Immune Response to Influenza Virus Infection

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Talk Outline

• Innate Immunity
• Adaptive Immunity
  – Cellular and humoral responses
  – Assays and detection of responses
  – Mechanisms of antibody control
• Escape from influenza immunity and cross-reactive immunity
• Immunity in aging
Kinetics of Immune Response to a Primary Influenza Infection

Days post-infection

1      3      5      7      9       11       13

INNATE ADAPTIVE

IFN    NK cell    Serum Antibody
TNF

CTL

Virus
First Line of Defense for early control
Activated in Hours
Primordial Receptors that recognize conserved molecular patterns (e.g. Lipids, carbohydrates RNA/DNA)
Self-Nonself discrimination is perfect
Epithelial Cells, Monocytes
Neutrophils, NK Cells, complement

Immunological Memory
Activated in Days-Weeks
Receptors Rearrange for Diversity and recognize complex structures (proteins, peptides)
Imperfect
T and B Cells
Innate Immune System Recognizes Pathogens through Pathogen-Associated Molecular Patterns (PAMPs)

• The body detects the presence of microorganisms by recognizing molecules unique to microorganisms that are not associated with human cells
  – Carbohydrates, proteins, lipids, nucleic acids

• PAMPs are recognized by Pathogen Recognition Receptors (PRRs)
Pathogen Recognition Receptors (PRRs)

**Pathogen sensors**

- **Extracellular-soluble**
  - Collectins and ficolins
    - SP A and D
    - MBL
    - Ficolins L, M, and H
  - Pentraxins
    - PTX3, PTX4, NPTX1, NPTX2
    - CRP, SAP
  - PLUNC
    - BPI
    - a protein
  - Complement

- **Membrane-bound**
  - Toll-like receptors
    - TLRs 1, 2, 4, 6, 8, 10
  - Lectin receptors
    - Mannose receptor
    - DC-SIGN
    - SIGNR1
    - Langerin
    - Dectin-1 and -2
  - Scavenger receptors
    - SR-AI, SR-AII, SR-AI, SR-AII
    - MARCO
  - Integrins
    - Mac-1

- **Vesicular**
  - Toll-like receptors
    - TLR-3
    - TLR-7/8
    - TLR-9

- **Cytoplasmic**
  - RNA sensors
    - PKR
  - RIG-I-like receptors
    - RIG-I
    - MDA5
  - Peptidoglycan sensors
    - NOD-like receptors
      - NOD1 and 2
      - Ipaf
      - cryopyrin
  - DNA sensors
    - DAI (DLM-1/ZBP1)
    - AIM2
Recognition of PAMPs in Influenza Virus Infected Cells

- Retinoic acid inducible gene-I (RIG-I)
  - Found in most cell types in cytosol
  - Recognizes ssRNA bearing 5’ triphosphate
  - Results in induction of type I interferons

- Toll-like Receptor (TLR)-7
  - Recognize genomic RNA in endosomes of dendritic cells
  - Plays important role in development of adaptive immunity
  - Both live and killed virus can induce type I IFN through TLR-7
Induction of type I Interferons and proinflammatory cytokines

- Infected epithelial cells produce type I interferons (IFN-α/β)
- PAMP - PRR interactions (TLR7) induce macrophages and immature DCs at site of infection to produce type I interferons and proinflammatory cytokines (IL-1, IL-6, IL-12, TNF-α and chemokines (MIP-1α/β, MCP-1, IL-8)
- Leads to amplification of the inflammatory response
Role of Type I interferons (IFNα/β)

- Protect uninfected cells from virus infection. Their binding to IFNR triggers antiviral functions in the cell.
- Induce the influx and activation of NK (natural killer) cells to kill infected epithelial cells before virus release.
- Upregulate MHC class I expression to make cells better targets for lysis.
Cytokine Responses in Volunteers Infected with H1N1 Virus
(Hayden et al., 1998; J Clin Invest 101:643)
The Inflammatory Response in Fatal Human Avian H5N1 Virus Infection
(deJong et al., Nat Med 2006;12:1203)

