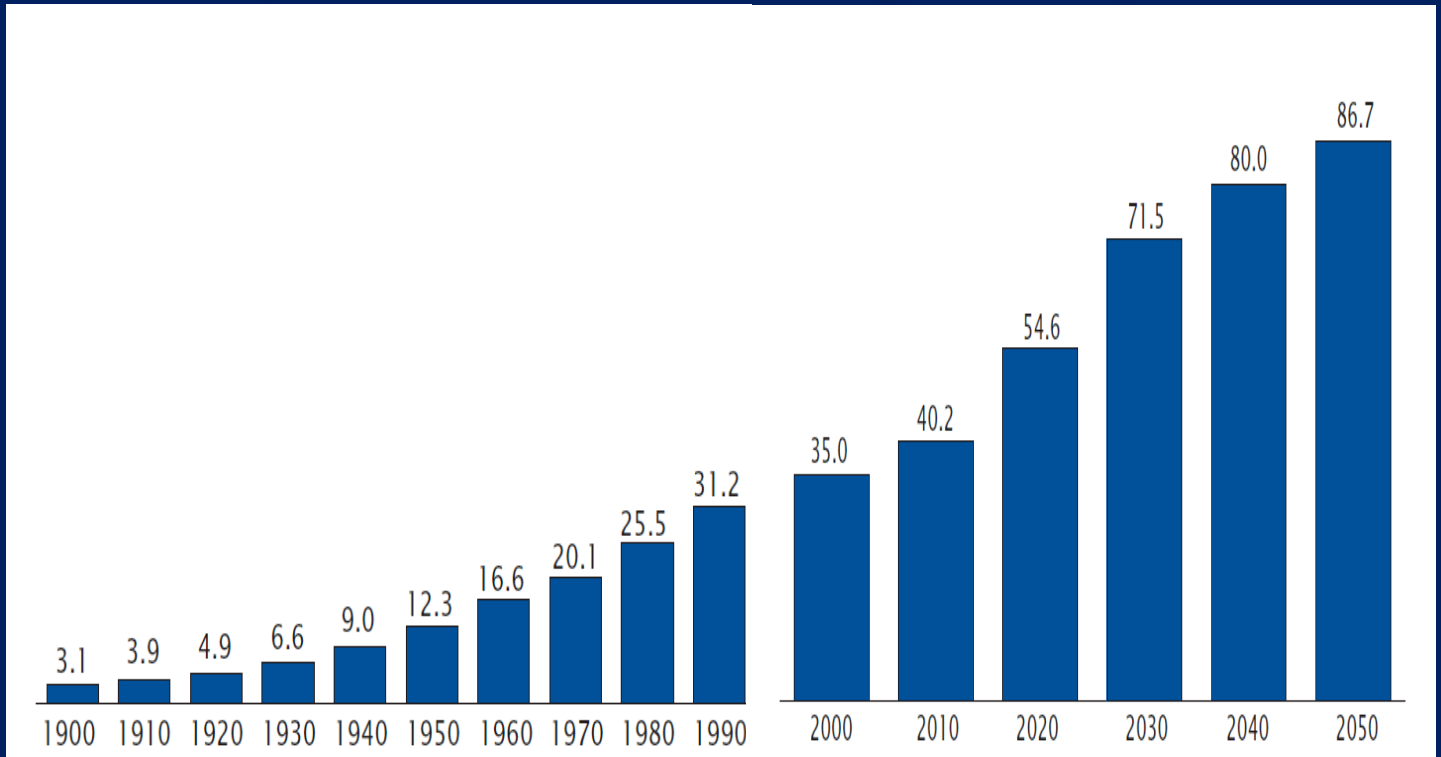
Overview of Human Immunosenescence

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)

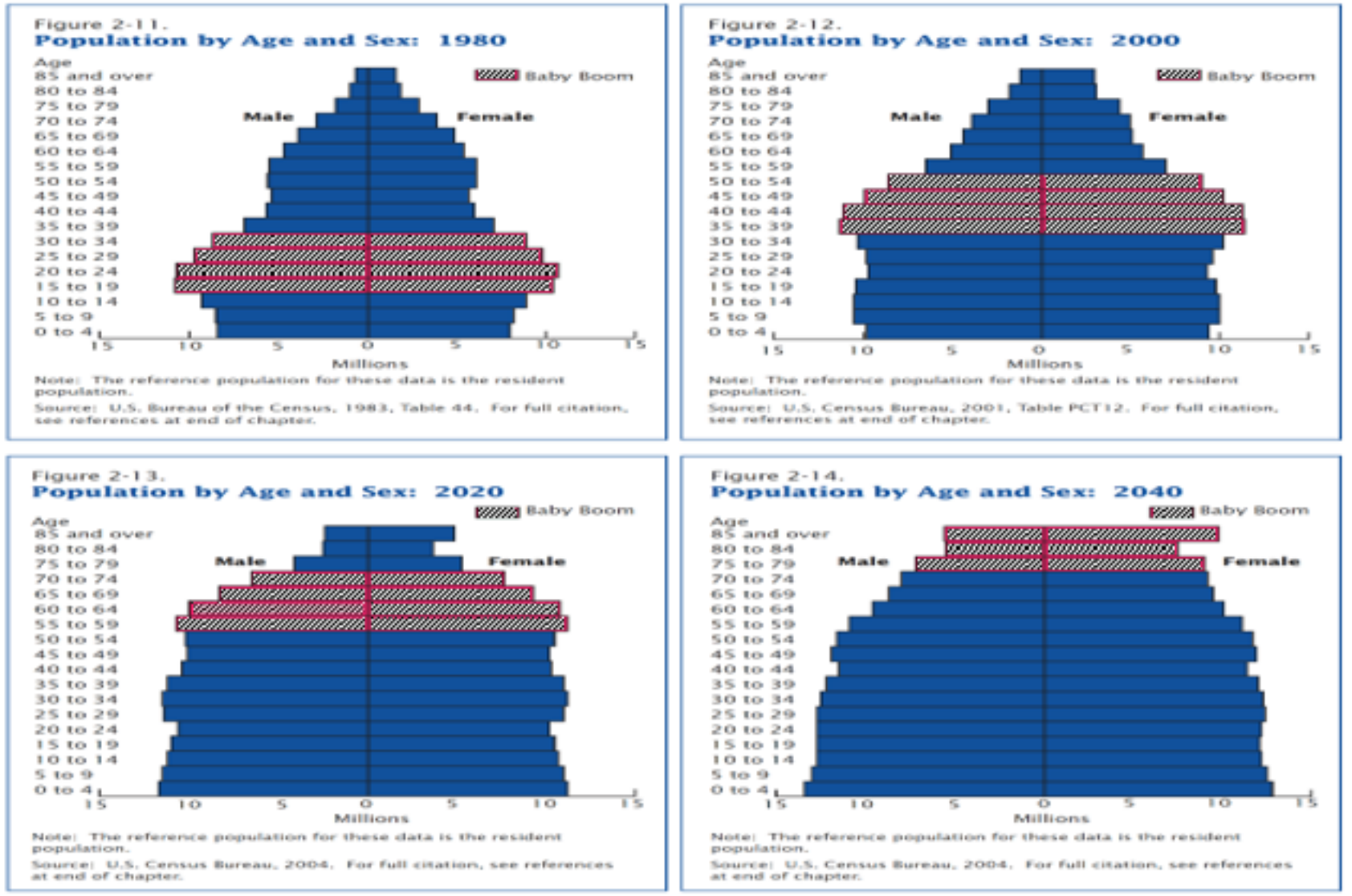


Note: The reference population for these data is the resident population.

Sources: 1900 to 1940, 1970, and 1980, U.S. Bureau of the Census, 1983, Table 42; 1950, U.S. Bureau of the Census, 1953, Table 38; 1960, U.S. Bureau of the Census, 1964, Table 155; 1990, U.S. Bureau of the Census, 1991, Table QT-P1; 2000, U.S. Census Bureau, 2001,

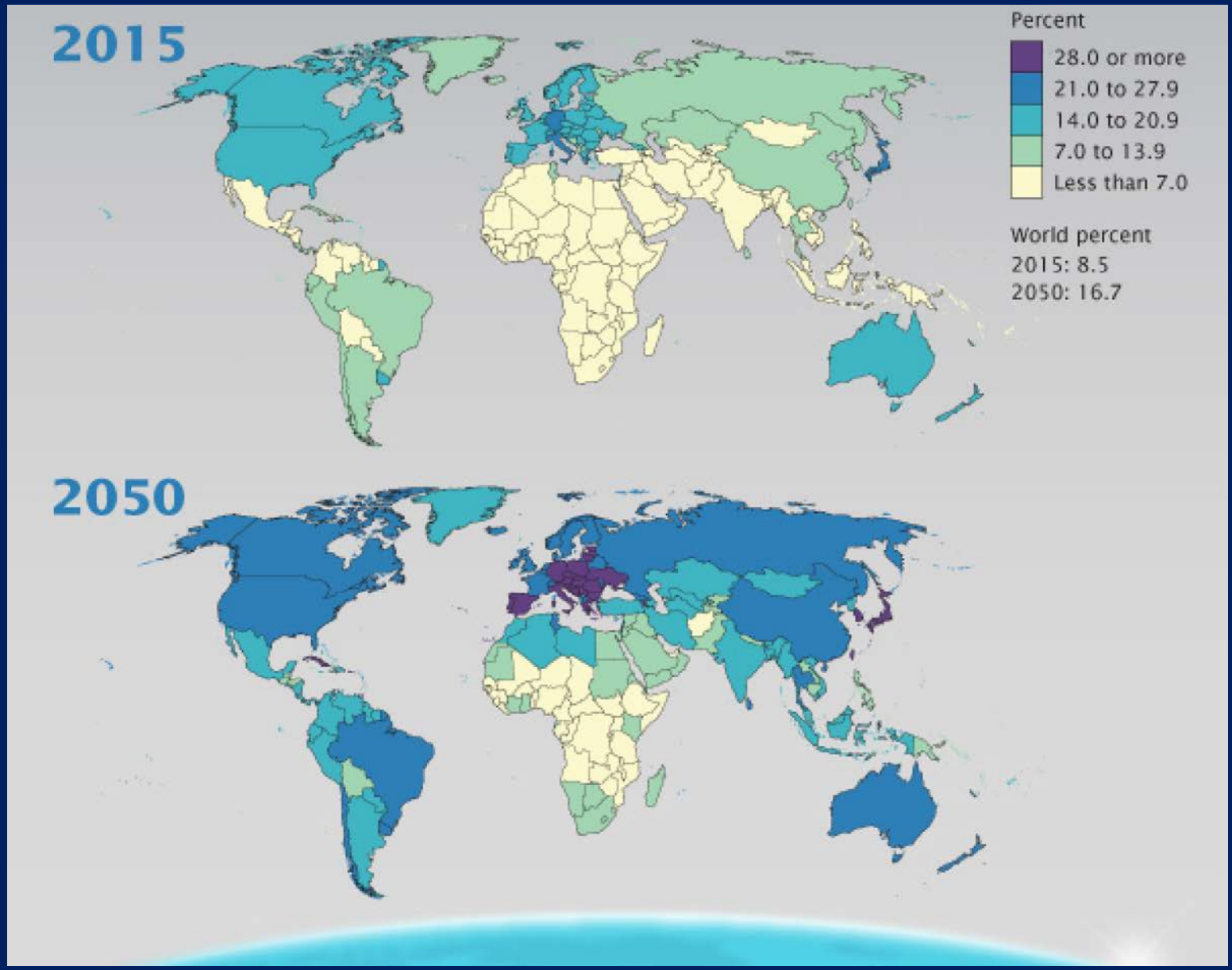
Individuals over age 65 who currently comprise about 15% of the US population account for over 35% of visits to general internists, 34% of prescription drug use, 50% of hospital stays, and 90% of nursing home residents (CDC, 2005).

Aging of the US Baby Boom Generation (1946-1965)



US Census Bureau, “65+ in the United States”, 2005

Increased Proportion of Adults ≥ 65 Years Worldwide

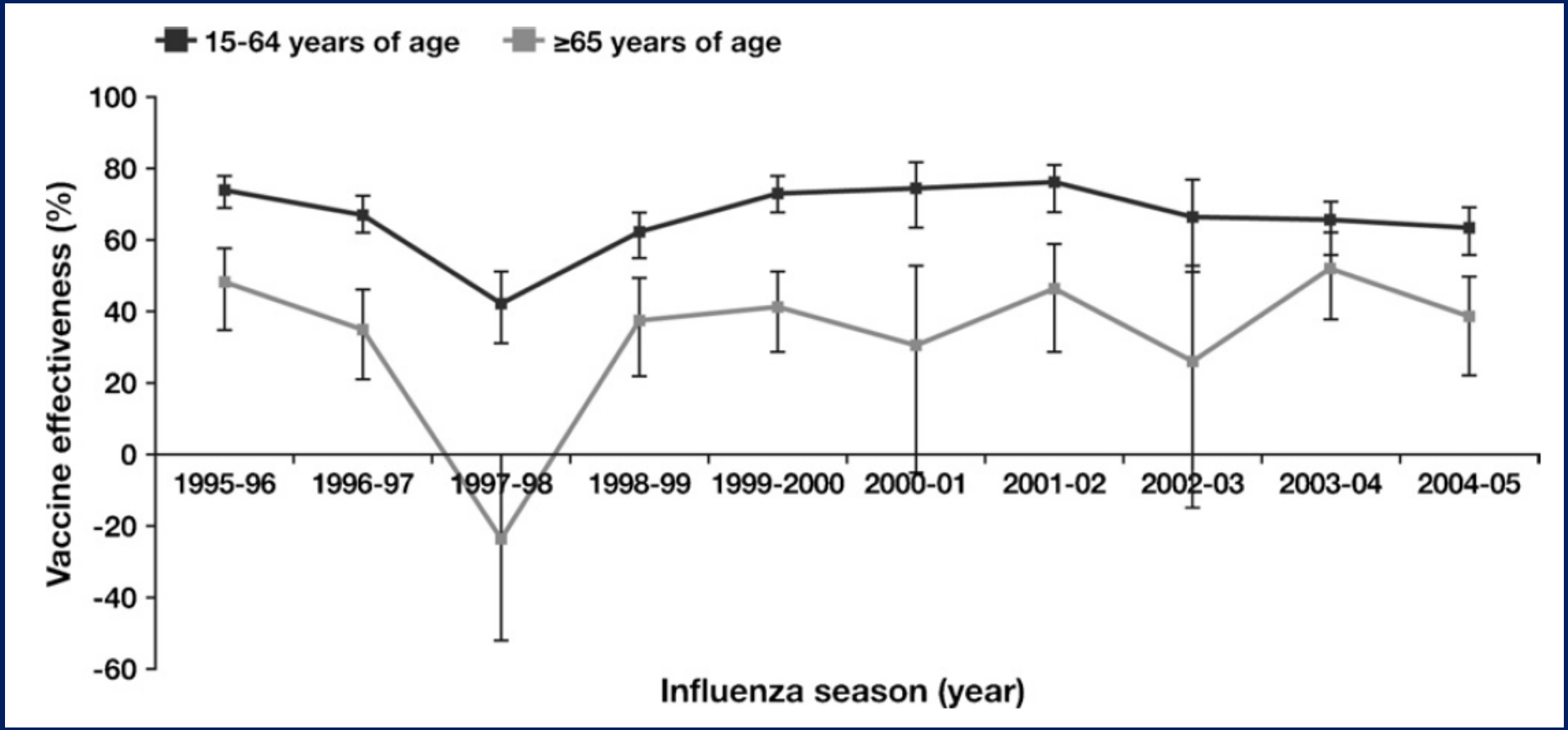


Relative Mortality Rates for Geriatric Infectious Diseases

	Relative mortality rate compared to young adults
Pneumonia	3
Urinary Tract Infection	5-10
Appendicitis	15-20
Cholecystitis	2-8
Sepsis	3
Meningitis	3
Endocarditis	2-3
Tuberculosis	10

