Overview of Human Immunosenescence

Al Shaw, M.D., Ph.D.
Professor of Medicine
Section of Infectious Diseases
Yale School of Medicine
The Geriatric Demographic Imperative: US Population over age 65 (millions)

Individuals over age 65 who currently comprise about 15% of the US population account for over 35% of visits to general internists, 34% of prescription drug use, 50% of hospital stays, and 90% of nursing home residents (CDC, 2005).
Aging of the US Baby Boom Generation (1946-1965)

US Census Bureau, “65+ in the United States”, 2005
Increased Proportion of Adults $\geq 65$ Years Worldwide
# Relative Mortality Rates for Geriatric Infectious Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Relative Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>3</td>
</tr>
<tr>
<td>Urinary Tract Infection</td>
<td>5-10</td>
</tr>
<tr>
<td>Appendicitis</td>
<td>15-20</td>
</tr>
<tr>
<td>Cholecystitis</td>
<td>2-8</td>
</tr>
<tr>
<td>Sepsis</td>
<td>3</td>
</tr>
<tr>
<td>Meningitis</td>
<td>3</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>2-3</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>10</td>
</tr>
</tbody>
</table>

Yoshikawa, 1997
Decreased Vaccine Effectiveness with Age

Monto et al., 2009
Innate Immunity

- Rapid onset—mediated by macrophages, NK cells, dendritic cells, mast cells
- Complement pathways, iron sequestration
- Phagocytosis
- Innate immune activation results in inflammatory responses
- Pattern recognition receptors, but not as specific as the slower onset adaptive immune response mediated by B and T cells
Pathogen Recognition Receptors in the Innate Immune System

Netea and van der Meer, 2011
Acute vs. Dysregulated Chronic Inflammation

- **Acute Inflammatory Reaction**
  - IL-6
  - CRP

- **Dysregulated Chronic Inflammation**
  - IL-6
  - CRP

- **Age-Related Pro-Inflammatory State**

- **Normal Recovery**

- **Time**: Stress/Infection → Response → Healing → Normal Recovery
Immune Activation in Aging: Inflamm-Aging

• Though overall immune function and defense against infection is impaired with aging, an age-associated pro-inflammatory milieu has been observed (Fagiolo et al., 1993; Franceschi et al., 2007).

• Elevated levels of cytokines (e.g. IL-1β, IL-6, IL-8, TNF-α), acute phase reactants (e.g. CRP) and clotting factors have been observed.

• Source for these inflammatory markers incompletely understood—possibilities include:
  – Control of chronic viral infections such as CMV.
  – Engagement of PRRs by endogenous damage-associated molecular patterns (DAMPs)
  – Release of pro-inflammatory cytokines following DNA damage.
  – Age-associated shift toward myeloid HSC differentiation.
Age-associated Increase in Basal Cytokine Production in Myeloid and Plasmacytoid DCs (n=104)

Panda, Qian et al., 2010
Age-Associated Alterations in Innate Immunity

Changes due to aging

- Impaired chemotaxis, phagocytosis and NET formation
- ↓ signal transduction e.g. to TLR-1, GM-CSF
- ↑ PI-3 kinase signal transduction

Neutrophils

- ↓ TLR1/2-dependent IL-6 and TNF-α production
- Impaired expression of co-stimulatory proteins
- ↑ TLR-5 induced cytokine production
- ↑ in IL-10 production

Monocytes

- ↓ TLR9 induced cytokine production
- ↓ IFN gene expression
- ↑ Basal cytokine production

Dendritic cells

- ↑ CD56dim CD16+ cytotoxic cells
- ↓ expression and function of cytotoxicity receptors

NK cells

Increased production of pro-inflammatory cytokines

- Uric acid
- Extracellular ATP
- β-amyloid
- SASP
- NLRP3
- NLRP3
- TLR9
- Non-cell assoc. DNA/mitochondrial DNA

Damage Response

Necrotic cells

Adaptive Immunity in Aging: B Cells


- Decreased B cell repertoire diversity with age
- Decreased AID expression and decreased Ig heavy chain class switching
Adaptive Immunity in Aging: T Cells

• DTH responses (e.g. PPD) clearly diminished in the elderly

• In human CD4 T cells, age-associated changes in signal transduction are seen, particularly in the ERK MAP kinase pathway.