- Fatal human H5N1 virus infection characterized by:
  - High viral load
  - High levels of some chemokines and cytokines in plasma

![Graphs showing IL-6, IP-10, and MCP-1 levels](image-url)
Natural Killer (NK) Cells and Influenza

- Important lymphocyte effector cells of the innate response
- Can directly recognize and bind virus-infected cells through receptors
  - Natural cytotoxicity receptors (NKp46) recognizes HA
- Kill virus infected cells through release of granules which cause cell death
- Activated NK cells produce a variety of cytokines (IFN-gamma) chemokines and are an important bridge between innate and adaptive immunity
- NK cells are also effectors for adaptive responses mediated by antibodies
  - Antibody dependent cell-mediated cytotoxicity (ADCC)
Dendritic cells are central to the initiation and regulation of the adaptive immunity. DC and microenvironment influence polarization of T cells.

Pulendran B, Nat Immunol, 2010
The innate response to primary influenza provides early control, slowing replication, but adaptive immunity ultimately clears the infection and allows recovery.
T cell-mediated immunity
Major Histocompatibility Class (MHC) I and II Antigen Structures and Interaction with CD8+ and CD4+ T cells, Respectively

MHC II:
Peptides are at least 13 aa long

MHC I:
Peptides are 8-10 aa long

Figure 3-25 Immunobiology, 7ed. (© Garland Science 2008)
CD4+ Helper T cell Response to Influenza Infection

- The B cell response to influenza virus HA is dependent on help from CD4+ T cells
- CD4+ T cells provide help for B cell activation and affinity maturation
- Epitopes recognized by CD4+ T cells are found on majority of influenza virus proteins
  - Recognition depends on HLA (MHC antigens) profile of an individual
- T cell memory is essentially life long
- In general, CD4+ T cells exert their effect by proving help for B cells and CD8+ T cells, and do not act directly to clear virus
  - Mice that lack B cells and CD8+ T cells succumb to infection
CD8+ T cell Effectors Mediate Lysis of Virus Infected Cells By Release of Cytotoxic Granules

From Adrian Reber
**Interferon-gamma T cell ELISPOT assay**

1. Coat plate with anti-IFN-g Ab
2. Add lymphocytes + antigen
3. Add enzyme-linked 2\textsuperscript{nd} anti-IFN-g Ab antibody and develop plates

6 – 12 hr
Specificity of Influenza Virus Specific T Cell Responses Following Experimental Challenge of Adults (Wilkinson et al., 2012 Nat Med)

T cell responses to NP and M1 are dominant
Inverse Correlation between Virus Shedding and CD8+ Cytotoxic T cell Responses in Seronegative Adults

McMichael et al., NEJM 1983 and Epstein et al., Exp Rev Vac 2010
B cell-mediated immunity
Influenza A virus Proteins as Antibody Targets

- Anti-HA Abs block attachment or fusion of virus to inhibit replication
- Anti-NA Abs prevent virus release and spread
- Abs to M2 external domain block virus budding/release and are target for ADCC
- Abs to NP reduce viral shedding in mice

....and other viral proteins
Serologic Assays for Detection of Human Antibodies

- **Hemagglutination-Inhibition (HI)**
  - Detects Ab that bind around receptor-binding site in globular head and block agglutination
  - Titer $\geq 40$ correlated with protection

- **Microneutralization (MN) or Virus Neutralization (VN)**
  - Detects Ab that bind around globular head and block virus attachment/entry
  - May detect cross-reactive antibody, including to HA stem
  - No correlate of protection established

- **Indirect ELISA using purified virus, protein or peptides**
  - Can detects any Ab that bind linear epitopes if protein is partially denatured
  - Suitable to detect IgG, IgM, IgA in serum and nasal washes
Hemagglutination Inhibition Assay

1. Treat serum, 2-fold serial dilutions
2. Add 4 HA units of virus, 30 min @ RT
3. Add 0.5% turkey RBC, 30 min @ RT
4. Record HI titer