Yoshikawa, 1997

Decreased Vaccine Effectiveness with Age

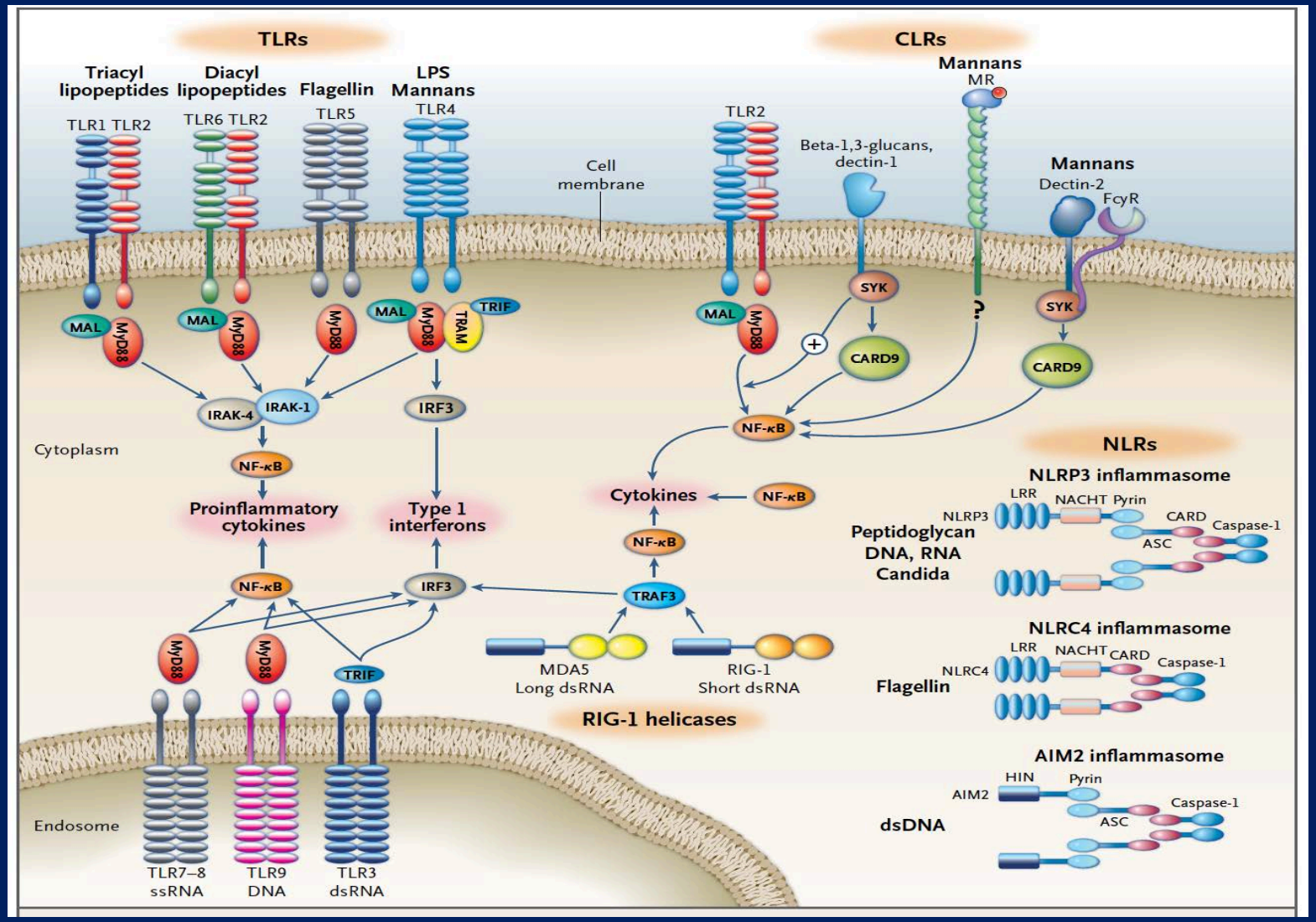


Monto et al., 2009

Innate Immunity

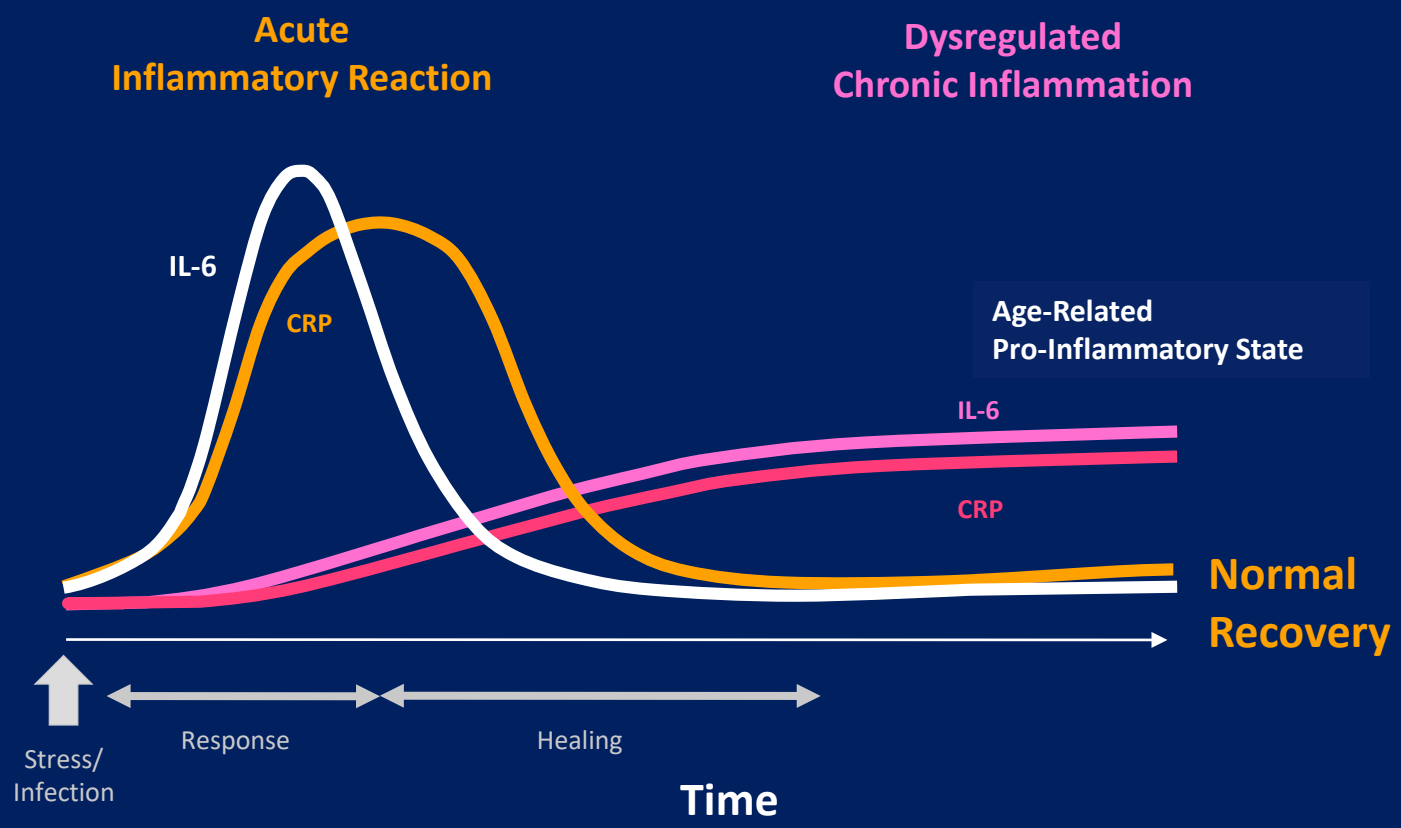
- Rapid onset--mediated by macrophages, NK cells, dendritic cells, mast cells
- Complement pathways, iron sequestration
- Phagocytosis
- Innate immune activation results in inflammatory responses
- Pattern recognition receptors, but not as specific as the slower onset adaptive immune response mediated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

Pathogen Recognition Receptors in the Innate Immune System



Netea and van der Meer, 2011

Acute vs. Dysregulated Chronic inflammation



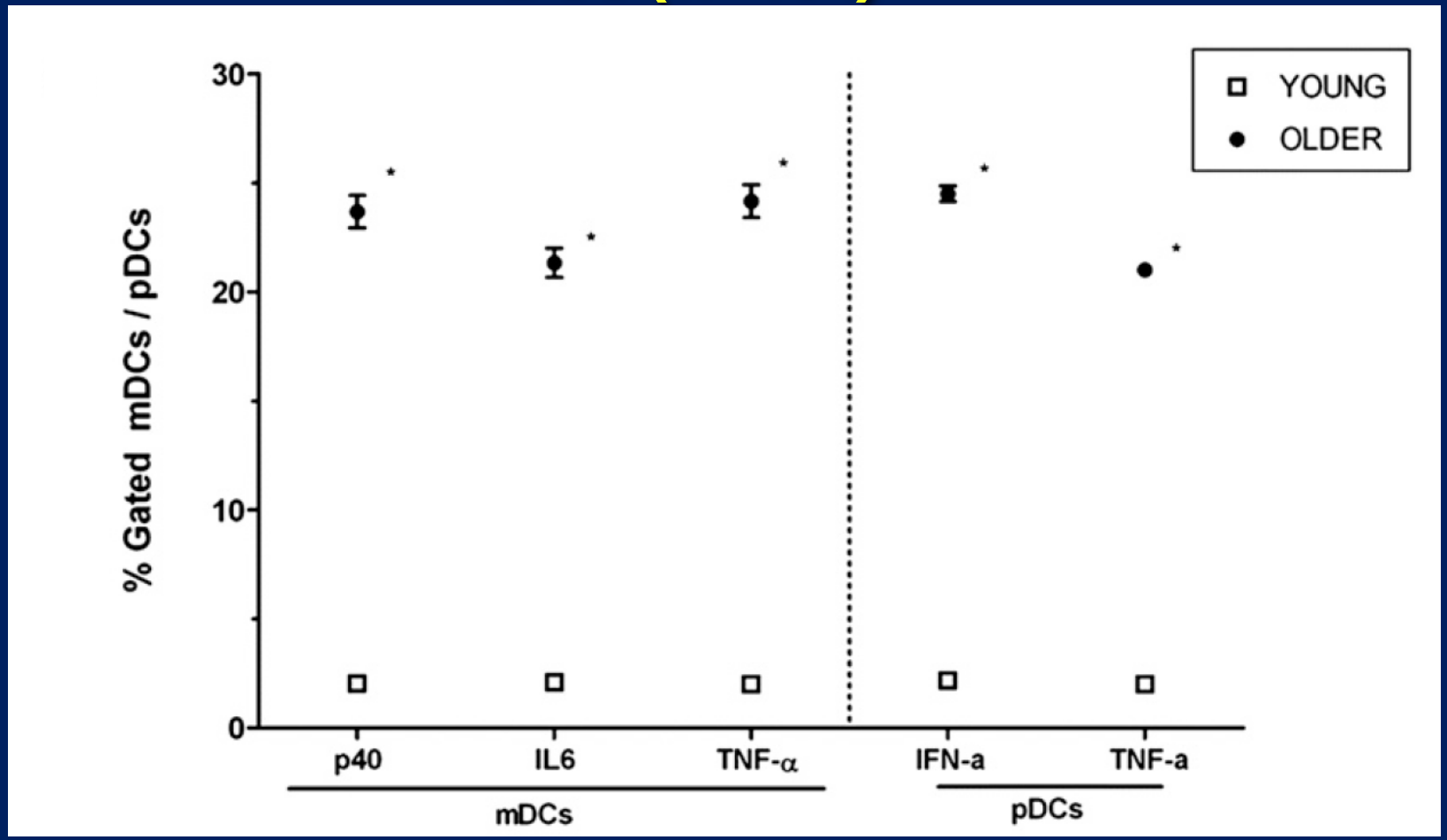
Immune Activation in Aging: Inflamm-Aging

- 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.

