“The neuroimmunomodulation theory of Alzheimer’s disease

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Presentation Route
“The neuroimmunomodulation hypothesis of Alzheimer’s disease (AD)”

1. Alzheimer’s disease: a major puzzle to Medicine
2. The multidisciplinary approach to AD
3. Major hypotheses in AD
4. Tau as a common final pathway in AD pathogenesis
5. AD as an autotoxic disease
6. The damage signals hypothesis of AD
7. Cytokines and signaling pathways in AD
Why is AD a Public Health Problem?

**Increasing Prevalence**

![Bar chart showing the increasing number of people with dementia in developing and developed countries from 2001 to 2040. The number of dementia patients is expected to increase significantly by 2040.]

2/3 of dementia patients live in developing and underdeveloped countries

Ferri et al., 2005
Kalaria et al., 2008

*Figure 2: Number of people with dementia in developed and developing countries*
Alzheimer’s Disease is a major Puzzle to Public Health, Medicine and Society.

Age distribution of the Chilean population

Prevalence of AD among Chilean population

Chile: over 175,000 people with AD (INE National Census 2002; Quiroga, 1995)

(Ferri et al., 2005)
MAIN CHALLENGES IN AD RESEARCH
PREVENTION, DIAGNOSIS AND TREATMENT

LIFE-STYLE
- Unhealthy diet
- Physical inactivity
- Alcohol misuse
- Decreased social activities
- Smoking
- Diminished mental stimulation

Diabetes
Hypercholesterolemia
Hypertension
Cardiovascular disease
Cerebrovascular disease

Brain reserve
Vascular insults
Neuronal damage

ApoE ε4: Poor repair/Protection

DEMENTIA

Unknown Structural/functional changes?
Our Laboratory tackles AD with a multidisciplinary approach
NEUROFIBRILLARY TANGLES: MAJOR PATHOGENOMONIC LESION IN ALZHEIMER’S DISEASE (AD)
The ‘amyloid cascade’ hypothesis

Conceptualized as resulting from the generation of the beta-amyloid (A/β) peptide from the amyloid precursor protein (APP), through multiple secondary steps, to cell death. It was the foundation for most (but not all) emerging options for the treatment of Alzheimer's disease.

Senile Plaques Formation

Amyloid precursor protein (APP) generating Aβ
Aβ (1-42) OLIGOMERS (ADDL’S)

- Glial activation
- Oxidative Stress
- Decrease of survival protein
- Increase of stress kinases
- Hyperphosphorylation of tau

- Insulin-like growth factor-1 receptor
- Erythropoietin
- Estrogen
- Interleukin-3 (Zambrano et al.)

These factors induce an increase in survival proteins

These factors protect against β-amyloid-induced neurotoxicity
Oxidative damage hypothesis of Perry and Smith

Lipid Peroxidation/Protein Adduction (4-HNE)

Protein Oxidation (Free Carbonyl Groups)

Nucleic Acids (8-OH-Guanosine)

Glycoxidation (Carboxymethyllysine)
There are six isoforms of human tau produced by alternative splicing of a single gene.
IN THE CONTEXT OF AD PATHOGENESIS

PATHOLOGICAL FOLDING OF TAU TO FORM PHF’s

TAU MONOMER  Epítope of MC-1 Ab

POLYMERS

Paired helical filaments (PHFs)
Distribution of Neurofibrillary Tangles

SAME AGE

Normal Age     MCI     Alzheimer's Disease
**Tauopathies**

Polymers of modified, or cleaved tau protein

NFTs

-Alzheimer’s disease
-Pick Disease
-DFTP-17
-Down syndrome
-Progressive Supranuclear Palsy (PSP)
Bridge Between Basic and Clinical Research.

TAU BIOMARKERS IN CSF


Anomalous brain tau.
THE NEURAL UNIT: glia/neurons/vessels

PROINFLAMMATORY MEDIATORS
Microglia are normally beneficial but are toxic when overactive

• Helpful actions include phagocytosis and killing pathogens

• Harmful actions include excess production of oxygen free radicals, complement proteins, inflammatory cytokines, prostaglandins, glutamate and many as yet unidentified products
Which signals are responsible for AD pathogenesis?

