Nutrition and Age-Associated Inflammation: Role of Nutritional Intervention

Simin Nikbin Meydani, DVM, PhD

simin.meydani@tufts.edu
Outline

1) Role of immune and inflammatory responses in age-related diseases

2) Brief description of mechanism of age-related increase in macrophage inflammatory products and its contribution to age-associated decline in T cell function and increase susceptibility to infectious diseases.

3) Age-associated adipose tissue inflammation and its implication for type 2 diabetes

4) Use of nutritional strategies to reverse macrophage inflammation and its impact for reducing the risk of infectious diseases.
Immune, Inflammatory Dysregulation and Age-Related Disease

Δ B Cell Function

Δ T Cell Function

Auto immune diseases
Antibody response

Infection
Cancer

Macrophage

Adipose Tissue

Inflammatory products
CVD
Alzheimer’s disease
Osteoporosis
Type II Diabetes
Cancer

Inflammatory products

Type II Diabetes
### Cytokine and PGE2 Production by LPS-stimulated Mϕ of Young and Old Mice (pg/μg protein)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>4 ± 1</td>
<td>20 ± 4*</td>
</tr>
<tr>
<td>IL-6</td>
<td>60 ± 24</td>
<td>82 ± 22*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.8 ± 0.4</td>
<td>20 ± 7*</td>
</tr>
<tr>
<td>PGE₂</td>
<td>85 ± 17</td>
<td>242 ± 50*</td>
</tr>
</tbody>
</table>

Mean ± SE, n=11-13/group  *Significant age effect

Ren et al., Unpublished data
PGE$_2$ and Immune Function in the Aged

• T cell function declines with aging in both animals and humans

• Increased PGE$_2$ production contributes to the age-associated suppression in T cell function

• PGE$_2$ is also implicated in pathogenesis of inflammatory, cardiovascular, and neoplastic diseases, incidence of which increases with age
Age-associated Increase in COX-2 mRNA Expression

What Is the Mechanism of Age-associated Increase in COX-2 Expression?
Regulators of COX-2 Gene Expression

<table>
<thead>
<tr>
<th>COX-2 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
</tr>
<tr>
<td>TNF-α</td>
</tr>
<tr>
<td>IL-6</td>
</tr>
<tr>
<td>ceramide</td>
</tr>
<tr>
<td>IL-10</td>
</tr>
<tr>
<td>glucocorticoid</td>
</tr>
<tr>
<td>GSH</td>
</tr>
</tbody>
</table>
Mediators of Age-associated COX-2 Expression

young

IL-1β
IL-6
TNF-α
IL-10

glucocorticoid
GSH
ceramide

old

IL-1β
IL-6
TNF-α
IL-10

↓ GSH
↑ ceramide
Age-associated Increase in COX-2 Expression is Due to Ceramide-induced Upregulating of NFκB Activation

- Old Mφ have higher ceramide levels and NFκB activity than young Mφ.
- Addition of Ceramide increases NFκB and COX-2 expression in young Mφ.
- NFκB inhibitors decrease COX-2 expression in old Mφ.

Claycombe et al., JBC 277:30784, 2002
Wu et al., JBC; 278: 10983, 2003
Mechanism for age-associated increase in COX-2 transcription

Claycombe et al., JBC 277:30784, 2002
Wu et al., JBC; 278: 10983, 2003
Immune, Inflammatory Dysregulation and Age-Related Disease

- Autoimmune diseases
- Antibody response
- Infection
- Cancer

△ B Cell Function

- Inflammatory products
- CVD
- Alzheimer’s disease
- Osteoporosis
- Type II Diabetes
- Cancer

△ T Cell Function

- Adipose Tissue

- Inflammatory products

- Type II Diabetes
It has been known for a long time that glucose intolerance, insulin resistance, and T2D incidence are increased with advancing age.
Obesity Results in Insulin Resistance Through Inducing Macrophage Infiltration and Their Production of Inflammatory Cytokines

Wellen and Hotamisligil, JCI, 112:1785, 2003
Sharp increase with age in diabetes incidence can’t be entirely explained by the change in body weight and body composition.


