Viral pathogenesis in the era of COVID

Guido Silvestri, MD
Emory University School of Medicine
Yerkes National Primate Research Center
Emory Center for AIDS Research
Origins of HIV
Monkey models:
The “Classical” Model of AIDS Pathogenesis

Anti-HIV CTL, Neutralizing Abs, ART → HIV → CD4+T cell pool → Progressive exhaustion.... AIDS
In support of the classical model:

- HIV infects and kills CD4+ T cells.

- CD4+ T cells decline progressively during infection and the level of CD4+ T cells predicts the risk of developing AIDS.

- Suppression of HIV replication by antiretroviral therapy (ART) is followed by increase of CD4+ T cells.
AIDS is more than an HIV-mediated loss of CD4+ T cells;

• Similar rate of cell death for CD4+ and CD8+ T cells

• Most of the CD4+ T cells that die during chronic HIV infection are NOT directly infected by the virus

• Chronic, generalized immune activation results in functional defects and increased apoptotic cell death of uninfected T cells (both CD4+ and CD8+)
Comparative AIDS Research:

- Virus replication
- Pathogenicity

- SIVmac
- SIVmac Chinese RMs
- SIVmac PTMs
- SIVmac RM EC
- HIV-1 humans
- HIV-2 humans
- HIV-1 humans LTNP/EC
- SM & AGM
- SIVsmE660 RMs
- SHIVsf162p3
- SIVcpz chimps
- HIV-1

Virus replication vs. Pathogenicity diagram with various primate species and HIV strains plotted.
How HIV and SIV infection cause AIDS: Lessons from natural SIV infections

Chronic immune activation & mucosal immune dysfunction

Virus replication in central and stem-cell memory CD4+ T cells

Progressive Infection and AIDS

- Silvestri et al. Immunity 2003
- Dunham et al. Blood 2006
- Brenchley et al., Immunity 2010
- Reddick et al., PLoS Path 2011
- Zhang et al., J Clin Invest 2011
- Delmau et al., J Clin Invest 2011
- Bosinger et al. PLoS Path 2013
- Palesch et al. Nature 2018
- Joas et al. Cell Reports 2020

- Garber et al. J Clin Invest 2004
- Schindler et al. Cell 2006
- Silvestri et al., J Clin Invest 2007
- Sodora et al. Nat Med 2009
- Keele et al. Nature 2009
- Paiardini et al. Nat Med 2011
- Ortiz et al. J Clin Invest 2011
- Chahroudi et al. Science 2012
- Chahroudi et al. PLoS Path 2014
- Cartwright et al., J Immunol 2015
- Chahroudi et al., Science Transl Med 2016
- Wetzel et al. PLoS Path 2019
- Lee et al. PLoS Path 2021
MTCT in natural SIV hosts

• MTCT is rare in all studied species of natural SIV hosts (Pandrea, J Virol 2008; Chahroudi, J Virol 2011).

• When infected, either naturally or experimentally, newborns of SMs and AGMs show low or undetectable viremia (Chahroudi, J Virol 2011; Brenchley, Blood 2012).

• Virus replication does not appear to be controlled by immune mechanisms, but rather by paucity of CD4+CCR5+ target cells (Chahroudi, PLoS Pathogens, 2014).

• Evolutionary pressure to protect newborns from SIV infection might have resulted in immunological adaptations that protect adults from pathogenesis (Chahroudi, Science 2013).
Immune responses to HIV: a fine balancing act

The key for an immunization strategy that successfully controls HIV transmission and/or early replication is to fully exploit the antiviral role of CD4+ T cells without the side effect of creating additional target cells for the virus.
Strategies to cure HIV/AIDS:

• Full suppression of virus replication

• Identification of persistent reservoirs
  - establishment
  - regulation/reactivation
  - persistence

• Elimination of reservoirs

*Modified from Dr. Steve Deeks*
Mechanisms of HIV Persistence under ART
CD8+ T lymphocytes mediate viral control in HIV/SIV infection

- Strong association between control of viremia and specific MHC class I alleles in both humans and macaques.

- Appearance of HIV/SIV-specific CTL responses is temporally coordinated with the postpeak decline in viral replication.

- Escape mutations in CTL-targeted epitopes develop early during infection.

- LTNP/EC have more polyfunctional HIV-specific CD8+ T-cells compared to progressors.