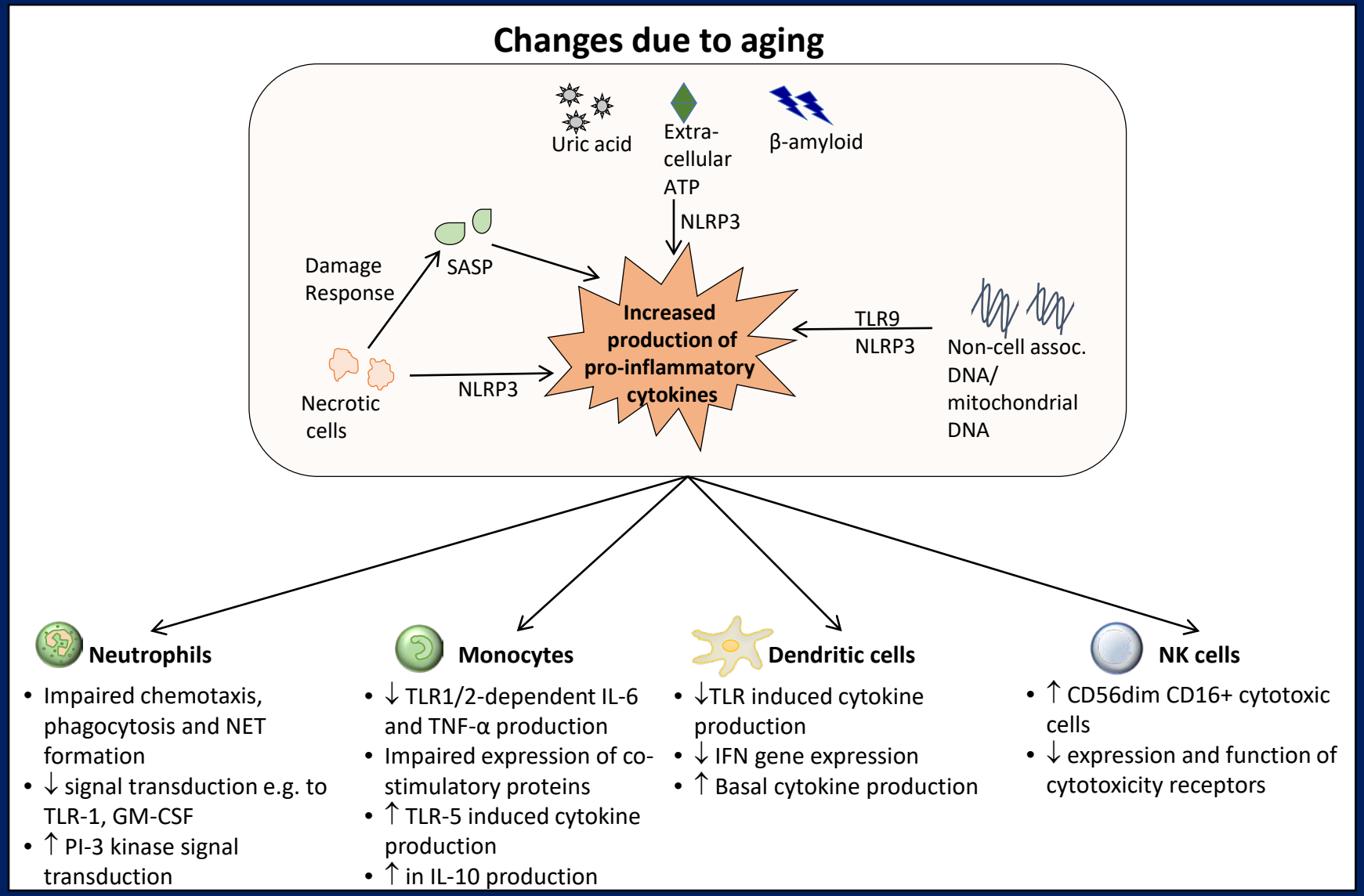
- Source for these inflammatory markers incompletely understood—possibilities include:
  - –Control of chronic viral infections such as CMV.
  - –Engagement of PRRs by endogenous damage-associated molecular patterns (DAMPs)
  - –Release of pro-inflammatory cytokines following DNA damage.
  - –Age-associated shift toward myeloid HSC differentiation.

Age-associated Increase in Basal Cytokine Production in Myeloid and Plasmacytoid DCs (n=104)



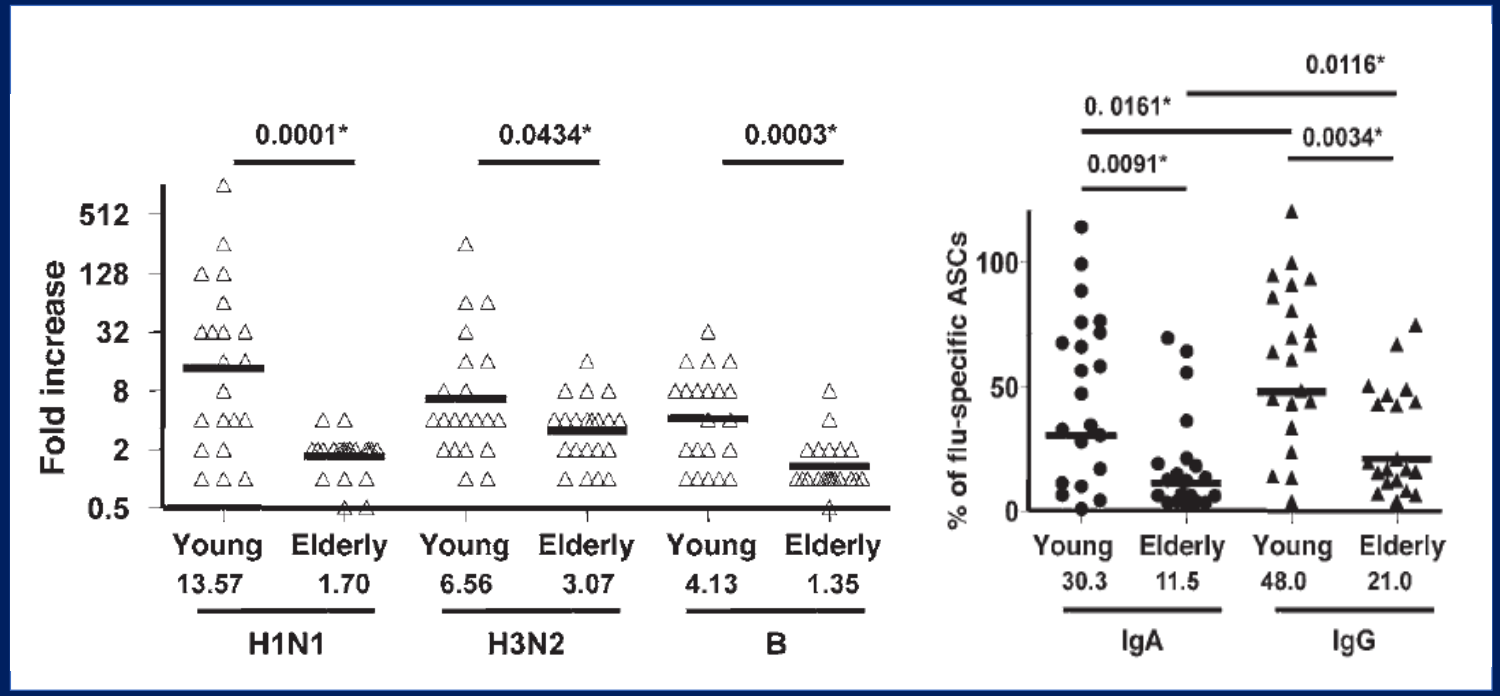
Panda, Qian et al., 2010

Age-Associated Alterations in Innate Immunity



Shaw and Bandaranayake, Clin. Geriatr. Med. 2016

Adaptive Immunity in Aging: B Cells

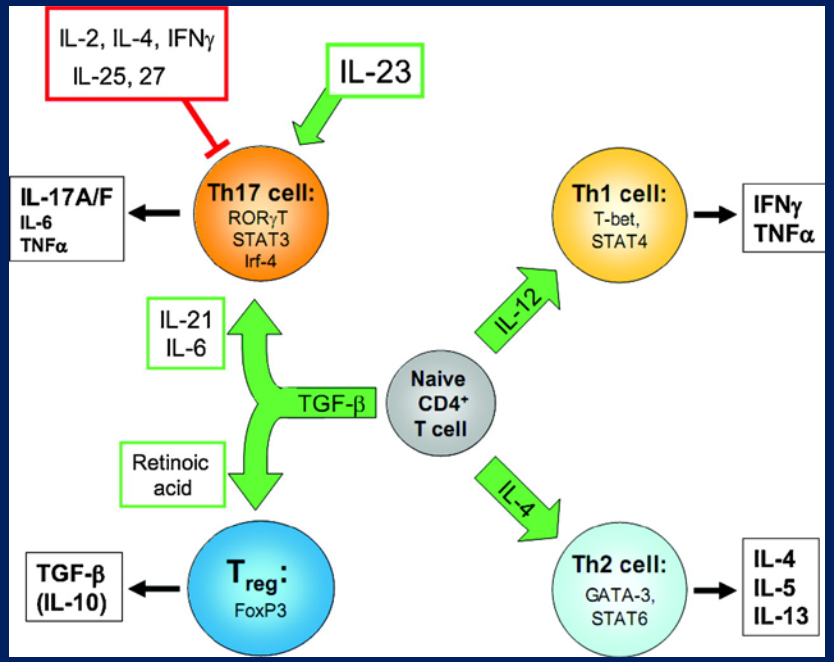


Sasaki et al., J. Clin. Invest. 2011

- Decreased B cell repertoire diversity with age
- Decreased AID expression and decreased Ig heavy chain class switching

Adaptive Immunity in Aging: T Cells

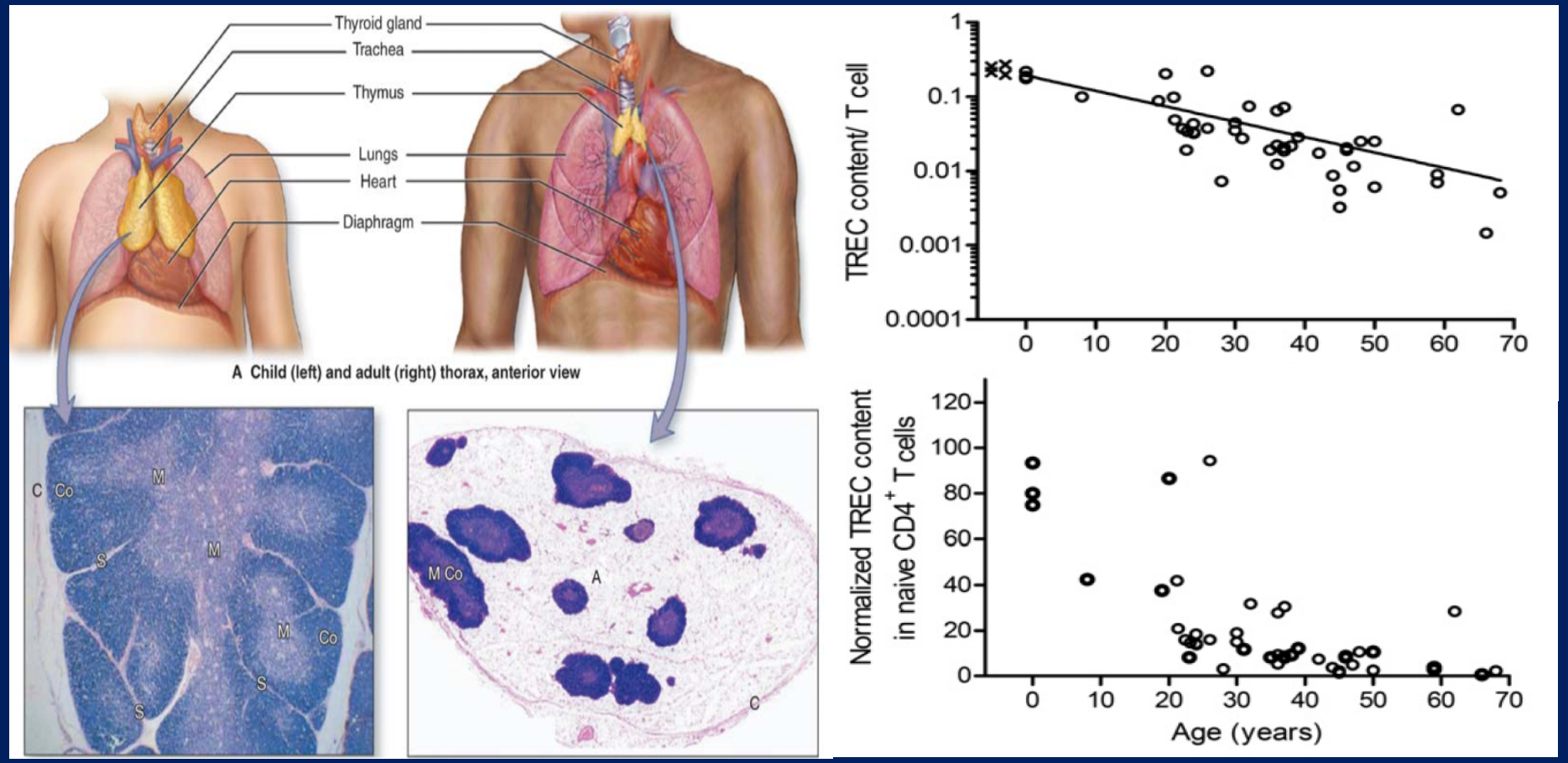
- DTH responses (e.g. PPD) clearly diminished in the elderly
- In human CD4 T cells, age-associated changes in signal transduction are seen, particularly in the ERK MAP kinase pathway.



- 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

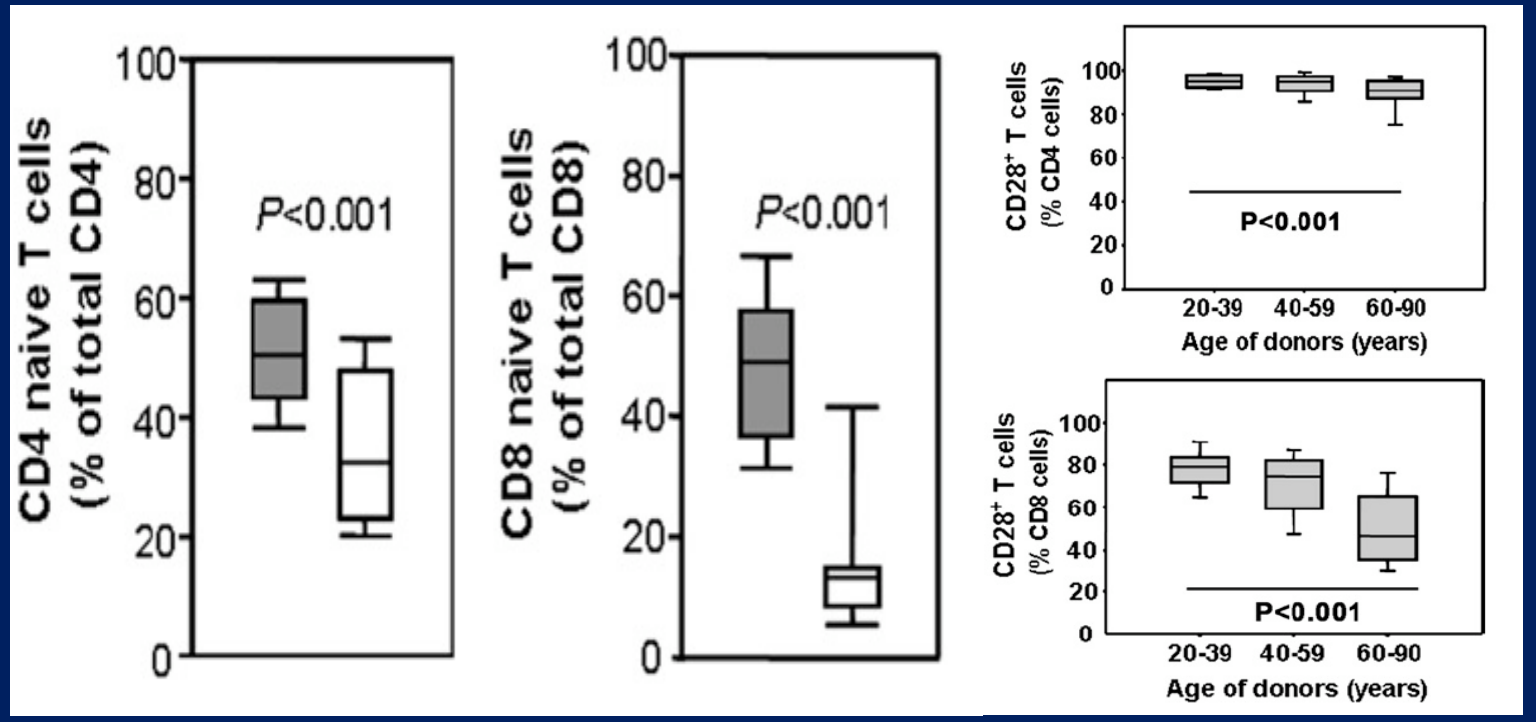
- With thymic involution, the human T cell compartment in adults is maintained almost exclusively (~90%) by peripheral expansion.



