Immunology of aging #1: Why older adults are at increased risk of vaccine-preventable diseases

Bonnie B. Blomberg
Dept. of Microbiology and Immunology
U Miami Miller School of Medicine

Immunization in the elderly
World Health Organization
March 22-23, 2017
WHO Objectives:
1) Discuss the immunologic basis for why susceptibility to vaccine preventable diseases increases with age.
2) Discuss the immunologic basis for why response to vaccination decreases with age.
3) Present possible mechanisms for these effects with our data on the molecular and cellular mechanisms affecting B cells and chronic inflammation in the elderly.
Acknowledgements

Daniela Frasca
Alain Diaz
Maria Romero
Thomas Vazquez
Richard Riley
Mike Antoni
Suzanne Lechner

All the subjects!

Franco Ferracci, Ryan Thomas, Nick Mendez Mitch Phillips, John Ryan, Robert Schwartz, Family Medicine
Sandra Chen, Env. Health & Safety
Bali Pulendran & Helder Nakaya (Emory)
Hana Golding & Surender Khurana (FDA)
Giuseppe del Guidice (Novartis)

NIH-NIA, R37 AG023717 (BBB)
NIH-NIA, R01 AG032576 (BBB) NIH-NIAID, R21 AI096446 (BBB+DF)
NIH (Penedo) NCI R01 CA206456 (collab. With Antoni and Northwestern) 7/01/2016 – 06/30/2021
- Introduction to Aging and Immune defects
- Summary of our previous results for Influenza vaccine responses
  - Biomarkers to predict the response: Sw Mem B, AID, low TNF-α
- Measures of inflammation/obesity and B cell response
- Conclusions, future directions
Aging Immune System - 1

• In both mice and humans:
  • Decline in T cell (cellular) functions:
    - Thymus atrophy (involution)
    - Decline in T cell functions such as frequency of CD4+ T cells and mitogen activation
• In both mice and humans:
• Decline in B cell (antibody) functions:
  - Diminished ability to generate high affinity protective antibody responses to immunization against infectious agents or experimental antigens.
  - Number of B cells stays about the same in mice, but their quality (function) is decreased (due to inflammation). In humans both the number and function decrease with age.
The Immune Risk Phenotype ("Inflamm-ageing": increased inflammation with age)

- CD4/CD8 ratio < 1
- CMV seropositivity
- Increased CD8+CD28-CD57+ cells
- Poor T cell proliferative response to mitogens
- Increased IL-6, TNF-α (decreased IGF-1, decreased muscle strength)
- High C-reactive protein
- Low levels of B cells
Factors that affect the immune system during aging

**Positive**
- Physical activity
- Psychosocial intervention / Stress reduction
- Group support / Motivation
- Hormonal influences e.g. endorphins
- Nutritional components
- Sleep

**Negative**
- Increased chronic inflammation
- Hormonal influences e.g. stress hormones
- Multiple infections / Illnesses
Immune (including B Cell-Associated) Deficiencies in Senescence/Aging

Bone Marrow

Spleen (mice) or PBMC (humans)

Pro-B → Pre-B → B

APC → TH

B → Ab
B CELL PERCENTAGES AND NUMBERS DECREASE WITH AGE

CD19+ cells (%)

Pearson's $r = -0.6198$
$p < 0.0001$

CD19+ cells (number/µl)

Pearson's $r = -0.6952$
$p < 0.0001$

38 young, 26 elderly
Age effects on B cell subsets

Switched memory: Spearman's rho = -0.61, p < 0.0001

IgM memory: Spearman's rho = -0.20, p = 0.17

Late/exhausted memory: Spearman's rho = 0.51, p = 0.0003

Naive: Spearman's rho = 0.32, p = 0.03

Total #s and % of B cells decrease with age

We call our “elderly” those 60 and older (where we see the changes)
1) The serologic response to influenza vaccine decreases with age

2) Successive annual vaccinations increase protection against influenza, suggesting that cellular and humoral immune mechanisms are important for protection in elderly individuals

3) Age-related decrease in antibody responses to influenza vaccination have previously been correlated with T cell function

4) Objectives of our work were to determine if autonomous biomarkers of human B cell function could help to predict the in vivo response to the influenza vaccine
EFFECT OF AGE ON Ig CLASS SWITCH

- Aging decreases E47 in activated B cells
- Aging decreases AID and CSR in activated B cells
- Aging decreases IgG antibody production by activated B cells

AID, activation-induced cytidine deaminase, regulates CSR and SHM, critical for high affinity antibodies

Frasca et al. Vaccine 2010
Frasca et al. Int Immunol 2012
**Molecular Pathways for Reduced B Cell Responses in Aging**