• Changes in T cell receptor signaling strength with age could influence engagement of downstream pathways

• Some evidence for increased IL-17, Th17 polarization)

• Decreased survival of memory T cells: age-associated increase in CD39 (ATPase) expression (Fang et al., Cell Reports 2016)
Adaptive Immunity in Aging: T Cells

- With thymic involution, the human T cell compartment in adults is maintained almost exclusively (~90%) by peripheral expansion.
In older individuals, more T cells show a “memory” phenotype (CD45RO+) than a “naïve” phenotype (CD45RA+).

Marked decrease in CD28 expression in CD4+ and (mainly) CD8+ T cells from elderly donors.

CD28- T cells have shortened telomeres.

CD28- T cells overproduce cytokines (e.g. IL-6).
Adaptive Immunity in Aging: T Cells

- Long-lived, clonal expansion of T cells (mostly CD8+) in healthy elderly individuals, possibly from chronic antigen stimulation

- ? Restriction of T cell repertoire

A Substantial Proportion of CD8+ CD28- T Cells Recognize CMV

- Age-associated accumulation of CMV-specific effector memory CD8+ T cells
- Likely reflects the broad tissue expression of CMV and the frequency of asymptomatic reactivation throughout life
Age-Associated Alterations in Adaptive Immunity

**Young**
- Memory B cell responses
- Production and secretion of antibodies in response to extracellular pathogens

**CD4+ cells**
- T helper functions, such as differentiation to Th1 cells for responses to intracellular pathogens
- Cytokine production to regulate inflammation and B cell function

**CD8+ cells**
- Cytotoxic T cells that lyse target cells e.g. virus-infected or tumor cells

**Older**
- ↓ production of antibody secreting cells
- ↓ class switching

*CMV*
- Impaired signal transduction
- ↑ memory cells
- ↓ production of naïve cells
- Impaired helper functions

**Shaw and Bandaranayake 2016**
Alternate vaccine formats:

- Higher dose vaccines (e.g. for influenza)
- Vaccine delivery (e.g. intradermal)
- Adjuvants
  - MF59 (Squalene derivative; Flued)
  - AS01B (MPL [TLR4 agonist] + QS-21 [saponin derivative, ?NLRP3; Shingrix])
- Agents to bolster immune responses in older adults (e.g. Rapamycin analogs, Metformin, NAD, senolytics)
mTOR Inhibitor Treatment Improves Influenza Vaccine Response and Decreases Respiratory Infections in Older Adults

Enrollment

Assessed for eligibility ($n = 340$)

Randomized ($n = 264$)

Screen failures ($n = 76$)

Allocation

RAD001 (0.1 mg daily) ($n = 52$)
- Received drug ($n = 52$)

RAD001 (0.5 mg daily) ($n = 54$)
- Received drug ($n = 54$)

BEZ235 (10 mg daily) ($n = 53$)
- Received drug ($n = 53$)

RAD (0.1 mg) + BEZ (10 mg daily) ($n = 53$)
- Received drug ($n = 53$)

Placebo daily ($n = 52$)
- Received drug ($n = 52$)

Increase in influenza GMT ratio relative to placebo for each of the influenza virus vaccine strains

Illumina vaccine strains

Influenza

Placebo

BEZ 10 mg

RAD 0.1 mg + BEZ 10 mg

RTIs/person/year

Mannick et al., 2018
Sex and gender as drivers for better design and efficacy of vaccines

Sabra L. Klein, Ph.D.
Molecular Microbiology and Immunology
Johns Hopkins Bloomberg School of Public Health
Baltimore, Maryland USA
Sex versus Gender

- Institute of Medicine report published in 2001 that concluded that ‘every cell has a sex from womb to tomb’

- **Sex** refers to biological differences associated with being male or female according to reproductive organs and sex chromosomes

- **Gender** refers to one’s sense of self as being male or female based on societal or cultural norms.
Biology and societal factors affect who we are and the diseases we acquire over the life course.
Why is this resolution important?

Because over our lifetime, the biological differences between males and females (referred to as sex) and the social or cultural constructs that define being male or female referred to as gender) can interact to impact exposures, susceptibilities, outcomes of disease, and the efficacy of treatments for disease.