Hazzard's Geriatric Medicine and Gerontology

den Braber et al. Immunity, 2012

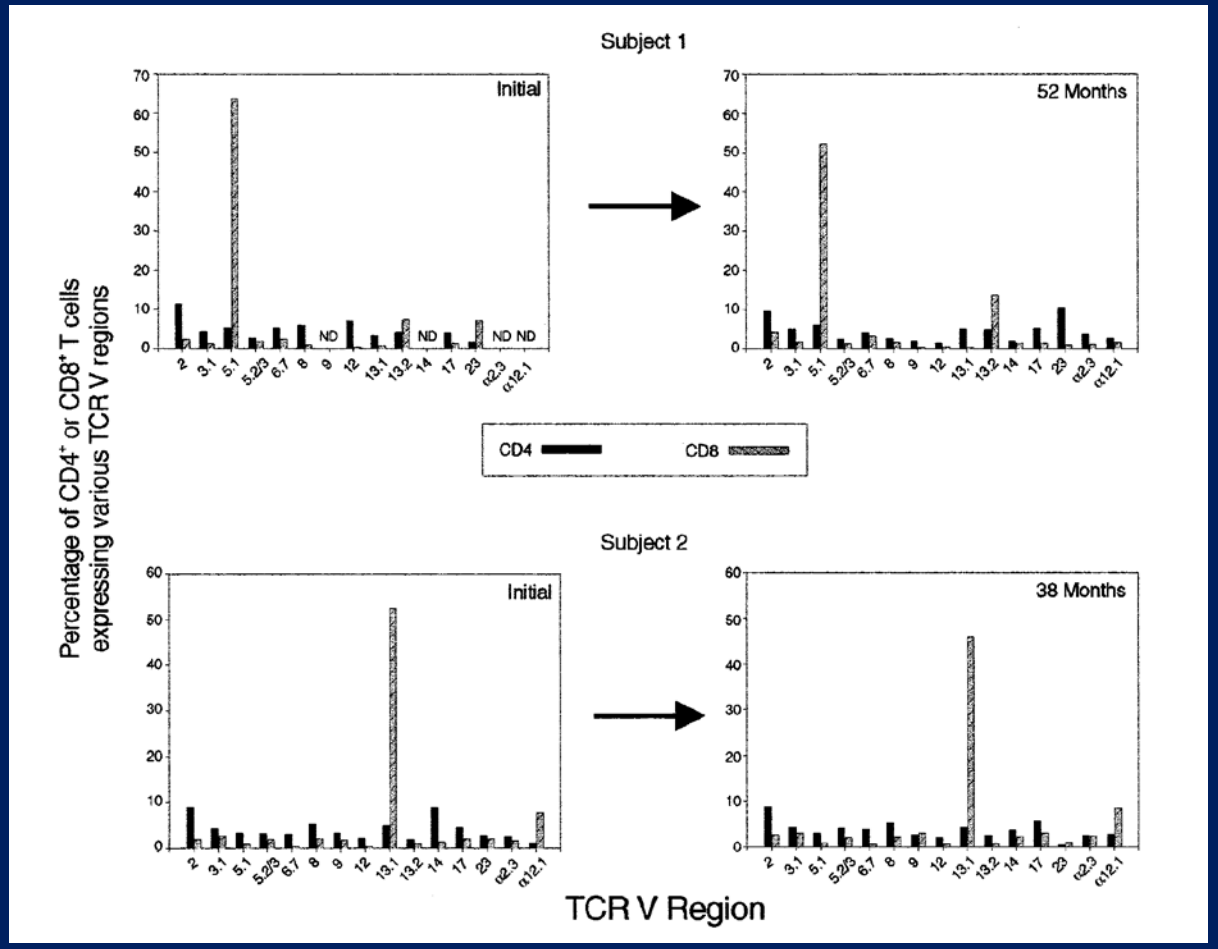
Adaptive Immunity in Aging: T Cells



Czesnikiewicz-Guzik et al., Clin. Immunol. 2008

- 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)

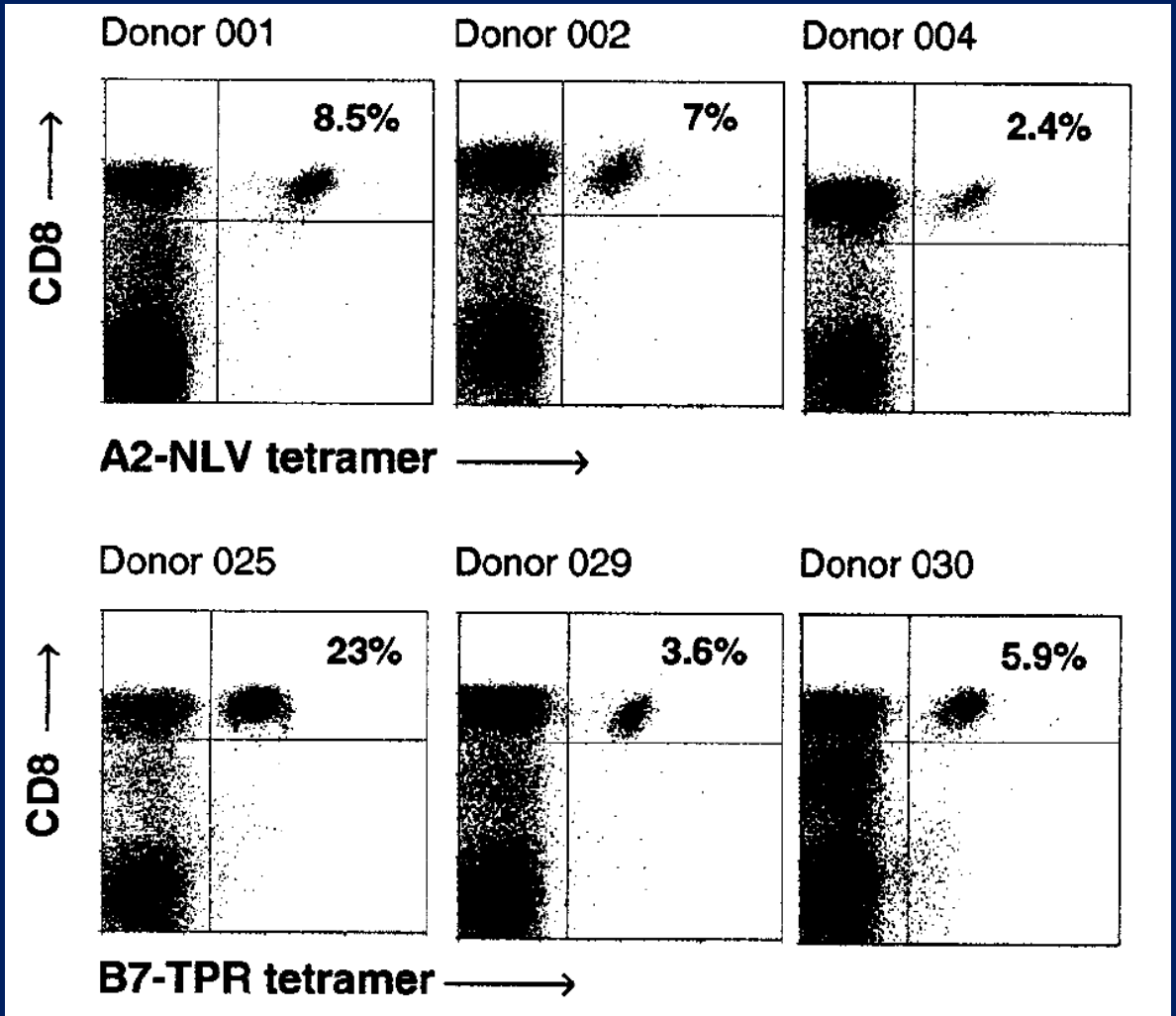
Adaptive Immunity in Aging: T Cells



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

Chamberlain et al., Clin. Immunol. 2000

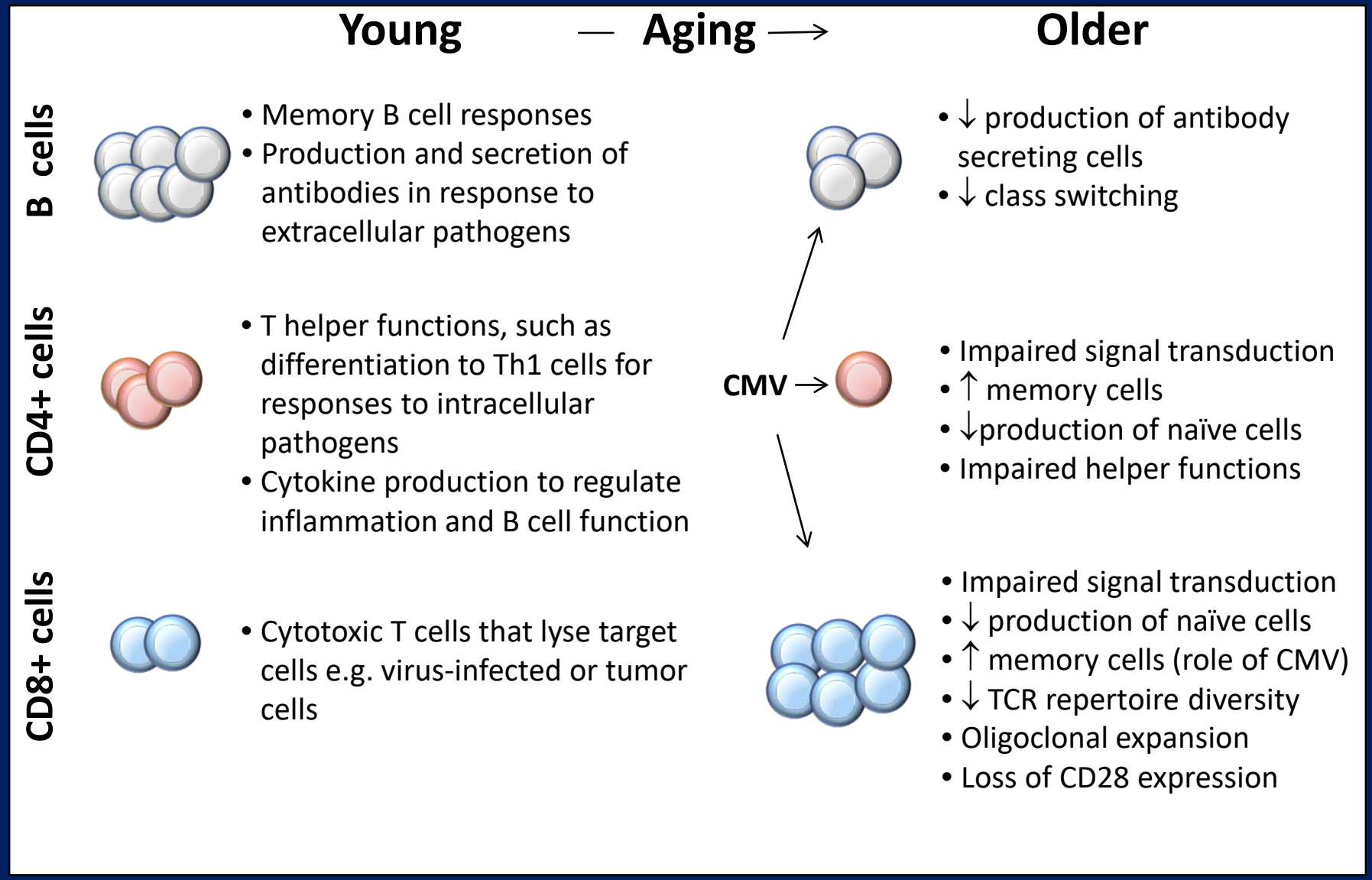
A Substantial Proportion of CD8+ CD28- T Cells Recognize CMV



- 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

Khan et al., J. Immunol. 2002

Age-Associated Alterations in Adaptive Immunity



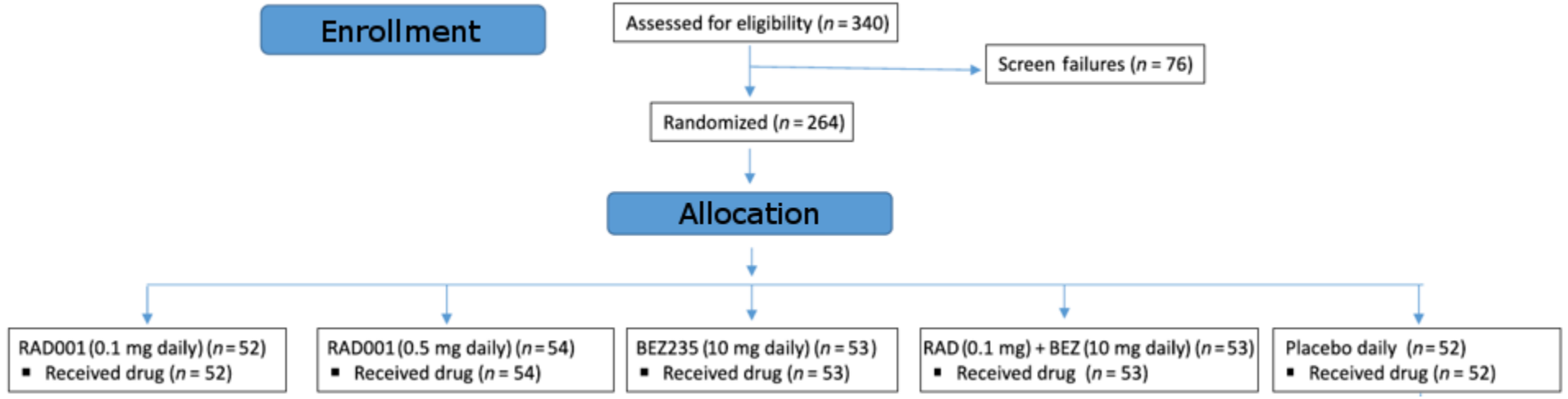
Shaw and Bandaranayake 2016

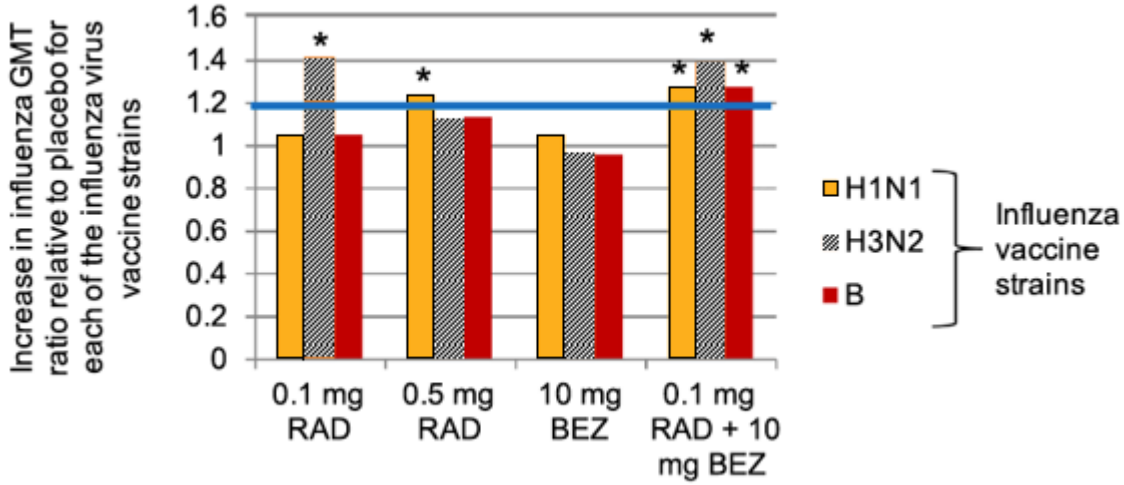
Improving Vaccine Responsiveness in Older Adults