Inflammatory cytokines

Microbiome

Fat

CMV

Old B cells are inflamed and refractory

Before stimulation

After stimulation

B cells → ↑↑↑ TNF-α → ↓ AID → ↓ CSR → ↓ IgG,E,A

<table>
<thead>
<tr>
<th>miRs</th>
<th>TTP</th>
<th>E47</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

Tristetraprolin

Activation-induced cytidine deaminase

Class switch recombination

AGE DEFECTS IN B CELLS CONTRIBUTE TO THE REDUCED RESPONSE OF THE ELDERLY TO INFLUENZA VACCINATION
(summary of our published results)

1. At t0, CpG (a B cell mitogen which mimics B cell stimulation by antigen/T cells)-induced AID predicts serum response before vaccination ➔ therefore AID is a biomarker to predict the immune response in both young and elderly

2. At t0, the percentages of switched memory B cells correlate with the serum antibody response to the vaccine and these are lower in the elderly therefore this is another biomarker to predict the response in both young and elderly

Frasca et al. Vaccine. 2010, 28:8077-84
Khurana et al. PLoS Pathog. 2012, Sep;8:e1002920
EXPERIMENTAL SCHEME

Seasonal influenza vaccine 2011-2012
(Novartis TIV Fluvirin and GSK TIV Fluarix)

Blood drawn at t0, t7 and t28:

Serum collected \((HAI, ELISA, \text{TNF-}\alpha)\)

\[\downarrow\]

Blood \((B \text{ cell subsets, } \text{icTNF}\alpha)\)

\[\downarrow\]

PBMC/B cells isolated

↓

Left unstimulated

↓

RNA extracted

↓

qPCR \((\text{miRs})\)↓

Stimulated \((\text{CpG or vaccine Ags})\)

↓

mRNA extracted

↓

qPCR \((\text{AID})\)

↓

fold-increase in AID from t0 to t28
The serum response to influenza vaccination decreases with age - HAI

H1N1

H3N2

B

37 young, 28 elderly
AID predicts the HAI response in 92% of young and 74% of elderly individuals.

- 92% individuals, p<0.001
- 74% individuals, p<0.05

Season 2010-2011 and 2011-2012
62 young, 39 elderly

+ = HAI ≥ 4 / AID ≥ 0.1
Switched memory B cells predict the HAI response in 87% of young and 79% of elderly individuals.

- 87% individuals, p<0.001
- 79% individuals, p<0.05

Season 2010-2011 and 2011-2012
62 young, 39 elderly

+ = HAI ≥ 4 / SM ≥ 2%
SUMMARY OF TNF-α DATA

1) Both serum and (unstimulated) B cell-derived TNF-α increase with age (and they are “refractory” to further stimulation)

2) Systemic (serum) and B cell-derived TNF-α are correlated

3) AID in vaccine-stimulated B cells is negatively correlated with intracellular levels of B cell-derived TNF-α protein

4) Anti-TNF-α antibody increases CpG-induced AID in aged B cell cultures (and in aged mice)
icTNF-\(\alpha\) in B cells at t0 predicts a low HAI response in 85% of young and 73% of elderly individuals.

Season 2010-2011 and 2011-2012

62 young, 39 elderly

85% individuals, \(p<0.0001\)

73% individuals, \(p<0.0001\)
What do we think contributes to inflammation?
Inflammatory cytokines
Microbiome
Fat
CMV

\[ \text{TTP} \rightarrow \downarrow \text{E47} \]

Before stimulation

\[ \uparrow \uparrow \uparrow \text{TNF-\(\alpha\)} \rightarrow \downarrow \text{AID} \rightarrow \downarrow \text{CSR} \rightarrow \downarrow \text{IgG, E, A} \]

After stimulation

Frasca et al., Exp Gerontol. 2007, 2014
Obesity is associated with increased inflammation and poorer vaccine responses

Frasca, D ... Blomberg, BB 2016 “Obesity decreases B cell responses in young and elderly individuals” Obesity 24:615
OBESE INDIVIDUALS SHOW LOWER INFLUENZA VACCINE RESPONSES AS COMPARED TO LEAN CONTROLS

TIV (2011-2012):
A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), B/Brisbane/60/2008 (B)
OBESITY IS ASSOCIATED WITH DECREASED AID AND E47 IN CULTURED B CELLS
WHICH ARE THE MECHANISMS THROUGH WHICH OBESITY AFFECTS B CELL RESPONSES?
**Hypothesis:**
Visceral adipose tissue contributes to inflammatory B cells (in aging and obesity)
Tissue-associated changes in the relative percentages of B cell subsets in the VAT as compared to the peripheral blood. B cells were isolated from the peripheral blood and from the abdominal VAT and stained. Results are gated on CD19+ B cells. Top. Representative staining to evaluate the major B cell subsets. Bottom. Comparison of percentages of B cell subsets from all individuals in blood versus VAT.
Changes in the relative percentages of B cell subsets in the VAT as compared to peripheral tissues are due to adipocytes.