Prevalence of obesity in US (2001)


CDC data (http://www.cdc.gov)
Age-related Increase in Adipose Tissue Inflammation and its Underlying Mechanisms

Ceramide

PKC-ζ

NF-κB

PPAR-γ

IL-6
TNF-α

Aging

Insulin resistance and other metabolic complications
**Animals**: C57BL mice Young (5-6 mo)  
Old (23-24 mo)

**Adipocyte and SVC isolation**: collagenase digestion

**mRNA analysis**: Real time RT-PCR

**Mφ in AT**: FACS and immunohistochemistry

**IL-6 and TNF-α assay**: ELISA

**PGE$_2$ assay**: RIA

**Cell viability**: MTS and LDH assays
Epididymal Adipose Tissue From Old Mice Have Higher Expression of Inflammatory Genes

*Significantly different from Young mice

Wu et al., JI, 179:4829, 2007
Mφ (F4/80+ cells) in epididymal adipose tissues of young and old mice

Wu et al., JI, 179:4829, 2007
IL-6 and PGE$_2$ Production Is Not Significantly Different Between Stromal Vascular Cells of Young and Old Mice

Mean + SE, n=6

Wu et al., JI, 179:4829, 2007
Adipocytes From Old Mice Have Higher IL-6 and PGE$_2$ Production Than Those of Young Mice

*Significantly different from young mice. Mean±SE, n=7-10

Wu et al., JI, 179:4829, 2007
Old Adipocytes Have Higher Levels of Ceramide and Sphingomyelin Compared to Those From Young

SM: sphingomyelin; Cer: ceramide; GlcCer: glucosylated ceramide; SO: sphingosine; S1P: sphingosin-1-phosphate; C1P: ceramide-1-phosphate.

Wu et al., JI, 179:4829, 2007
Ceramide Increases IL-6 Production by Adipocytes of Young and Old Mice

*Significant age difference within each treatment.
Mean±SE, n=6

Wu et al., JI, 179:4829, 2007
Ceramide Synthesis, Related Enzymes and Enzyme Inhibitors

- Palmitoyl CoA + L-serine
- Serine palmitoyltransferase (SPT)
- 3-keto sphinganine
- Ketosphinganine reductase
- Sphinganine
- Dihydroceramide synthase
- Dihydroceramide desaturase
- Sphingomyelin synthase
- Sphingomyelinase (SMase)
- Sphingomyelin
- Sphingosine 1-phosphate
- Sphingosine kinase
- Sphingosine
- Ceramide synthase
- Ceramidase
- Ceramide
- Ceramide kinase
- Ceramide 1-phosphate
- Sphingomyelin synthase
- Sphingomyelinase (SMase)
- Sphingomyelin

Inhibitors:
- Myriocin
- FB1
- GW4869
- Me-SM
Effect of inhibiting de novo ceramide synthesis and nSMase on LPS-stimulated IL-6 and TNF-α production by adipocytes

Mean ± SE, n=10
Different letters within each age denote significant difference at p<0.05.
There is a significant difference between age group in every treatment.
Inhibiting NF-κB Activation Reduces IL-6 Production by Adipocytes of Young and Old Mice

Wu et al., JI, 179:4829, 2007
Inhibiting NF-κB Activation Reduces Sphingomyelinase-induced IL-6 Production by Mouse Adipocytes

Wu et al., JI, 179:4829, 2007
Adipose tissue expression of pro-inflammatory mediators is upregulated while anti-inflammatory and insulin-sensitizing factor PPAR-γ is downregulated with aging.

Unlike the changes observed in obesity, there is no apparent age-related difference in either Mφ infiltration into adipose tissue or MCP-1 production by adipocytes.

Adipocytes and not non-adipocytes (stromal vascular cells) in adipose tissue may be the major player responsible for age-related upregulation of inflammatory products, suggesting a different mechanism from that in obesity.
Age-related Increase in Adipose Tissue Inflammation and its Underlying Mechanisms

Ceramide
PKC-ζ

PPAR-γ

NF-κB

Aging

IL-6
TNF-α

Insulin resistance and other metabolic complications
Nutritional Intervention and Age-associated Inflammation

- Vitamin E
- n-3 PUFA
- Calorie restriction
Vitamin E and PGE$_2$ Production

Wu, et al., AJP 1998;275:C661
Vitamin E and Immune Response in the Older Adults

Vitamin E supplementation of healthy elderly significantly improves in vivo and in vitro indices of T cell-mediated function.