Unfortunately, these differences have been marginalized and often ignored in the biomedical sciences, which is why I’m here today to begin our discussion about women’s health and focus on my research area: women’s health and our immune system.
A woman’s immune system fights off infection better than a man’s

A few infectious disease for which females control the infection better than males:
1. HIV
2. Hepatitis B/C virus
3. SARS/MERS
4. Ebolavirus
5. Tuberculosis
6. Bacterial pneumonia
7. Malaria
8. Toxoplasmosis
9. Schistosomiasis
10. Entamoeba histolytica

Protective immunity against infections, including responses to vaccines, are greater in women
Vaccines protect us against a long list of infectious diseases.

Women develop significantly higher immune responses to vaccines than men. In our studies with the flu vaccine, females mount higher immune responses to the vaccine and are better protected following exposure to the virus. Unfortunately, in human clinical trials, even though females mount higher immune responses to the flu, hepatitis B, HPV and shingles vaccines, rarely are outcome data partitioned and analyzed to compare the sexes.
Which vaccines?

Women produce greater immune responses to vaccines against:
• Flu (influenza)
• Hepatitis B
• Human papilloma virus
• Rabies
• Shingles
• Smallpox

Women also experience more adverse reactions to vaccines against:
• Flu
• Measles
• Human papilloma virus
• Tetanus
Human Papilloma Virus Vaccine in the USA

- Uptake, including completion of scheduled doses, of the vaccine is greater in adolescent and young adult females than males (gender).

- Passive reporting of adverse events, including non-serious events (e.g., dizziness, headache, injection site swelling, nausea, erythema), is greater for adolescent and young adult females than males (sex and gender).

- Immunogenicity of the HPV vaccine is similar between adolescent (9-15 years of age) females and males (sex).

Koplas et al. 2018 *J Am Coll Health* doi.org/10.1080/07448481
Suragh et al. 2018 *Br J Clin Pharmacol* 84:2928
Van Damme et al. 2015 *Pediatrics* 136:e28
Gender differences in reporting of adverse reactions following receipt of the seasonal influenza vaccine

Engler et al. 2008 Arch Intern Med 168:2405
Local erythema/induration following influenza trivalent inactivated vaccine (TIV) is greater in females

Cate et al. 1983 Rev Infect Dis 5:737
Sex differences in response to the seasonal H1N1 vaccine antigen

Engler et al. 2008 Arch Intern Med 168:2405
Aging reduces influenza immunity in females

Potluri et al. 2019 npj Vaccines 4:29; doi.org/10.1038/s41541-019-012406
In humans, age and sex are predictors of influenza immunity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DF</th>
<th>F value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroidism</td>
<td>1, 158</td>
<td>0.0841</td>
<td>0.7722</td>
</tr>
<tr>
<td>Hysterectomy/Vasectomy/Post-menopausal</td>
<td>1, 158</td>
<td>0.0004</td>
<td>0.9849</td>
</tr>
<tr>
<td>Oral Contraceptive Use</td>
<td>1, 158</td>
<td>0.2542</td>
<td>0.6148</td>
</tr>
<tr>
<td>Depression and/or Anxiety</td>
<td>1, 158</td>
<td>0.3081</td>
<td>0.5796</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>1, 158</td>
<td>0.1883</td>
<td>0.6649</td>
</tr>
<tr>
<td>Age</td>
<td>35, 158</td>
<td>2.1267</td>
<td>0.0009*</td>
</tr>
<tr>
<td>Sex</td>
<td>1, 158</td>
<td>4.5181</td>
<td>0.0351*</td>
</tr>
<tr>
<td>Chronic Respiratory Disease or Smoker</td>
<td>1, 158</td>
<td>1.9411</td>
<td>0.1655</td>
</tr>
<tr>
<td>Hysterectomy/Vasectomy/Post-menopausal x Oral Contraceptive Use</td>
<td>1, 158</td>
<td>0.0058</td>
<td>0.9397</td>
</tr>
<tr>
<td>Age x Sex</td>
<td>16, 158</td>
<td>3.6545</td>
<td>1.15E-05*</td>
</tr>
<tr>
<td>Age x Chronic Respiratory Disease or Smoker</td>
<td>4, 158</td>
<td>3.5479</td>
<td>0.0084*</td>
</tr>
</tbody>
</table>
Hormones. Females have higher concentrations of estrogen and males have higher concentrations of testosterone and these concentrations can change (or decline) with aging.