**MICE**

**HUMANS**
Conclusions

➢ There is an increased percentage of pro-inflammatory B cells in the VAT of healthy obese individuals compared to peripheral blood.

➢ Blood B cells co-cultured with adipocytes generate more LM (late memory/inflammatory) B cells

➢ The pro-inflammatory late memory B cells were elevated in the tumor-adjacent VAT and the TIL of a patient with larynx tumor in comparison with the average level in peripheral blood of healthy individuals (not shown).

➢ Adipocytes secrete several chemokines and the expression of the corresponding chemokine receptors is up-regulated (for some) in VAT-infiltrating B cells compared to peripheral blood B cells (not shown).

➢ Adipocyte-derived soluble factors induce secretion of pro-inflammatory cytokines by peripheral blood B cells from lean individuals (not shown).
Summary, Conclusions and Future Directions

- There is an age-related increase in epididymal VAT size and infiltration by immune cells. The increased VAT size with age is negatively correlated with the expression of AID in stimulated B cells.

- Epididymal VAT B cells show an inflammatory profile with increased presence of the ABC subset and higher levels of lipolysis and inflammation markers compared to splenic B cells.

- Adipocytes can induce a pro-inflammatory profile on splenic B cells in vitro by decreasing FO B cells and/or increasing ABC cells.

- A significant expression of chemokines in adipocytes and the corresponding receptors on VAT B cells suggest a mechanism for recruitment of B cells to the VAT.

- B cells from VAT secrete autoimmune IgM antibodies and IgG, most of which is IgG2c (previously shown to be autoimmune).

- In conclusion, the adipose tissue can be a major contributor to B cell inflammation, which we have previously shown negatively impacts the antibody response and here have shown can also impact T cell inflammation.
Aging significantly reduces the number of individuals with a responding phenotype in different measures

<table>
<thead>
<tr>
<th>MARKERS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>YOUNG</th>
<th>ELDERLY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of individuals with positive responses (%)</td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>21/33 (64)</td>
<td>6/26 (23)</td>
</tr>
<tr>
<td>CpG-induced AID&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27/33 (82)</td>
<td>2/26 (8)</td>
</tr>
<tr>
<td>Vaccine-induced AID&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27/33 (82)</td>
<td>5/26 (19)</td>
</tr>
<tr>
<td>E47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31/33 (94)</td>
<td>2/26 (8)</td>
</tr>
<tr>
<td>swIg percentages&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17/33 (52)</td>
<td>0/26 (0)</td>
</tr>
<tr>
<td>LM percentages&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31/33 (94)</td>
<td>3/26 (12)</td>
</tr>
<tr>
<td>TNF-&lt;sub&gt;α&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20/33 (61)</td>
<td>5/26 (19)</td>
</tr>
<tr>
<td>miR-155&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24/33 (73)</td>
<td>0/26 (0)</td>
</tr>
<tr>
<td>miR-16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24/33 (73)</td>
<td>0/26 (0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The 9 markers identifying a responding phenotype are the following. Fold-increase in the reciprocal of the titers ≥4; qPCR of CpG-induced AID mRNA expression at t0 ≥0.1; fold-increase in qPCR values of vaccine-induced AID mRNA expression at t0 ≥2; qPCR of CpG-induced E47 mRNA expression at t0 ≥0.05; percentage of switched memory B cells (swIg) at t0 ≥2; percentages of late memory B cells (LM) at t0 ≤3; qPCR of RNA expression of TNF-α ≤4, miR-155 ≤0.05, miR-16 ≤0.1 at t0.

<sup>b</sup>qPCR results are 2<sup>ΔΔCt</sup> values of sample RNAs (AID, E47, TNF-α, miR-155, miR-16) normalized to GAPDH.

<sup>c</sup>Results are percentages of peripheral blood CD19+ cells.

Results are from 3 consecutive influenza vaccine seasons (2011-2014).

Young: 33 individuals, Elderly: 26 individuals, all healthy.
CONCLUSIONS

Many molecular and cellular markers can be used to predict the ability to respond well (e.g. to the influenza vaccine) including

1) #s and %s of SwM and LM B cells
2) Amount of qPCR for AID (or E47) after B cell or PBMC in vitro stimulation
3) Amount of qPCR (or intracellular stain) for TNFα in B cells and serum TNFα and other miRNA markers (155 and 16)
4) CTRA (conserved transcriptional response) – to be done for influenza vaccine response in older breast cancer subjects (ongoing) – see below for immune/CBSM studies
Also in cancer, increased inflammation and associated decreased Ig (immunoglobulin) molecular markers (mRNA microarray) are associated with poor Ig response and poor prognosis.
Background: CBSM and CTRA

• CBSM may improve disease outcomes in BCa patients (e.g., disease-free interval)

• Biobehavioral mechanisms? reducing circulating leukocyte gene expression, since these cells may act as stromal influences on disease progression and metastasis

• Use leukocyte gene expression composite to predict clinical outcomes?