HA
hemagglutination

HI
hemagglutination inhibition

Photos courtesy of A. Balish
Miconeutralization Assay

1. Add heat inactivated sera
2. Add virus 100 or 200 TCID/well
3. Add MDCK cells 1.5 x 10^4 cells/well
4. Wash/Fix
5. ELISA α-NP Ab

1 hr @ 37°C
18-20 hr @ 37°C

VC BT CC
Kinetics of Antibody Responses to H3N2 Virus Infection in Children (Murphy et al., 1982)

Neutralizing (anti-HA) antibody

Anti-NA antibody
Kinetics of Neutralizing Antibody Response in Humans infected with Avian H5N1 Virus in 1997
Primary anti-HA Immunoglobulin Response to Influenza Live Attenuated H3N2 Virus Infection in Children

(Murphy et al., Infect Immun 1982)

Serum

Nasal Wash
Function of Virus-specific Antibodies

• IgM
  – Earliest antibody and hallmark of primary infection
  – Initiates complement-mediated virus neutralization
• IgG
  – Antibodies from serum transudate into respiratory tract and provide long-lasting protection
  – Predominant antibody for protection of lower respiratory tract
• IgA
  – Produced locally at mucosal site of infection
  – Provides local protection of upper airway epithelial cells
Secretory IgA responses are an important defense against mucosal pathogens

- Secretory or polymeric IgA is produced by B cells at mucosal surfaces of upper respiratory tract
- IgA binds to receptor on epithelial cells and is endocytosed
- Internal IgA can bind viral proteins in internal compartments
- Free IgA binds virus in lumen

- IgA is produced at mucosal surfaces following influenza infection
  - Can be detected for at least 3 months

Antibodies to the HA Globular Head Block Binding to Receptor on Host Cell

Provided by James Stevens, CDC

Subbarao and Joseph,
Nat Rev Immunol 2007: 7:267
HA Stem Region Antibodies

- Based on recent identification of human MAbs that recognize conserved stalk region of HA that encompasses fusion peptide (*Throsby et al., PLoS One* 2008; *Ekiert et al., Science* 2009; *Sui et al., Nat Struct Mol Biol* 2009)
  - 2 structural groups for 16 HA subtypes
  - Antibodies inhibit membrane fusion
  - Low levels of these heterosubtypic Abs detected in human sera (*Sui et al., CID* 2011)
  - Vaccination may boost these responses (*Corti et al., JCI* 2010;120:1663)
Non-neutralizing antibodies can inhibit release of virus from infected cell

MDCK cells infected with H3N2 virus
In the presence and absence of anti-M2 Ab

Subbarao and Joseph,
Nat Rev Immunol 2007: 7:267

Hughey et al., Virology 1995;212:411-21
**Antibody-dependent Cell-mediated Cytotoxicity (ADCC)**

- Effector NK cell
- Perforin
- Granzymes
- Fc receptor (CD16)
- Fc domain
- M2 antigen
- Cell lysis

From Weimin Zhong
Anti-M2 Antibody Response

• Transient responses to M2 detected following influenza A virus infection in humans
  – In one study, approximately one third of adults demonstrated a rise in anti-M2 response (Black et al., J Gen Virol 1994)

• In sera collected prior to 2009 pandemic, a portion of adults but not children had detectable anti-M2 antibodies
  – Detected anti-M2 antibody in ~50% of lab confirmed 2009 H1N1 infected persons
B cell ELISPOT assay

1. Coat plate with antigen
2. Add PBMC
3. Incubate overnight
4. Add enzyme-linked antibody to human Ig and develop plates
Circulating B cell Responses Following Immunization with seasonal LAIV or TIV (H3N2 response)  
(Sasaki et al., J Virol 2007;81:215)

- ~80% of adults and older children made circulating IgG ASC responses to LAIV or TIV on days 7-12 post infection
- IgG ASC were low in children aged 6mo - 4 yr
- Baseline levels of virus specific memory B cells increased significantly with age
Serum Antibody Responses to Influenza Virus Infection is Long-lived

- In 1977-78, H1N1 reemerged after absence of more than 20 years
  - Only children and young adults susceptible

- Memory B cells specific for 1918 virus isolated from elderly persons exposed to 1918 virus
  - Pre-existing serum antibody cross-reactive with 2009 H1N1pdm virus found in older adults
Specificity of HA Antibody Response

- Following natural infection of children, the majority of antibody induced is highly specific for infecting strain.