It turns out that every immune cell in your body has receptors that can recognize estrogen or testosterone. These hormones regulate the activity and functioning of our immune cells, with testosterone generally suppressing immune cell activity and estrogen generally enhancing immune cell activity.

Differences in the concentrations of our hormones cause our immune cells to respond differently when they are exposed to allergens, self-antigens, viruses, and even vaccines. My group has shown estrogens enhance and testosterone suppresses immune responses to the flu vaccine in both mice and humans. Others and we have also shown that these hormones regulate immune responses during infection, allergy-induced asthma, and autoimmune diseases, including multiple sclerosis.
Hormone levels correlate with influenza immunity in humans

Potluri et al. 2019 npj Vaccines 4:29; doi.org/10.1038/s41541-019-012406
Aging reduces influenza immunity in female mice

(a) A study design showing the timeline of events:
- Adult Males (AdM)
- Adult Females (AdF)
- Aged Males (AgM)
- Aged Females (AgF)

Day 0: Inactivated mouse-adapted A/Cal09 H1N1 vaccine
Day 21: Plasma Collection
Day 28
Day 35

(b) Graph showing Anti-2009 H1N1 HAI titer (x10^2) at days 28 and 35 post-vaccination.

(c) Graph showing Anti-2009 H1N1 neutralizing antibody titer (x10^3) at days 28 and 35 post-vaccination.

Potluri et al. 2019 npj Vaccines 4:29; doi.org/10.1038/s41541-019-012406
Estrogen causes elevated immunity in female mice
Sex-specific vaccine design

Determinants of sex differences in immune function
- X-linked genes
- Gene polymorphisms
- Endogenous hormones
- Exogenous hormone exposure
- Reproductive status
- Age
- Environmental exposures

Sex differences in vaccine efficacy
- Inflammation
- Innate immune cell activation
- Cytokine responses
- Antibody responses
- Cell-mediated responses
- Memory responses

Sex-specific vaccine design
- Adjuvant
- Dose
- Formulation
- Route of administration

Maximize immunogenicity and minimize reactogenicity in both sexes

Klein & Pekosz 2014 J Inf Dis 209:S114
Does equal mean same?
In medicine and even public health, we like to find simple, effective solutions to medical problems. Most times that means finding a ‘one size fits all’ treatment that is presumed to work equally well in all of us.

Today, I want to pose a question: What if in order to care for us equally, we need to be treated differently? What if one of the variables contributing to differential efficacy of treatments is our biological sex? In other words, the sex chromosomes, sex hormones, and reproductive tissues that often defines us as male or female may impact that efficacy of treatments.

Today, I will discuss evidence that the immune systems of males and females behave differently and should be considered in treatments of diseases associated with our immune system ranging from inflammatory diseases to infectious diseases.
Acknowledgments:

Ashley Fink, PhD  
Tanvi Potluri, ScM  
Kyra Engle, ScM  
Harish Narasimhan, MHS  
Wan-Yee Tang, PhD

Kristyn Sylvia, PhD  
Santosh Dhakal, DVM, PhD  
Rebecca Ursin, MS  
Sharvari Deshpande  
Landon vom Steeg, PhD

Financial support provided by:
NIH/ORWH/NIA SCORE U54 AG062333 and NIH/NIAID Center of Excellence in Influenza Research and Surveillance contract HHSN272201400007C
NIAID’s Vaccine Adjuvant Discovery and Development Programs

Improving Vaccine Efficacy with Adjuvants

Wolfgang W. Leitner, MSc, PhD
Chief, Innate Immunity Section; Basic Immunology Branch
Division of Allergy, Immunology and Transplantation
National Institute of Allergy and Infectious Diseases
National Institutes of Health
September 17, 2019
Goals of the NIAID Adjuvant Program

- Support of all stages of vaccine adjuvant research
  - Discovery, product development, preclinical testing, clinical evaluation
- Elucidate adjuvant mechanism-of-action
- Determine rules for matching adjuvant and vaccine, adjuvant and target population
- Improve vaccines against infectious diseases, autoimmune diseases, allergy, opioid addiction
- Described in 2018 Strategic Plan
How Adjuvants Improve Vaccine Efficacy