• Which Genes are known to be sensitive to stress/adversity?

The Conserved Transcriptional Response to Adversity (CTRA) (upregulated inflammatory and down-regulated anti-viral and antibody-making) can be operationalized as a composite of gene expression in leukocytes during periods of stress/adversity.
CTRA – Conserved Transcriptional Response to Adversity (Fredrickson et al, 2013)

- 53 Genes
  - Upregulated inflammatory (19)
    - IL1A, IL1B, IL6, IL8, TNF, PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA, RELB
  - Down-regulated anti-viral (31)
    - GBP1, IFI16, IFI27, IFI27L1-2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1-3, IFIT5, IFIT1L, IFITM1-3, IFITM4P, IFITM5, IFNB1, IRF2, IRF7-8, MX1-2, OAS1-3, OASL
  - Down-regulated antibody making (3)
    - IGJ, IGLL1, IGLL3
- Composite = avg of quantile-normed, log2 and z score

Cox Proportional Hazard survival: low vs. high 12 mo CTRA expression change and DFS.

Note. Low and high CTRA gene expression change groups are indicated by -/+ 1 S.D. above average level of change over time. Functions represented are adjusted for age, disease stage, treatment with chemotherapy, radiation therapy, and hormone therapy and condition.

Antoni, … Blomberg (2016) PNEC
Summary Results

• UNADJUSTED: Change in CTRA 53-gene expression composite over the initial 6-12 month period predicted 11-year disease recurrence risk:
  – Weibull AFT: -0.83, $\chi^2 = 5.0$, $p = .0254$
  – Cox: HR = 4.02 [1.17-13.83], $p = .0275$

• ADJUSTED: Effects after controlling for age, race, tumor stage, ER/PR, chemotherapy, radiation:
  – Weibull AFT: -1.09, $\chi^2 = 7.33$, $p = .0068$
  – Cox: HR = 6.32 [1.41-28.33], $p = .0160$
Factors that affect the immune system during aging

Positive

Physical activity  
Psychosocial intervention /  
Stress reduction  
Group support /  
Motivation  
Hormonal influences  
e.g. β-endorphins  
Nutritional components  
Sleep

Negative

Increased chronic inflammation  
Hormonal influences  
e.g. stress hormones  
Multiple infections /  
Illnesses
Acknowledgements

Daniela Frasca
Alain Diaz
Maria Romero
Thomas Vazquez
Richard Riley
Mike Antoni
Suzanne Lechner

All the subjects!

Franco Ferracci, Ryan Thomas, Nick Mendez Mitch Phillips, John Ryan, Robert Schwartz, Family Medicine
Sandra Chen, Env. Health & Safety
Bali Pulendran & Helder Nakaya (Emory)
Hana Golding & Surender Khurana (FDA)
Giuseppe del Guidice (Novartis)

NIH-NIA, R37 AG023717 (BBB)
NIH-NIA, R01 AG032576 (BBB)
NIH-NIAID, R21 AI096446 (BBB+DF)
NIH (Penedo) NCI R01 CA206456 (collab. With Antoni and Northwestern) 7/01/2016 – 06/30/2021
Young and Elderly CMV+ respond less well to influenza H1N1
CMV more profoundly effects elderly than young individuals

The percentages of young and elderly responders were defined as those who had HAI \( \geq 4 \), CpG-AID qPCR at t0 \( \geq 0.1 \), switched memory B cell percentages at t0 \( \geq 2 \) and icTNF-\( \alpha \) at t0 \( \leq 4 \).
Antibody affinity maturation after vaccination increases for HA1 domain

Surface Plasmon Resonance to measure steady-state equilibrium binding of pre- and post-vaccine plasma polyclonal antibodies with a ProteOn SPR biosensor (BioRad), at 25°C

*Khurana, Frasca, Blomberg, Golding, PloS Pathogens, 2012*
Video-Conferenced Stress Management and Relaxation Training for Older Women with Breast Cancer
AIMS

PRIMARY AIM

- **Specific Aim 1**: To test the effects of R-CBSM intervention on HAI-Fold Increase in antibody response to influenza vaccine (IV) and to the strains contained in the vaccine.

SECONDARY AIMS

- **Specific Aim 2**: To test the effects of R-CBSM on Affective Status (decreases in Negative Affect and Depressive Symptoms and Increases in Positive Affect).

- **Specific Aim 3**: To examine changes in immune (numbers switched B-cells [swB cells] and B-cell functional responses [AID response to CpG stimulation]) and inflammatory serum measures over the initial intervention (pre-IV) period and inflammation measures and swB cells before and during the IV response period, and associate these with the in vivo vaccine response.