THE NEED OF A UNIFYING HYPOTHESIS.
Testing Neuroimmunomodulation hypothesis of AD. Search for Treatment Avenues.

Endogenous Damage Signals

Microglia & astrocytes

RAGE

TLR4

HMBG1

S100

AGES

Aβ peptide oligomers

ox-LDL

Oxyradicals

Mechanical Damage

Plasma membrane

Cell cycle activation without proliferation

Neuron Damage

Hyperphosphorylated tau protein

Degenerating neuron

External Factors

Deleterious

Head injury

High fat Intake

Deficiency in B vitamins

Infections

Iron Overload

Protective

Statins

NSAIDs

Long-term exposure to cholinergic agonists (?)

Maccioni et al Ann NY Acad Sci 2009
Autotoxic diseases are different from autoimmune diseases

• Autoimmune diseases involve the adaptive immune system. They are aggressive and strike the young.

• Autotoxic diseases are less aggressive and strike the elderly. They are much more prevalent than autoimmune diseases.

• We postulate that the major driving force in AD is neuroinflammation. Activation of the innate immune system.
Treatment with Antiinflammatory drugs reduces the risk of Alzheimer Disease

- NSAIDS > 6 mos (.65) - Yip et al 2005
- NSAIDS chronic (.5) - Landi et al 2004
- NSAIDS >2y (.42) - Zandi et al 2002
- NSAIDS > 2y (.4) - Stewart et al 1997
- NSAIDS > 2y (.2) - In’t Veld et al 2001
- Rheumatoid Arthritis (.16) - McGeer et al 1990
PREVALENCE OF ALZHEIMER DISEASE IN THE 65 AND OVER AGE GROUP

- General population (average of 5 studies)
- In 923 patients in rheumatoid arthritic clinics
- In 7490 hospital patients with rheumatoid arthritis

From McGeer et al., Lancet 335:1037, 1991
POSITIVE EVENTS

Neuritic Growth
Neuronal Differentiation

NEGATIVE EVENTS

Apoptosis
Neuronal Dysfunction
Cultured Microglial cells

Microglia

$\text{H}_2\text{O}_2$

Microglia Over-activated

Interleukins IL-6; IL-1 TNF$\alpha$

Conditional Media

Neurodegenerated cells

Neurofibrillary degeneration

Cultured Neuron
IL-6 INDUCES p35 IN RAT HIPPOCAMPAL NEURONS

A
Control      IL-6
36.4 kD
24.7 kD
p35
actin

B
Control      IL-6
924 bp
158 bp
p35
actin
RT-PCR
IL-6 (5ng/ml) x 48h

n=6
IL-6 PROMOTES TAU HYPERPHOSPHORYLATION IN AD EPITOPES

**A**

Control | IL-6
---|---
AT8
61.3 kD
61.3 kD
49.0 kD

**B**

Control | IL-6
---|---
Tau-1
61.3 kD
61.3 kD
49.0 kD

IL-6 (5ng/ml) x 48h

n = 6

*Denotes significant difference.

n = 6
IL-6 INDUCED TAU PHOSPHORYLATION IS DEPENDENT ON cdk5/p35

A

Control  IL-6  IL-6+But  But

61.3 kD  

AT8

61.3 kD  

Tau 5

49.0 kD  

B

Control  IL-6  IL-6+But  But

61.3 kD  

PHF-1

61.3 kD  

Tau 5

49.0 kD  

IL-6 (5ng/ml) x 48h  But(-) cdks
SCHEMATIC REPRESENTATION OF cdk5 and JNK SIGNALING CASCADES IN NEURODEGENERATION

Aβ, cytokines, free radicals

cdk5

p35

Rac

MEKK1/2/3/4

JNK/p38

Tau

Apoptosis

Nucleus

Activation of Gene Expression

PHFs

GSK-3β
IL-6 induced signal transduction in calcium influx as analyzed with Fluo3 AM