- Antibody-mediated CD8+ lymphocytes depletion in SIV-infected macaques results in increased viremia and rapid disease progression.
CD8 depletion is followed by dramatic increase of viremia in “controller” SIV-infected RMs

Chowdhury et al., J Virol 2015
KEY QUESTIONS:

What is the role of CD8+ lymphocytes in controlling viremia on ART?

What is the role of CD8+ lymphocytes in the establishment and maintenance of the persistent reservoir of latently infected cells under ART?
CD8+ Lymphocytes Control Viral Replication in SIVmac239-Infected Rhesus Macaques without Decreasing the Lifespan of Productively Infected Cells

Nichole R. Klatt¹,², Emi Shudo³, Alex M. Ortiz¹, Jessica C. Engram¹, Mirko Paiardini¹, Benton Lawson², Michael D. Miller⁴, James Else², Ivona Pandrea⁵, Jacob D. Estes⁶, Cristian Apetrei⁵, Joern E. Schmitz⁷, Ruy M. Ribeiro³, Alan S. Perelson³, Guido Silvestri¹,²*

A

Early phase lifespan of infected cells

B

Late phase lifespan of infected cells

No difference in lifespan of productively infected cells in the presence or absence of CD8+ lymphocytes in both early or late chronic infection.

Similar result by Wong, Dandekar et al., PLoS Pathogens 2010.

We concluded that non-cytolytic mechanisms may contribute to CD8+ lymphocyte-mediated suppression of virus replication.
CD8 depletion is followed by increase in VL under highly suppressive ART

CD8+ lymphocytes are required to maintain undetectable viremia in ART-treated SIV-infected macaques (Cartwright et al., Immunity, 2016)
Is the virus production observed after CD8+ lymphocyte depletion in ART-treated SIV-infected macaques just due to increased levels of CD4+ T cell activation?
CD4+ T cell depletion is followed by a significant increase of proliferating, Ki67+ CD4+ T cells

Kumar et al., J Virol, 2018
... but is NOT associated with increase in viremia

Plasma Viral loads

Weeks post-ART

Total n = 8 animals

Kumar et al., J Virol, 2018
Study Design

McBrien et al., Nature 2020
N-803 does not induce virus reactivation in ART-treated SIV-infected macaques

Group 1: N-803 Alone

SIV RNA copies/mL plasma

ART

N-803

LOD=3

-12W -8W -4W W0 W1 W2 W3 W4 W5 W6

/7 RM

PRE-INTERVENTION
CD8 depletion is still associated with increased virus production even after 12 months of ART

Group 2: CD8 Depletion Alone

CD8 depletion is still associated with increased virus production even after 12 months of ART.
N-803 induce strong and persistent virus reactivation in ART-treated CD8-depleted macaques
N-803 induce strong and persistent virus reactivation in ART-treated CD8-depleted macaques
N-803 + CD8 depletion induces robust virus reactivation in HIV-infected ART-treated BLT humanized mice

McBrien et al., Nature 2020
Co-culture of latently infected CD4+ T cells with autologous unprimed CD8+ T cells results in decreased expression of HIV Gag after LRA treatment.

Mc Brien et al., Nature, 2020
Unprimed CD8+ T cells suppress HIV replication in a dose-dependent fashion

**CD8+/CD4+ co-culture assay:**

- Positive control wells with infected CD4+ cells alone
- Co-culture wells with infected CD4+ plus CD8+ at 1:1 and 5:1 effector-to-target cell (E:T) ratios
- Negative control wells (MOCK) with uninfected CD4+ cells and/or CD8+ cells
- For cell density control, the total number of cells per well remained constant

M. Zanoni, PLoS Pathogens 2020
In vitro HIV infection of memory CD4+ T cells (mCD4) shows the impact of CD8+ T cell suppression activity.