- In contrast, infection or vaccination of adults induces anti-HA antibody that is predominantly cross-reactive for antigenically related viruses within the subtype.
Original Antigenic Sin

- Concept based on patterns of serum antibody responses among persons with varying histories of influenza virus infection or vaccination (Francis)
- Exposure to initial influenza virus leaves a lasting “imprint” on the immune response
- Subsequent re-exposure to variant of same subtype results in immune response dominated by antibodies that cross-react with earlier strain
- Pre-existing memory B cells preferentially expand upon re-infection
  - Reduced expansion of naïve B cells recognizing new epitopes
Escape from Influenza Immunity

- **Innate immune response**
  - NS1 protein inhibits type I interferon by multiple mechanisms
  - Other viral proteins also interfere with host response
- **Antibody response**
  - Antibodies prevalent in humans due to infection/vaccination fail to neutralize novel viruses
    - Antigenic drift or shift
- **T cell responses**
  - Epitopes acquire mutations at critical residues
  - Evolution of H3N2 viruses associated with escape from CD8+ T cells
Cross-Protective Immunity to Influenza A Viruses

• Heterosubtypic immunity
  – Protection by virus or antigen of one influenza A virus against a virus of a different subtype

• Heterotypic immunity
  – Protection within a subtype; e.g. across H5N1 clades

• Induction of broadly cross-protective immunity is the goal of “Universal Vaccines”
Heterosubtypic Immunity in Animal Models

- Mild infection of animals with one strain protects against subsequent severe challenge with virus bearing different HA and NA
  - Infection is not prevented
  - Reduction of virus shedding and less severe disease

- Both T and B cell-mediated immunity involved
  - CD8+ CTL recognize epitopes on conserved internal proteins
  - Anti-M2 antibody and HA stem region antibody
  - Role for CD4+ T cells may be to support and enhance CTL responses and provide help for B cell responses
Evidence for Heterosubtypic Immunity in Human During 1957 H2N2 Pandemic

(Epstein and Price, Expert Rev Vaccines 2010; 9(11):1325-41)
T cells established by seasonal human influenza A virus infection cross-react with H5N1 Virus in Healthy Adults (Lee et al., J Clin Invest 2008)

- Memory CD4+ and CD8+ T cells from a majority of people showed cross-reactivity with at least one H5N1 virus internal protein
- M1 and NP dominant proteins recognized
Older adults experience more severe and fatal influenza illness
Effectiveness of vaccines is reduced

Immunosenescence: age-related changes in immune function
Greater heterogeneity of responses in older adults
Affects both innate and adaptive arms of immune response in terms of decreases in number or function of cells
Decline in B and T cell Responses to Influenza Vaccination with Aging

- Numbers of naïve B and T cells are reduced
- Poorer stimulation of T cells
  - Decrease in Th1 relative to Th2
- Reduced antibody responses to vaccination

McElhaney, 2005

Sasaki et al, 2011
Summary and Conclusions

• Innate immunity is important to shape adaptive immune responses to influenza
  – Pathogen recognition receptors trigger early antiviral and inflammatory responses

• Influenza infection induces long-lasting B and T cell immunity
  – More variable HA and NA are major targets of B cell responses
  – More conserved internal proteins are major targets of T cell response

• Subtype cross-reactive responses are comprised of T cell responses and antibody responses to conserved proteins/peptides

• Influenza viruses can escape both innate and adaptive (B and T cell) responses

• Aging is associated with a decline in immunological function, evident in vaccine responses
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