- Overcome age-related immune differences/deficits

- Drive appropriate adaptive immune response
  - Promote specific CD4⁺ T helper cell subsets (and associated antibody profiles)
  - Induce CD8⁺ cytotoxic T cell activation

- Induce long-lasting immune memory
Challenges for Infant Vaccines

- Infants and neonates are a major target population for vaccines due to high susceptibility to infection
  - Respond weakly to most vaccines and many vaccine adjuvants compared to older children/adults (exception: TLR7 agonists)
  - Have skewed Th2 T cell and cytokine expression

Examples of NIAID-supported vaccine adjuvants for infants and neonates
- Combination adjuvant that induces TLR9-expression
- TLR7/8 adjuvant that overcomes the immunosuppressed state shortly after birth
Adjuvant Formulation Improves Murine Neonatal Responses to Influenza Vaccine

Advax = Inulin, a plant-derived carbohydrate crystal

Advax/CpG55.2 = Inulin + TLR9 agonist; Inulin induces TLR9 expression
Inducing the Optimum Immune Response

- Protection against various pathogens requires antibodies AND specific T cell subsets
- Adjuvant selection and route of vaccine administration can affect immune profiles
- Alum adjuvants promote Th2 immune response, but not Th1, Th17, or cytotoxic T cells
A TLR-Agonist Induces Robust Immunity in Neonates and Overrides Alum’s T cell Profile

Human cytokine response of adult vs. neonatal leukocytes to TLR7-agonist

Mouse neonatal Th1-driven antibody response to acellular Pertussis vaccine (aP; Infanrix®)

TLR7-agonist (10μg)

Anti-FHA IgG2c (ng/ml)

Saline

aP/alum

aP/alum + TLR7 agonist (1μg)

aP/alum + TLR7 agonist (10μg)
Nanoemulsion (NE) Adjuvant Changes Immunity Established by Acellular Pertussis Vaccine

- Alum-adjuvanted acellular pertussis vaccine (aP) induces Th2 immune profile; the whole cell vaccine (wP) induces a protective Th17 profile
- Boosting aP-vaccinated animals with NE-formulated vaccine “rescues” the Th17 profile and promotes production of more effective antibodies

**IL17 in Lung Tissues**

- IM Alum aP
- IN NE aP
- IM Alum aP + IN NE aP

**Bactericidal activity of serum antibodies**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reduction (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM Alum aP</td>
<td>80%</td>
</tr>
<tr>
<td>IN NE aP</td>
<td>80%</td>
</tr>
<tr>
<td>IM Alum aP + IN NE aP</td>
<td>100%</td>
</tr>
</tbody>
</table>

Week: 8 18 24 26

4x IM Alum-aP  4x IN NE-aP  2x Alum-aP + 2x NE-aP
Short- vs. Long-term Protection

- Many adjuvants induce good effector T cells (short term) but few memory T cells (long term)

- Combination adjuvants can trigger multiple immune pathways to improve long-term protection
  - Need to identify appropriate pairing for vaccine indication

- Examples of combination adjuvant building blocks
  - Adjuplex: polymeric complex with soy lecithin
  - GLA (PHAD): synthetic derivative of bacterial endotoxin (TLR4 agonist)
  - CpG DNA: mimics bacterial DNA (TLR9 agonist)
Adjuvant Combinations Trigger Durable Immune Protection in Mice

Flu-specific CTL in Lungs 8 days post vaccination

Memory vs. Effector T cells 8 days post vaccination

Viral burden after infection 100 days after vaccination

NP-flu vaccine plus adjuvant
Adjuplex and GLA:

- induce **the most** CTLs and helper T cells
- Imprints a memory rather than effector T cell phenotype
- Provides stronger protection against flu infection
- Provides better protection against mis-matched flu strains
- Provides longer-lasting protection
Summary: Role of Vaccine Adjuvants

- Overcome reduced immunogenicity in vulnerable populations
- Improve vaccine efficacy
  - Increase magnitude and quality of immune response
  - Induce the desired type of immune response
  - Faster onset and longer duration of protection
- Allow vaccine delivery by alternative routes
  - Intranasal, sublingual, oral, transdermal
- Promote dose sparing