Franchitti et al., in preparation
Transcriptomics analyses showed reduced HIV RNA expression in sorted CD4+ T cells from *in vitro* CD8+ T cell co-cultures.
Metabolism, effector function and apoptosis pathways are downregulated in CD4+ T cells from CD4:CD8 co-cultures.
Quiescence, memory maintenance pathways are upregulated in CD4+ T cells from CD4:CD8 co-cultures.
Wnt signaling plays an important role in the regulation of lymphocyte differentiation, survival and stemness
Conclusions

• *In vitro* data using peripheral blood from HIV-naïve individuals reveals a CD8-specific suppression of HIV expression in CD4+ T cells

• This reduction of HIV expression is not derived from a decrease in the frequency of infected cells, but reduced virus transcription

• CD8+ T cell modulation of the Wnt signaling axis (that includes NFKB/IL-10/Bcl-2/Dead-box) may play a key role in modulating stemness and pro-survival pathways that contribute to HIV persistence

• Ongoing experiments are centered on identifying and manipulating the Wnt/NFKB/IL-10/Bcl-2/Dead-box pathways
## Antiviral activities of CD8+ T cells

<table>
<thead>
<tr>
<th></th>
<th>CTL activity</th>
<th>HIV silencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppression of virus</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>replication in absence of ART</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC-I restriction</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Present in HIV-neg subjects</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Requires cell-cell contact</td>
<td>YES</td>
<td>Partly</td>
</tr>
<tr>
<td>Mediating factors</td>
<td>Perforin, granzyme B</td>
<td>Unknown</td>
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<tr>
<td>Subject to escape and exhaustion</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Impact on virus reservoir</td>
<td>clearance</td>
<td>stabilization</td>
</tr>
<tr>
<td>under ART</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impact on CD4 activation</td>
<td>none</td>
<td>down-modulation</td>
</tr>
<tr>
<td>Effect on latency reversal</td>
<td>none</td>
<td>inhibition</td>
</tr>
</tbody>
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What about SARS-CoV-2 and COVID-19?
Immune responses & Immunopathology

Exposure to SARS-CoV-2

- No infection
- Infection

Symptomatic

Asymptomatic
- Recovery

Mild
- Recovery

Moderate
- Recovery

Severe
- Death

PROTECTIVE IMMUNITY
- Neutralizing anti-S antibodies
- Cytotoxic CD8 cells
- Th1 responses

IMMUNE DYSREGULATION
- Acute inflammation → cytokine storm
- acute lung injury → ARDS → cuagulopathy
- multiorgan system dysfunction

Garcia et al., Front Immunol 2020
Antiviral and/or Immuno-modulatory Interventions

1. EARLY STAGES INTERVENTIONS: antivirals (remdesivir, molnupiravir, paxlovi); monoclonal antibodies.

2. LATE STAGES INTERVENTIONS: Immuno-modulatory interventions, blockade of IL-6, JAK/STAT, IL-1 etc.

3. The “therapeutic” role of vaccines – vaccinated individuals are better at early control of viremia
Immuno-modulatory interventions

**Study Design and Analyses**

- **SARS-CoV-2 Infection**
- **Baricitinib**

**Main Findings**

**SARS-CoV-2**
1. Pro-inflammatory environment
2. Increased neutrophil and macrophage recruitment
3. NETosis activity
4. Activated T cells

**SARS-CoV-2 + Baricitinib**
1. ↓ Inflammatory cytokines and chemokines
2. ↓ Neutrophil and macrophage recruitment
3. ↓ NETosis activity
4. ↓ Activated T cells
5. Preserved innate antiviral responses

- Infected type II alveolar cell
- Neutrophil
- Macrophage
- T cell
- Activated T cell
Hsoang T, ... Silvestri G, Bosinger S, Paiardini M. Baricitinib treatment resolves lower airway inflammation and neutrophil recruitment in SARS-CoV-2-infected rhesus macaques.
Reduced SARS-CoV-2 T cell responses in MIS-C

SARS-CoV-2-Specific T-cell Responses in MIS-C, convalescent COVID-19, and healthy children shown as AIM+ (CD4+OX40+41BB+ or CD8+CD69+41BB+ T-cells) in response to Spike and non-Spike peptide mega-pool stimulation.

Singh V. et al., JCI insight
Auto-reactive IgM in severe COVID-19 patients

Wong et al., Cell Reports Med 2021

Broad auto-reactive IgM responses are common in critically ill patients, including those with COVID-19

Graphical abstract

Authors
Andrew Kam Ho Wong, Isaac Woodhouse, Frank Schneider, Deanna A. Kulpa, Guido Silvestri, Cheryl L. Maier

Correspondence
cheryl.maier@emory.edu

In brief
Critical illness can be associated with immune dysregulation; yet, mediators contributing to disease severity in COVID-19 are unclear. Wong et al. show a high percentage of critically ill patients possess auto-reactive IgM, which, in SARS-CoV-2 infection, are capable of binding diverse targets across key organs and inflicting complement-dependent cytotoxicity.