Meydani et al. AJCN 1990; 52:557-563
Meydani et al. JAMA 1997; 277:1380-1386.
<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin E</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All respiratory infections</td>
<td>74%</td>
<td>65%</td>
<td>0.65* (0.43-0.97)</td>
</tr>
<tr>
<td>Upper respiratory infections</td>
<td>62%</td>
<td>50%</td>
<td>0.62* (0.43-0.91)</td>
</tr>
<tr>
<td>Lower respiratory infections</td>
<td>32%</td>
<td>33%</td>
<td>1.03 (0.69-1.53)</td>
</tr>
</tbody>
</table>

*Significantly different from Placebo at p<0.05

• Vitamin E treatment did not have an overall effect on TNF\(\alpha\) production
Genetics influence cytokine production

• There is a high degree of variability in cytokine production between healthy individuals
  – Genetic factors may explain variability in cytokine production

• SNPs may account for individual variability
  – Single nucleotide polymorphisms (SNPs) are single base pair changes in the DNA.
  – Identified at genes that encode cytokine proteins.

SNP→ influence cytokine response→ impact infection
The Effect E on TNF-α Production Depends on TNF-α -308G>A

- Interaction: vitamin E and TNF-a -308G>A
  p=0.039*

*Adjusted for baseline TNF-α production
Placebo n=56 (G/G =46; A/G and A/A =10); Vitamin E group n=39 (G/G=22; A/G and A/A =17)

Belisle et al., JN, 2009
Conclusions

• These observations suggest that individual immune responses to vitamin E supplementation are in part mediated by genetic factors.

• Because A allele at TNF$\alpha$ is associated with higher TNF$\alpha$ levels, our observation suggest that the antiinflammatory effect of vitamin E is specific to those genetically predispose to higher inflammation.
Fish Oil Was Shown to Improve Clinical Symptoms In:

- Cardiovascular diseases
- Rheumatoid arthritis
- Psoriasis
- Multiple sclerosis
- Systemic lupus erythematosus
- Atopic dermatitis
- Ulcerative colitis
Modulatory Effect of EPA on Eicosanoid Synthesis

Diet → AA → membrane phospholipids → COX → LTB₅, PGE₃ → Less inflammatory

Diet → AA → membrane phospholipids → LOX → LTB₄, PGE₂ → Inflammatory

EPA

PLA₂
**Effect of Low-fat Diets High and Low in Fish on Ex-vivo Cytokine and PGE$_2$ Production**

<table>
<thead>
<tr>
<th></th>
<th>Low fat, high fish</th>
<th>low fat, low fish</th>
<th>% change #</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1$\beta$</td>
<td>-40*</td>
<td>62*</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>-35*</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-34*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PGE2</td>
<td>-63*</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

* Compared to their own baseline  

Meydani et al., JCI; 92:105-1, 1993
Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy

CALERIE

Jean Mayer USDA HNRCA at TUFTS UNIVERSITY
BOSTON, MA
Design: Randomized, controlled, single-blind

Subjects: Men and women, 25-45 years old, with BMI of 25-29 Kg/m²

Intervention: 10 or 30% calorie restriction.

DTH: Baseline and after 6 mo.

In vitro immune measures: Baseline and after 6 mo.
Effect of Calorie Restriction on LPS-Stimulated PGE$_2$ Production

* Significantly different from baseline  Ahmed et al. J. Gerontology, 2009
Effect of Calorie Restriction on Delayed Type Hypersensitivity Skin Response

* Significantly different from baseline

Ahmed et al. J. Gerontology, 2009
Effects of Calorie Restriction on Lymphocyte Proliferation

**Anti CD3**

- Baseline
- 6-month

**ConA**

- 10% restricted
- 30% restricted

**PHA**

- 10% restricted
- 30% restricted

* Significantly different from baseline

Ahmed et al. J. Gerontology, 2009