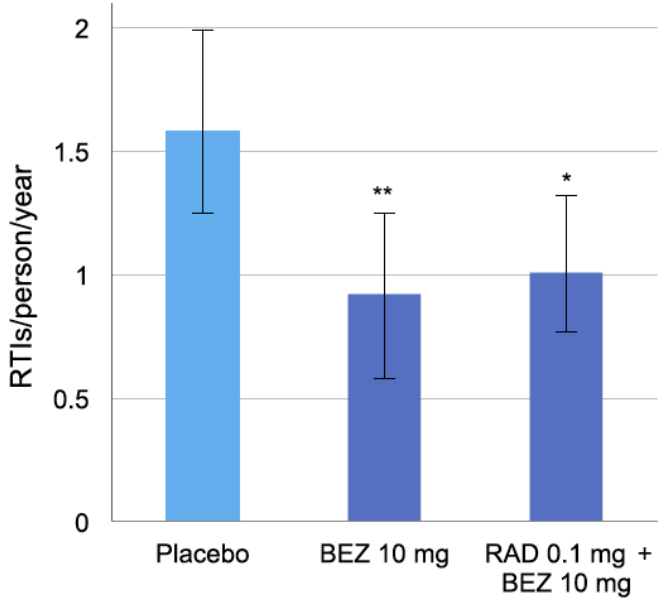
Alternate vaccine formats:

- Higher dose vaccines (e.g. for influenza)
- Vaccine delivery (e.g. intradermal)
- Adjuvants
  - –MF59 (Squalene derivative; Fluad)
  - –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







Mannick et al., 2018

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Specialized Center of Research Excellence
in Sex and Age Differences
in Immunity to Influenza

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

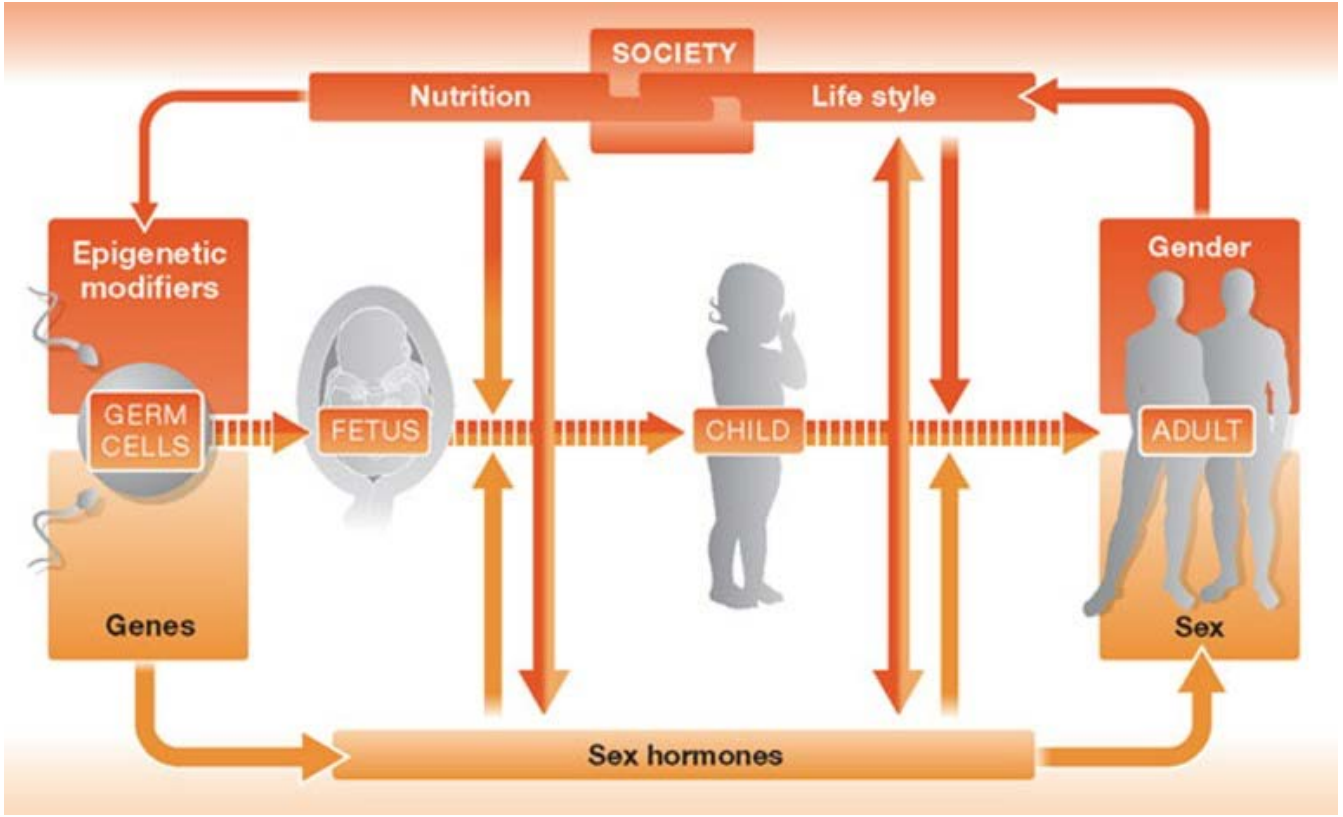
Sex versus Gender

- 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



Regitz-Zagrosek 2012 *EMBO Rep.* 13:596

Why is this resolution important?

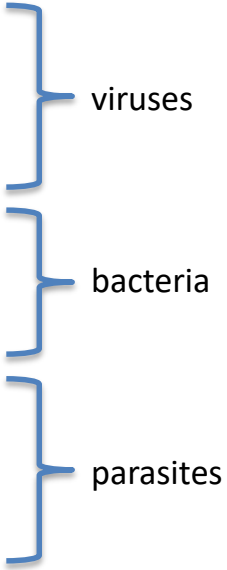
Because over our lifetime, the biological differences between males and females (referred to as sex) and the social or cultural constructs that define being male or female referred to as gender) can interact to impact exposures, susceptibilities, outcomes of disease, and the efficacy of treatments for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



vom Steeg & Klein 2016 *PLoS Pathog* 12(2): e1005374

Protective immunity against infections, including responses to vaccines, are greater in women

Vaccines protect us again 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:

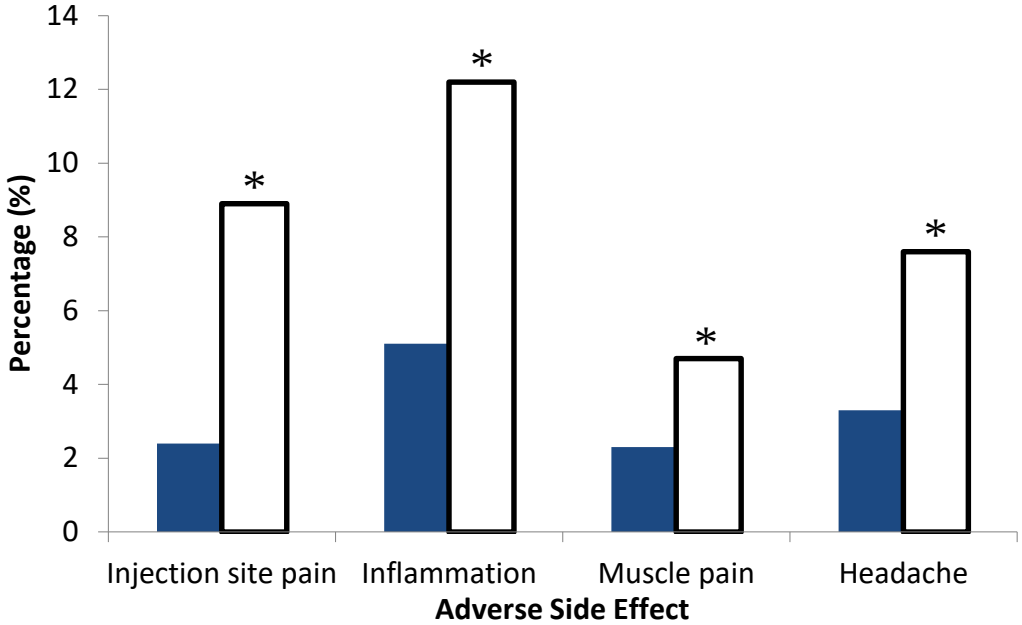
- Flu
- Measles
- Human papilloma virus
- Tetanus

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)

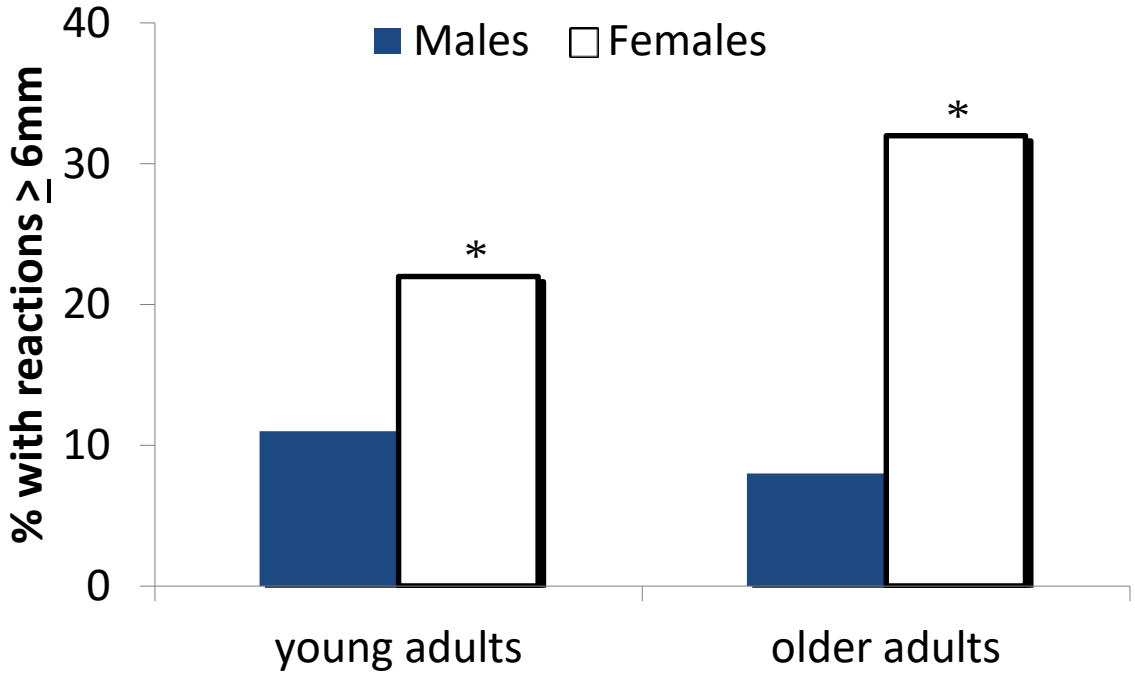
Koplas et al. 2018 *J Am Coll Health* doi.org/10.1080/07448481
Suragh et al. 2018 *Br J Clin Pharmacol* 84:2928
Van Damme et al. 2015 *Pediatrics* 136:e28

Gender differences in reporting of adverse reactions following receipt of the seasonal influenza vaccine



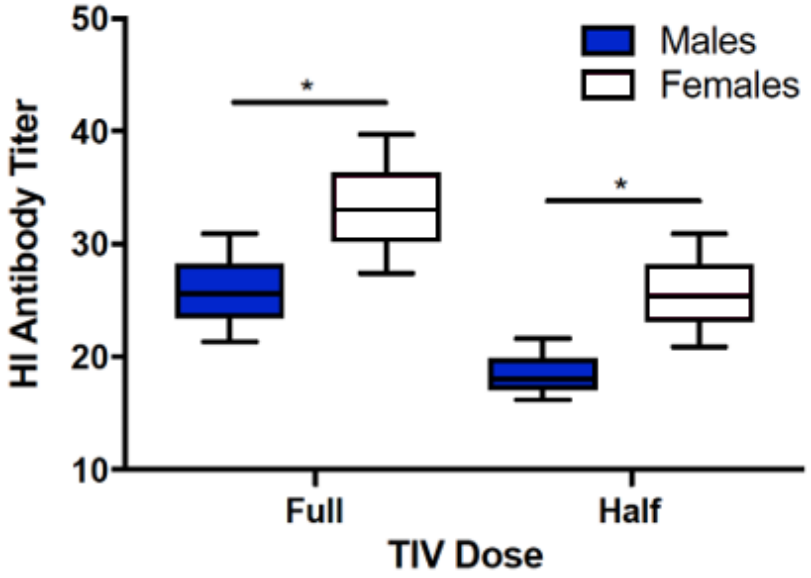
Engler et al. 2008 *Arch Intern Med* 168:2405

Local erythema/induration following influenza trivalent inactivated vaccine (TIV) is greater in females