Highlights
- More than 90% of critically ill COVID-19 patients have auto-reactive IgM antibodies
- Auto-reactive IgM binds diverse targets across multiple organ types
- IgM and complement component C4d are abundant in COVID-19 non-survivor lung tissue
- COVID-19-associated auto-IgM fixes complement to induce cell death in vitro

Wong et al., 2021, Cell Reports Medicine 2, 100321
June 15, 2021 © 2021 The Author(s).
https://doi.org/10.1016/j.xcrm.2021.100321
Severe COVID-19 as an endotheliopathy

ACE / ACE2 imbalance in the pathogenesis of the COVID-19-associated cytokine storm

Hirano & Murakami, Immunity 2020
Severe COVID-19 as an endotheliopathy
Lipidomics/metabolomics analysis in plasma from severe COVID patients reveals changes of the following pathways: arginine and prolin metabolism; arginine biosynthesis; glycine, serine, and threonine metabolism; biosynthesis of unsaturated fatty acids, linoleic acid and glycerophospholipids; glutathione metabolism.
Severe COVID-19 as an endotheliopathy: lipidomics and metabolomics analysis in MIS-C
Severe COVID-19 as an endotheliopathy: proteomics analysis of the PLASMA of COVID patients (severe vs non severe), non-COVID ICU patients, and controls.

COVID-associated proteomic changes involve pathways such as:
(i) C’ and coagulation cascades
(ii) IL-6/IL-6R pathway
(iii) IL-17 pathway
(iv) CD4 monocytes
(v) neutrophil trap formation
(vi) renin-angiotensin system
(vii) fluid shear stress and atherosclerosis
(viii) focal adhesion
(ix) N-glycan byosynthesis
(x) AGE-RAGE pathway
(xi) EMC-receptor interactions
Multiplatform analyses reveal coagulopathy and endotheliopathy as key drivers of systemic pathogenesis in severe COVID-19

Druzek S, Iffrig E et al., in revision
Key unknowns about COVID-19 pathogenesis

1. What is the role of genetic factors in influencing transmission and/or pathogenesis?

2. What is the role of pre-existing cross-reactive immunity against other human Coronaviruses?

3. Why does COVID-19 have a disproportionate impact on the elderly and people with specific pre-existing conditions?

4. How can severe COVID-19 be treated from the point of view of patho-physiology?

Viral pathogenesis is a COMPLICATED business!
Immune responses to HIV: a fine balancing act

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Immune responses & Immunopathology

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Mild → Moderate → Severe

Recovery

Recovery

Recovery

Death

PROTECTIVE IMMUNITY
Neutralizing anti-S antibodies
Cytotoxic CD8 cells
Th1 responses

IMMUNE DYSREGULATION
Acute inflammation → cytokine storm
→ acute lung injury → ARDS → coagulopathy
→ multiorgan system dysfunction

Garcia et al., Front Immunol 2020
Other examples of viruses that are less pathogenic in natural hosts “endemic” vs. non-natural “recent” hosts include Hantaviruses, Rabies, Nepah viruses, Ebola, Marburg, and many others.

Seal et al., Evolution of pathogen tolerance and emerging infections: A missing experimental paradigm. Elife 2021

“Researchers worldwide are repeatedly warning us against future zoonotic diseases resulting from humankind's insurgence into natural ecosystems. The same zoonotic pathogens that cause severe infections in a human host frequently fail to produce any disease outcome in their natural hosts. What precise features of the immune system enable natural reservoirs to carry these pathogens so efficiently? To understand these effects, we highlight the importance of tracing the evolutionary basis of pathogen tolerance in reservoir hosts, while drawing implications from their diverse physiological and life-history traits, and ecological contexts of host-pathogen interactions. Long-term co-evolution might allow reservoir hosts to modulate immunity and evolve tolerance to zoonotic pathogens, increasing their circulation and infectious period. Such processes can also create a genetically diverse pathogen pool by allowing more mutations and genetic exchanges between circulating strains, thereby harboring rare alive-on-arrival variants with extended infectivity to new hosts (i.e., spillover). Finally, we end by underscoring the indispensability of a large multidisciplinary empirical framework to explore the proposed link between evolved tolerance, pathogen prevalence, and spillover in the wild.”
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