Characterization of peripheral B-cells in aged individuals

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Impaired Humoral Response of Aged Individuals

• 90% of deaths from influenza occur in individuals over 65 years of age [1,2]

• Aged individuals not adequately protected by vaccination [3-5]
  — Partly due to B-cell dysfunction in aging

Can principles of HIV’s immune dysregulation be applied to aging B cells?

**AGING**
- Decreased production of B-cell precursors
- Shift in B-cell subsets
- Reduced expression of CSR molecule AID
- Reduced BCR diversity

**HIV**
- Shift in B-cell subsets
- Dysregulated B-cell TLR expression/responsiveness
- Elevated expression of inhibitory molecules on B cells
- Dysregulation of B-cell co-stimulatory molecules
- Impaired BCR signaling

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- Poor vaccine response
- Poor antibody specificity
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Hypothesis: mechanism underlying the observed impaired B-cell response of aged individuals is multifactorial

- Dysregulation of inhibitory molecules (CD22, CD72, CD85J)
- Disruption in B cell subsets (Bregs, RM, TLM)
- Defective BCR signaling/synergism
- Increased inflammatory cytokines (IL-6)

B-cell exhaustion

Impaired humoral response seen in aged individuals
# Inclusion Criteria

## Aged
- >65 years old (median age 81.5)
- Reported by clinician to have no signs of illness
- No medications known to alter immune system (e.g., statins, anti-inflammatories)
- Recruited from Rush Alzheimer’s Disease Center and Rush University Senior Care

## Young
- 22-45 years old (median age 28)
- No signs of chronic illness
B-cell Subsets Disrupted in Aged Subjects

- Fewer total B cells in aged subjects (p=0.006)
- Aged subjects have significantly (p=0.024) fewer resting memory B cells

CD10- CD20+ $\rightarrow$ CD21

CD27

<table>
<thead>
<tr>
<th>Naive</th>
<th>Resting Memory</th>
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<tbody>
<tr>
<td>Tissue-like Memory</td>
<td>Activated Memory</td>
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B-Cell Phenotypic Characterization

**Inhibitory molecules by flow cytometry**
- CD22
- CD72
- CD85j
- CD305
- PD-L1

**BCR inhibitory molecules by RT-qPCR**
- FcRL1
- FcRL2
- FcRL3
- FcRL4
- FcRL5

**Co-stimulatory molecules by flow cytometry**
- TLR1/6
- TLR2
- TLR5
- TLR9
- CD40
- CD80
- CD86
- HLA-DR

**TLRs by RT-qPCR**
- TLR2/6
- TLR10
Expression of Inhibitory Markers in Aged Subjects

CD22—also mediates B-cell signaling via clathrin-mediated endocytosis

CD72—constitutively active—likely sets threshold of BCR signaling

CD85j—can prevent isotype switching

These BCR inhibitory molecules are upregulated in HIV infection[1]

1. Moir et al. 2008 JEM. 205 (8): 1797
Expression of BCR Inhibitory Molecules

FcRL Expression of Sorted B Cells

-ΔΔCt (Aged v Young)
TLR Response

- HIV-infected individuals have Blunted B cell TLR response
- TLR response could be impaired in B cells of aged individuals as well

Expression of TLR4 and TLR5 Decreased in Aged Subjects

TLR Expression of Sorted B Cells

-ΔΔCt (Aged v Young)

-1.5

0.0

-1.0

-0.5

TLR4

p=0.048

TLR5

p=0.006
Role of TLR Signaling and Inflammation in Impaired B-cell Response of Aged Subjects

- Expression of 16S DNA from Serum
- Serum Concentration of IL-6

CpG Induces CD85j Expression

- Fold Change Relative to Young
- MFI of CD85j
Class Switch Recombination
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