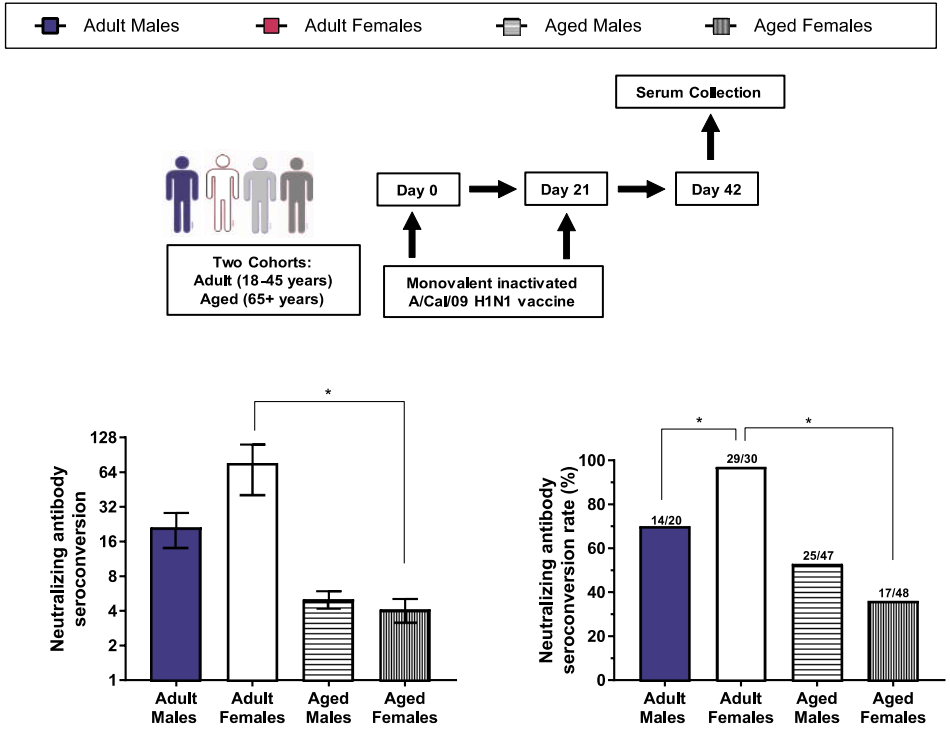
Cate et al. 1983 *Rev Infect Dis* 5:737

Sex differences in response to the seasonal H1N1 vaccine antigen



Engler et al. 2008 *Arch Intern Med* 168:2405

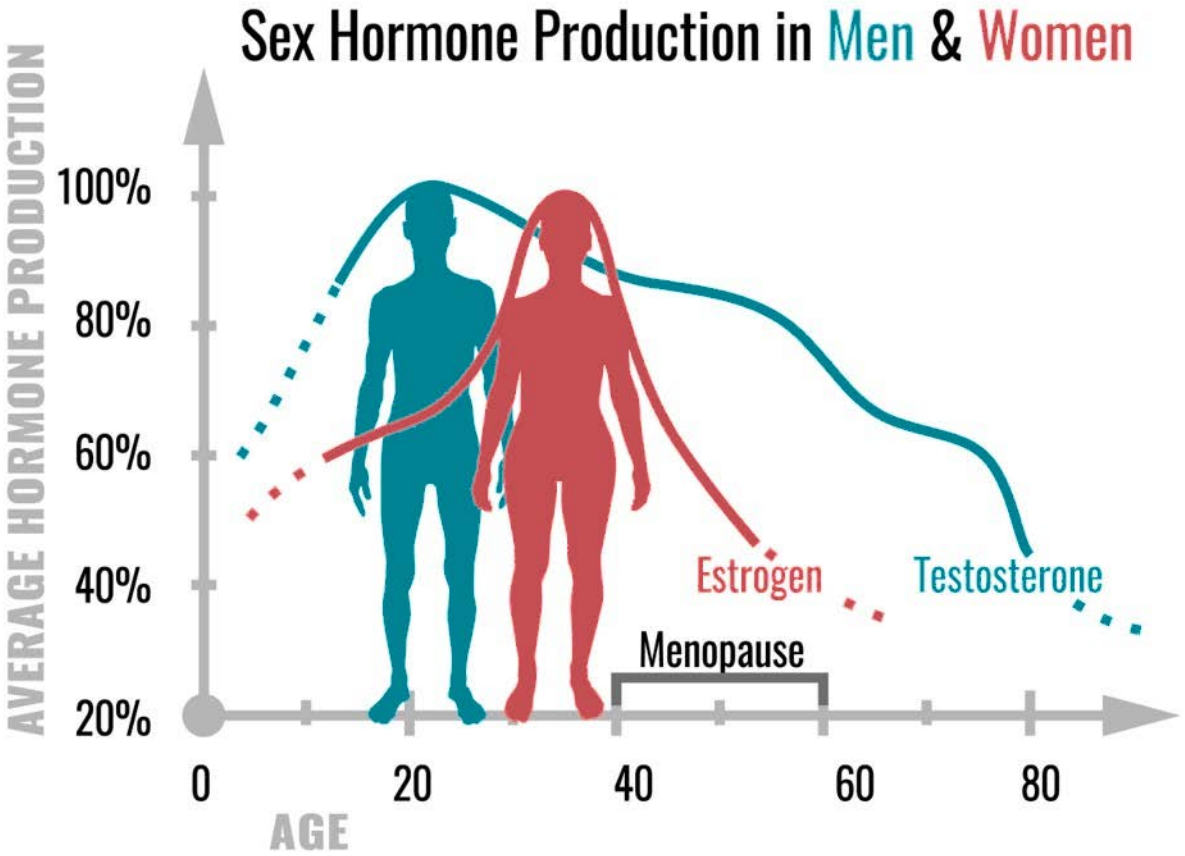
Aging reduces influenza immunity in females



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

Variable	DF	F value	p-value
Hypothyroidism	1, 158	0.0841	0.7722
Hysterectomy/Vasectomy/Post-menopausal	1, 158	0.0004	0.9849
Oral Contraceptive Use	1, 158	0.2542	0.6148
Depression and/or Anxiety	1, 158	0.3081	0.5796
Corticosteroids	1, 158	0.1883	0.6649
Age	**35, 158**	**2.1267**	**0.0009***
Sex	**1, 158**	**4.5181**	**0.0351***
Chronic Respiratory Disease or Smoker	1, 158	1.9411	0.1655
Hysterectomy/Vasectomy/Post-menopausal x Oral Contraceptive Use	1, 158	0.0058	0.9397
Age x Sex	**16, 158**	**3.6545**	**1.15E-05***
Age x Chronic Respiratory Disease or Smoker	**4, 158**	**3.5479**	**0.0084***

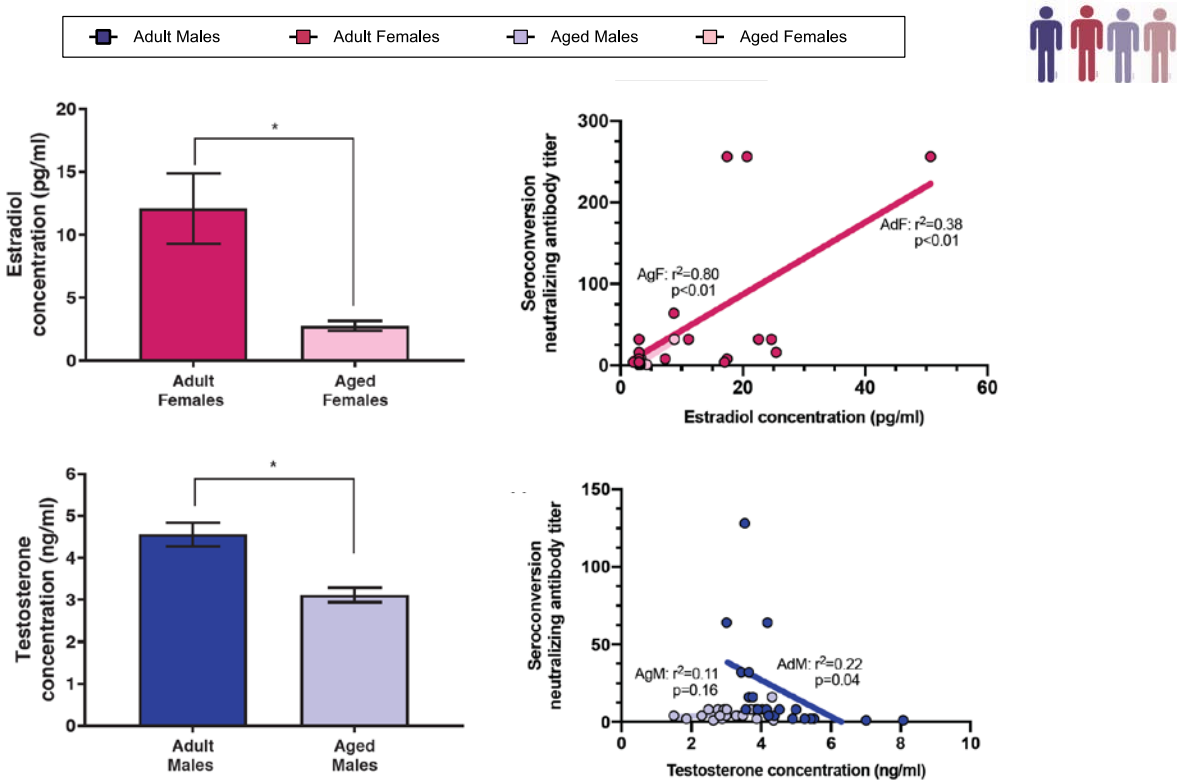


Hormones. Females have higher concentrations of estrogen and males have higher concentrations of testosterone and these concentrations can change (or decline) with aging.

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.

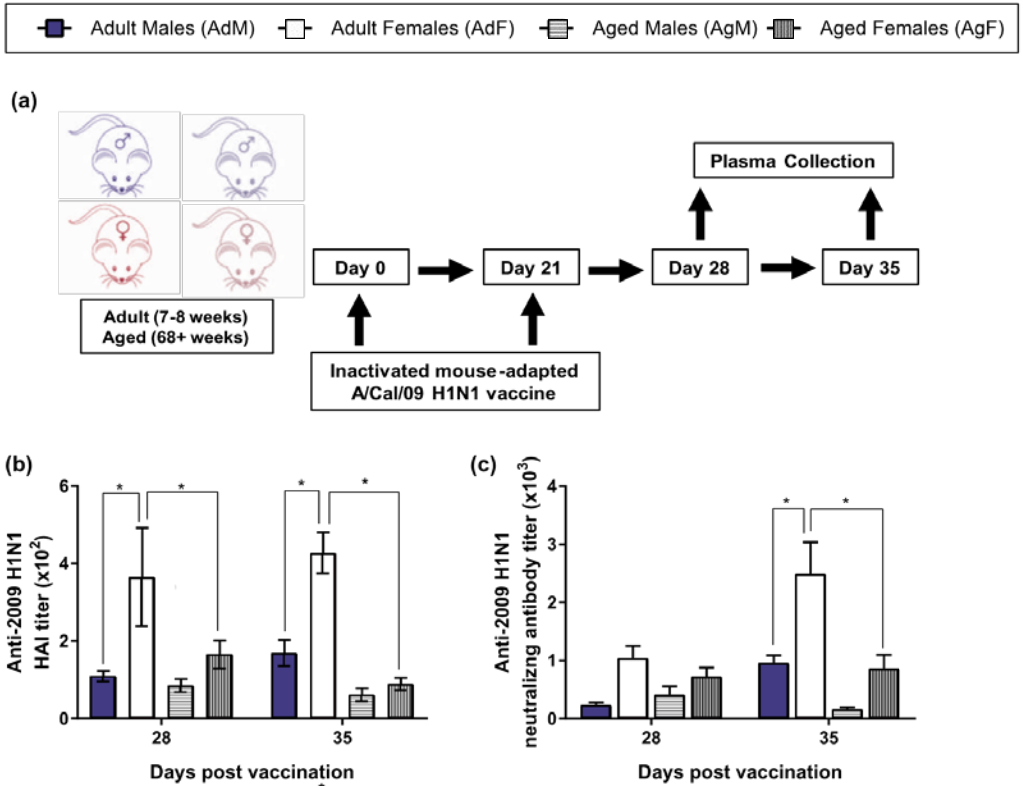
Differences in the concentrations of our hormones cause our immune cells to respond differently when they are exposed to allergens, self-antigens, viruses, and even vaccines. My group has shown estrogens enhance and testosterone suppresses immune responses to the flu vaccine in both mice and humans. 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



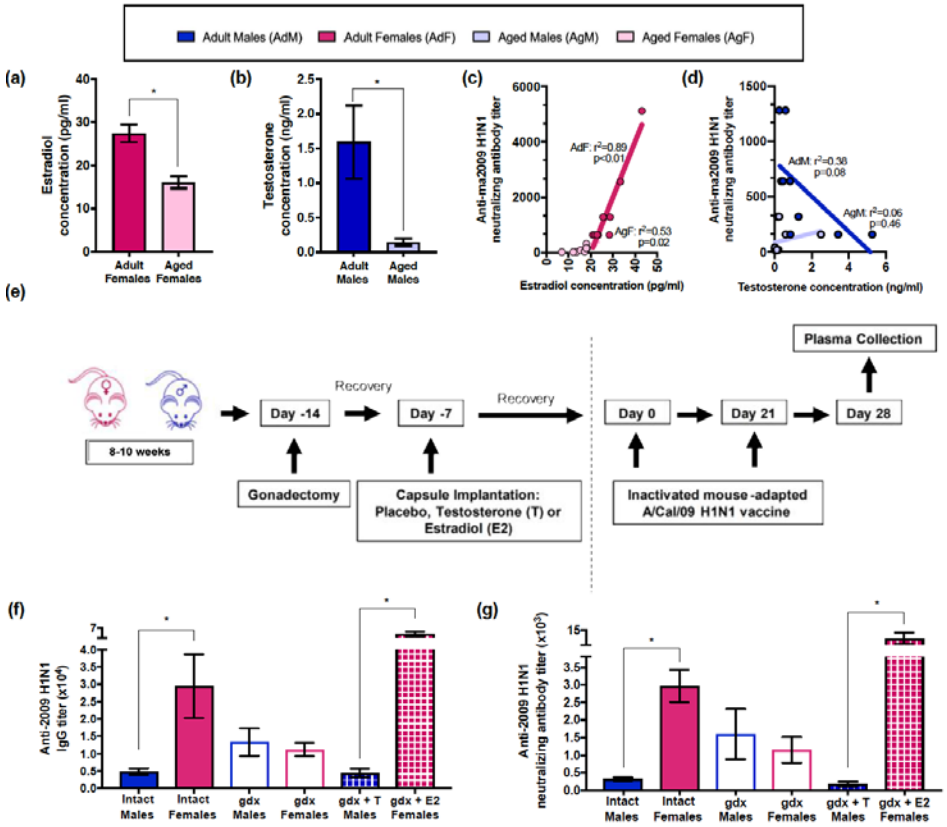
Potluri et al. 2019 *npj Vaccines* 4:29; doi.org/10.1038/s41541-019-012406

