD-VIMC

National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)
Division of AIDS (DAIDS)
U.S. Department of Health and Human Services (HHS)

Center for HIV/AIDS Vaccine Immunology (U19AI067854)
U.S. Military HIV Research Program (MHRP)
Henry Jackson Foundation
CALL FOR ABSTRACTS

4th Symposium of Infectious Diseases in Africa: “Immune escape from HIV-1, Malaria and TB: implications for vaccine design” 30 August – 1 September, 2013

&


For more information and abstract submissions, please contact or send to the meeting secretariat at carinak@nicd.ac.za Deadline: 10 June, 2013

IDA Symposium Co-Organizers: Dr. Clive Gray, University of Cape Town
Dr. Guido Ferrari, Duke University

ICS Workshop Co-Organizers: Dr. Wendy Burgers, University of Cape Town
Dr. Tom Scriba, University of Cape Town
Topic of the Symposium

Immune escape from HIV-1, Malaria and TB: implications for vaccine design.
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Immune escape from HIV-1, Malaria and TB: implications for vaccine design.

Travel support

12 full scholarships will be awarded to young African scientists to attend the Symposium and the Workshop.
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Dr. Richard Koup – Vaccine Research Center, USA
Dr. Anthony Moody – Duke University Medical Center, USA
Dr. Henry C Mwandumba – Welcome Trust-Liverpool-Malawi Program, Malawi

Dr. Eleanor Riley – London School Of Hygiene, UK
Dr. Mario Roederer – Vaccine Research Center, USA
Dr. Tom Scriba – University of Cape Town, ZA