A

B

(n=5)
IL-6 INCREASES Egr-1 FACTOR IN HIPPOCAMPAL NEURONS

**Egr-1 ANTI Egr1 C 588**

**actin**

**IL-6 (5ng/ml) x 48h**

**SB: a p38 inhibitor**

**SB3089**

**PD: MAPK inhibitor**

**PD 98059**
MAIN FINDINGS

• IL-3 IS A NEUROPROTECTOR VIA ACTIVATION OF NEURONAL SURVIVAL FACTORS.

• IL-6 and IL-1 INDUCE DEREGULATION OF THE cdk5/p35 COMPLEX AND LEAD TO TAU HYPERPHOSPHORYLATION IN HIPPOCAMPAL CELLS

• JAK/STATs and MAPK-p38 PATHWAYS ARE INVOLVED IN IL-6 AND IL-1 TAU MODIFICATIONS

• IL-6 INDUCES AN INCREMENT IN THE CYTOSOLIC CALCIUM THUS AFFECTING TAU PHOSPHORYLATION PATTERNS
The Damage Signals Hypothesis of Alzheimer’s Disease Pathogenesis

Jorge A. Fernández, Leonel Rojo, Rodrigo O. Kuljis, and Ricardo B. Maccioni

Endogenous Damage Signals

- HMBG1
- AGES
- S100

External Factors

- Deleterious
  - Head injury
  - High fat intake
  - Deficiency in B vitamins
  - Infections
  - Iron overload

- Protective
  - Statins
  - NSAIDs
  - Long-term exposure to cholinergic agonists (?)

Microglia and astrocytes

NFκB

TNFα
IL-1β
IL-6

RAGE

TLR4

Aβ peptide oligomers

ox-LDL

Oxyradicals

Mechanical Damage

Plasma membrane

Degenerating neuron

Hyperphosphorylated tau protein

Neuron Damage

Cell cycle activation without proliferation

THERAPEUTIC AVENUES: Search for NOVEL Anti-Alzheimer Drugs (Application for NDI approval at FDA).

TARGET 1: TAU

Tau aggregates disassembly

THQ-55/THQ-4S

TARGET 2: IMMUNE SYSTEM

Neuroimmunomodulation

Anti-TNFα molecules

Integrative approaches based on our unified AD theory
(Fernandez et al., 2008)
Approaches to anti-inflammatory therapy of AD

1. Block the secreted inflammatory mediators: eg prostaglandins, inflammatory cytokines, complement proteins

2. Block the intracellular inflammatory pathways: eg Erk1,2; NFkB; JAK-STAT 1,2,3

3. Stimulate the antiinflammatory intracellular pathways: eg convert microglia from the Th-1 type to Th-2 type

These approaches do not overlap so that multiple agents may have synergistic effects
Etanercept is an antiinflammatory drug clinically successful for AD treatment, supporting the neuroimmunomodulation hypothesis.

Drug is used via perispinal injection, since it does not pass the blood brain barrier.

**USE OF DRUG DELIVERING MICELLES.**
POLYMER MICELLES FOR DRUG DELIVERY: ETANERCEPT

- Hydrophilic outer shell composed of flexible tethered polymer strands
  - Excellent biocompatibility
  - Stimuli sensitivity

- Micelle core as a drug reservoir
  - Stable incorporation of a variety of drugs
  - Controlled drug release
  - Stimuli-responsive drug release

- Uniform size with a range comparable to viruses
  - High extravasating and tissue penetrating ability

- Corona
  - Targeting ligands
  - pH sensitivity
  - Thermal sensitivity

- Core
  - pH sensitivity
  - Chemical sensitivity

- Introduction of functional groups on the micelle surface
  - Targetability

- Graph showing the number of articles related to DDS and micelles from 1994 to 2003.
RESEARCH TEAM

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Endeavors toward quality of life of our citizens.