Aging reduces influenza immunity in female mice



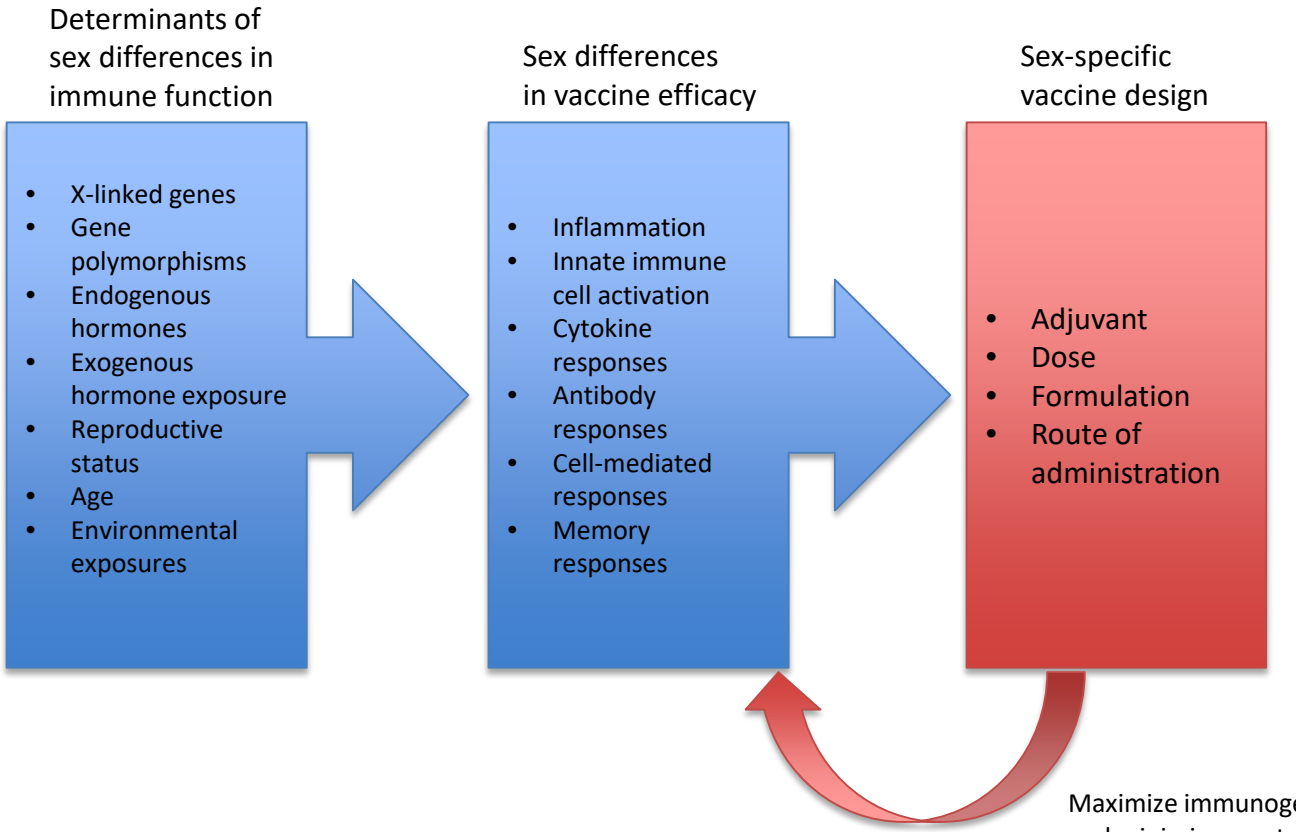
Potluri et al. 2019 *npj Vaccines* 4:29; doi.org/10.1038/s41541-019-012406

Estrogen causes elevated immunity in female mice

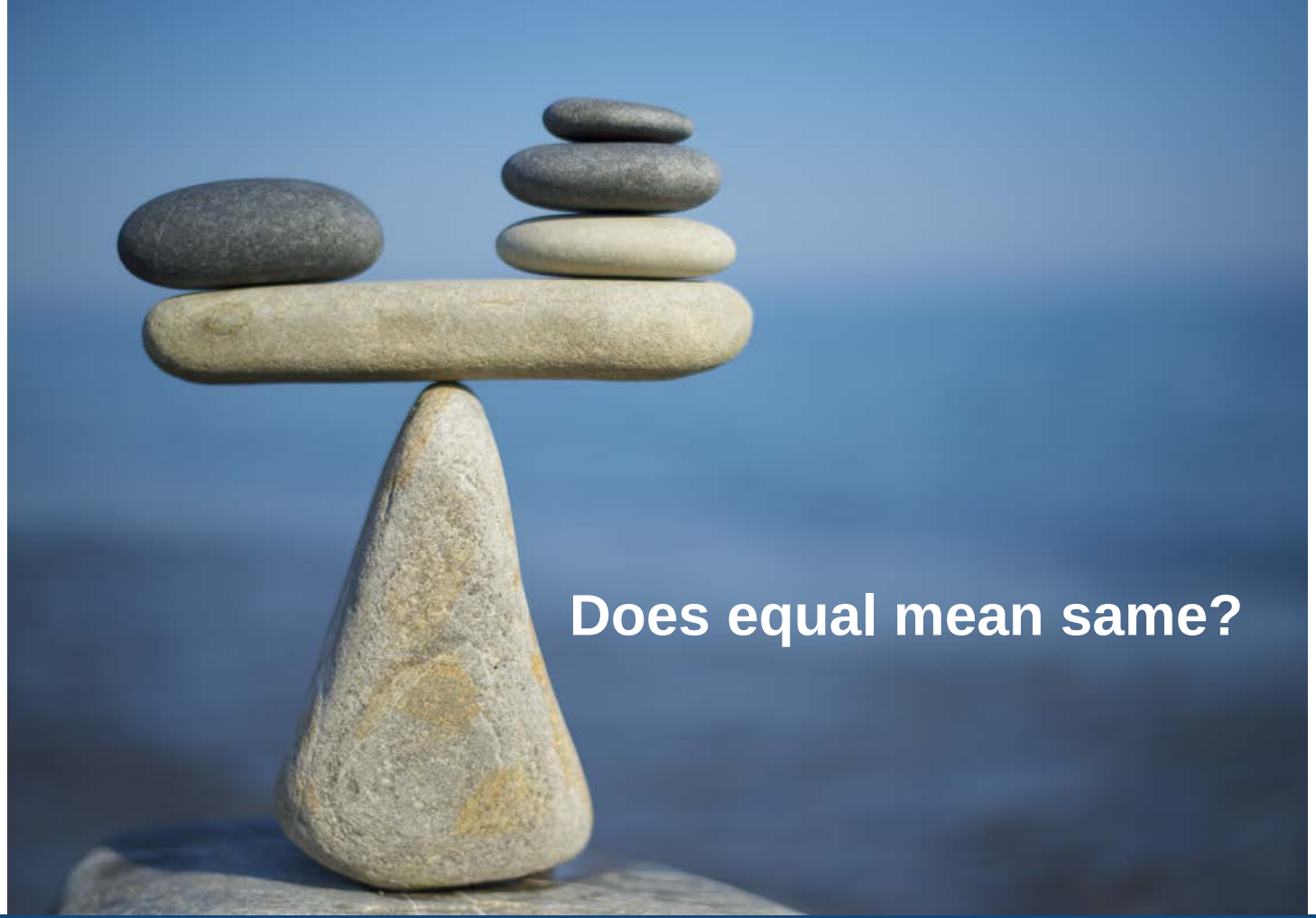


Potluri et al. 2019 *npj Vaccines* 4:29; doi.org/10.1038/s41541-019-012406

Sex-specific vaccine design



Klein & Pekosz 2014 *J Inf Dis* 209:S114



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.

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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

National Institute of Allergy and Infectious Diseases

Goals of the NIAID Adjuvant Program

National Institute of Allergy and Infectious Diseases

- 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
  - https://www.niaid.nih.gov/sites/default/files/NIAIDStrategicPlanVaccineAdjuvants2018.pdf

How Adjuvants Improve Vaccine Efficacy

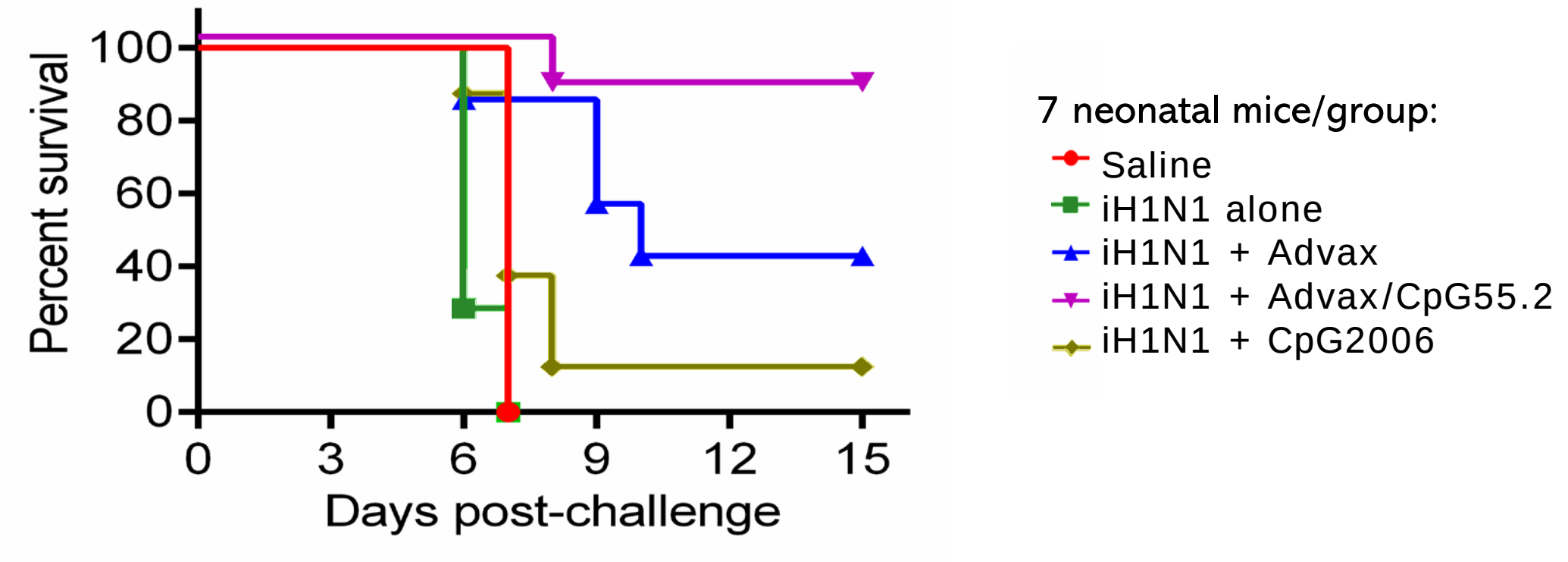
- Overcome age-related immune differences/deficits
- Drive appropriate adaptive immune response
  - Promote specific $CD4^+$ T helper cell subsets (and associated antibody profiles)
  - Induce $CD8^+$ cytotoxic T cell activation
- Induce long-lasting immune memory

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 Reponses to Influenza Vaccine

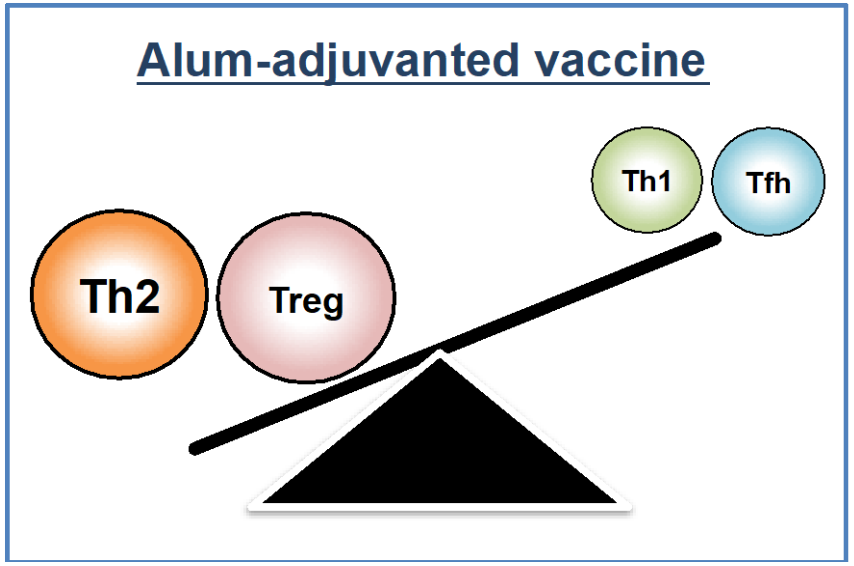


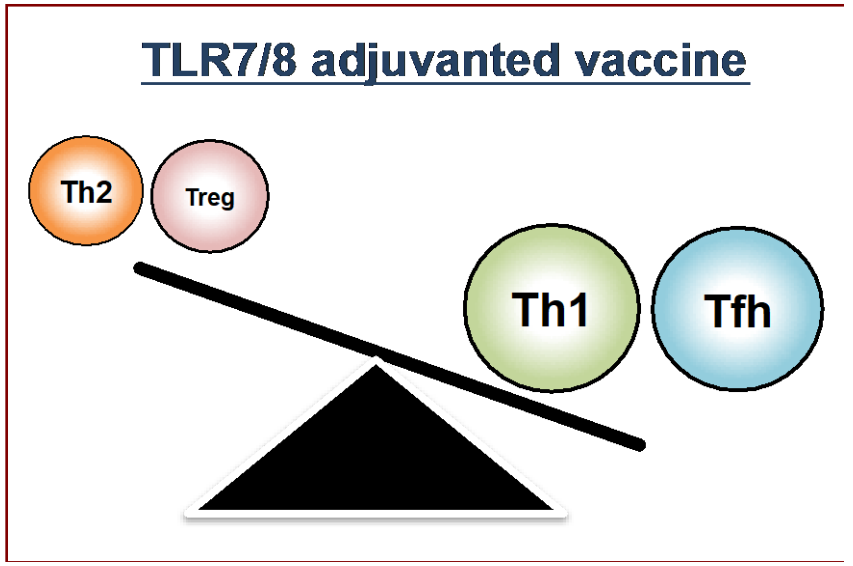
Advax = Inulin, a plant-derived carbohydrate crystal

Advax/CpG55.2 = Inulin + TLR9 agonist; Inulin induces TLR9 expression

Inducing the Optimum Immune Response

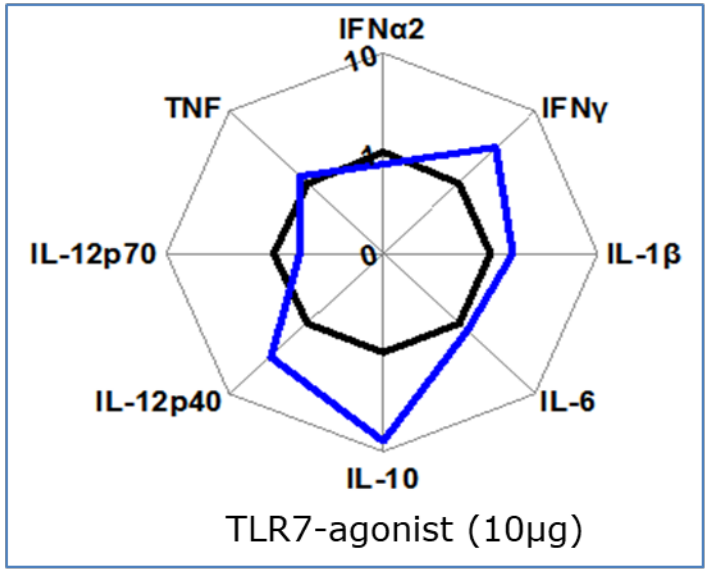
- Protection against various pathogens requires antibodies AND specific T cell subsets
- Adjuvant selection and route of vaccine administration can affect immune profiles
- Alum adjuvants promote Th2 immune response, but not Th1, Th17, or cytotoxic T cells



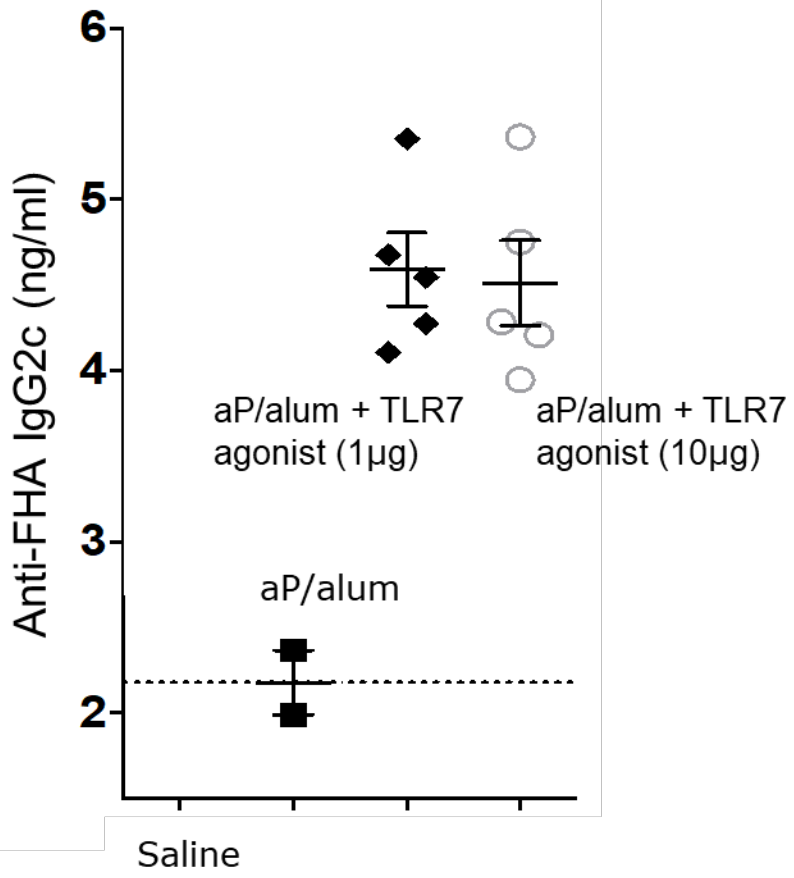


A TLR-Agonist Induces Robust Immunity in Neonates and Overrides Alum's T cell Profile

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

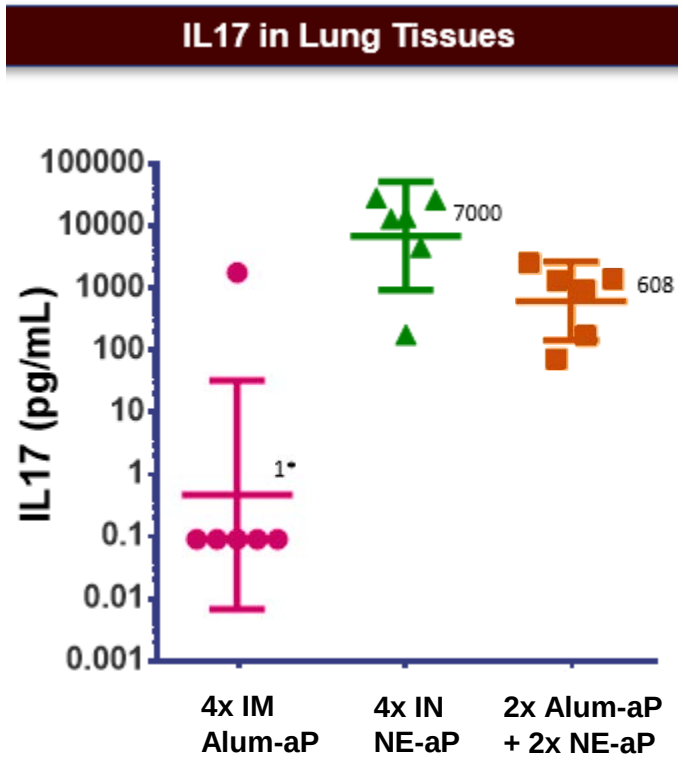


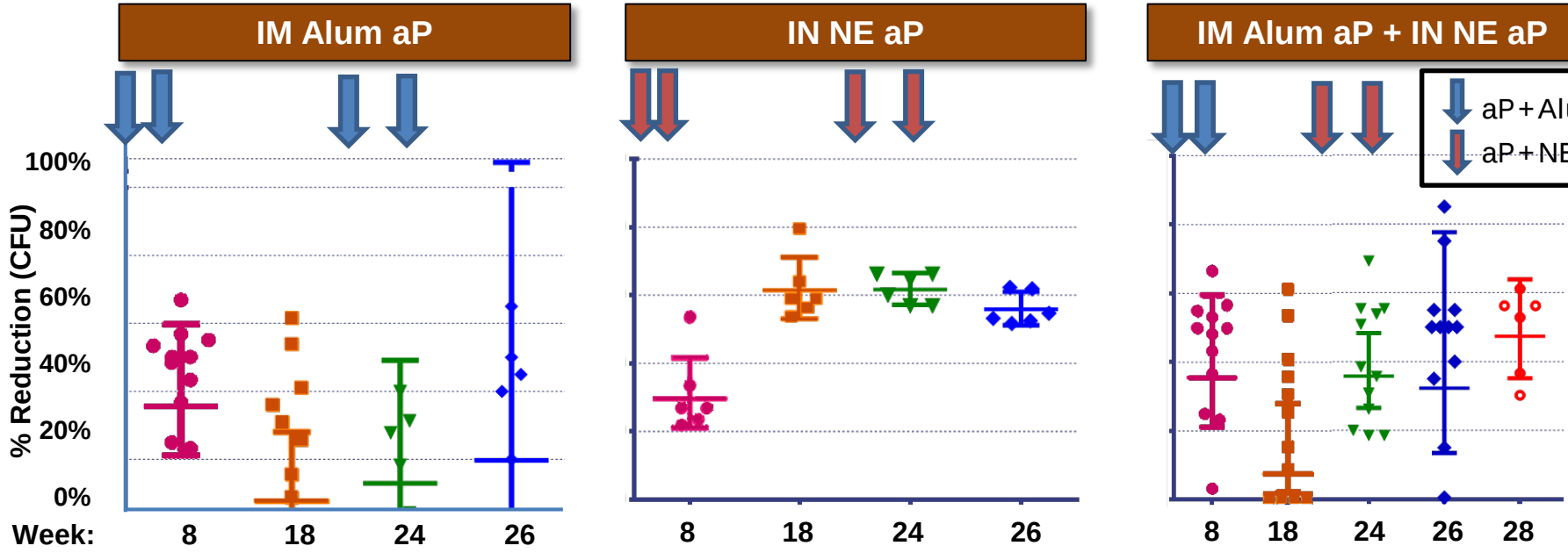
Mouse neonatal Th1-driven antibody response to acellular Pertussis vaccine (aP; Infanrix®)



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





Short- *vs.* Long-term Protection

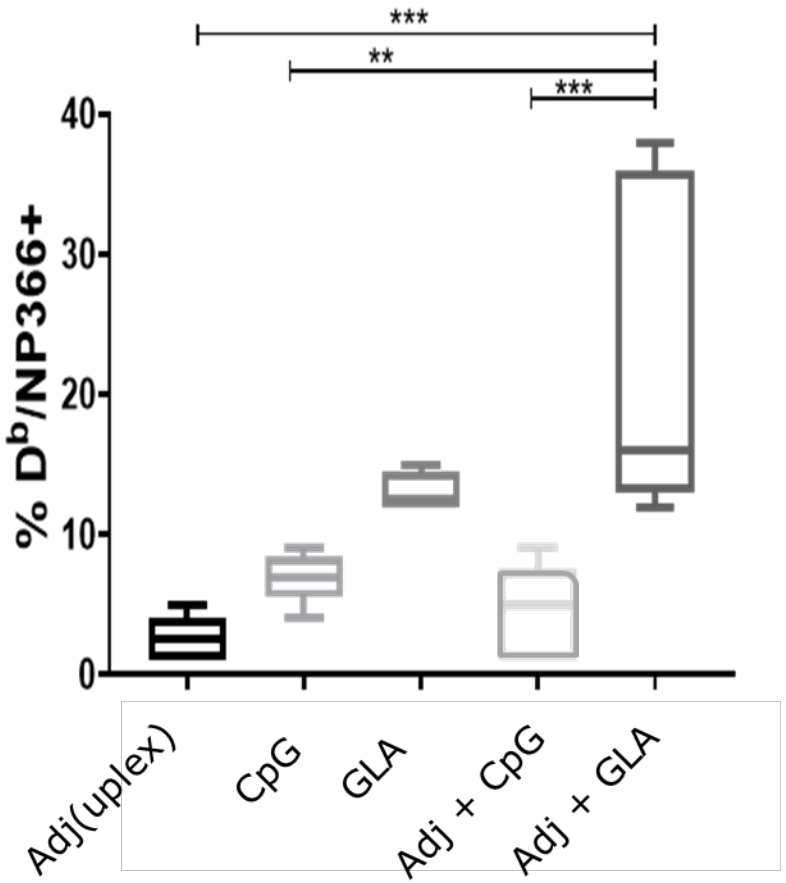
- Many adjuvants induce good effector T cells (short term) but few memory T cells (long term)

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

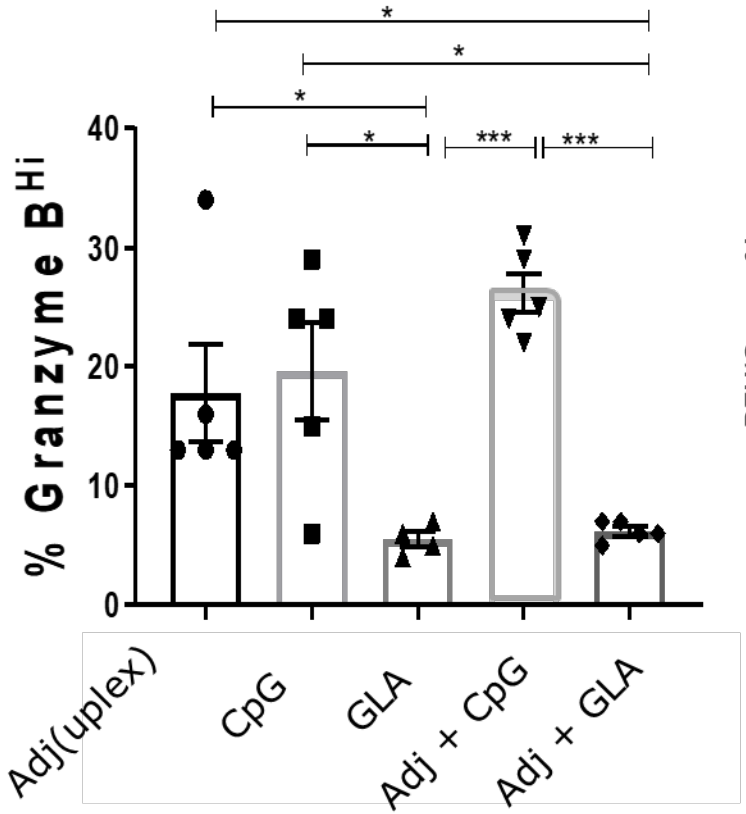
- Examples of combination adjuvant building blocks
  - Adjuplex: polymeric complex with soy lecithin
  - GLA (PHAD): synthetic derivative of bacterial endotoxin (TLR4 agonist)
  - CpG DNA: mimics bacterial DNA (TLR9 agonist)

Adjuvant Combinations Trigger Durable Immune Protection in Mice

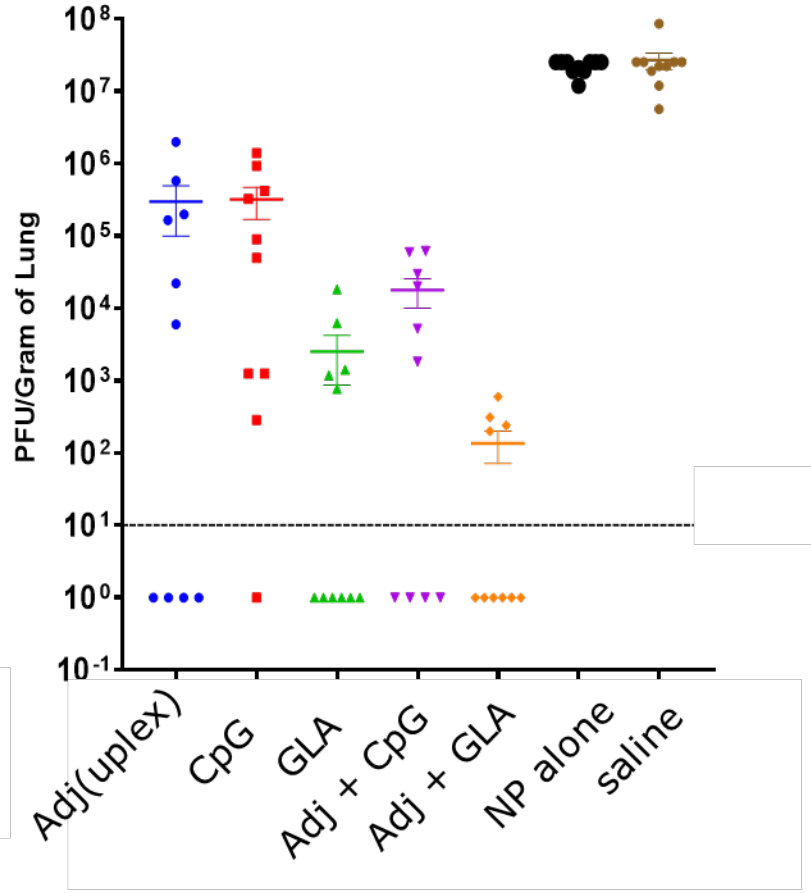
Flu-specific CTL in Lungs
8 days post vaccination



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