NEUTROPHILS INDUCE ALZHEIMER'S-LIKE DISEASE VIA LFA-1-INTEGRIN

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Alzheimer’s disease (AD)

Age-related neurodegenerative disorder
(over 1 million people only in Italy)

- accumulation of Amyloid-beta (Aβ) peptide
- hyperphosphorylation of Tau protein
- neuronal death and synaptic loss
- neuroinflammation
Experimental models of Alzheimer’s Disease

5xFAD  
*(Oakley et al., 2006; Ohno et al., 2006)*

- amyloid pathology
- coexpress 5 Familial Alzheimer’s Disease mutations on APP and PSEN1 that lead to accelerated plaque formation and increased Aβ42 levels
- memory impairments in behavioral paradigms begins at 4 months of age

3xTg-AD  
*(Billings et al., 2005; Oddo et al., 2003)*

- amyloid plaques and neurofibrillary pathology
- coexpress 3 independent transgenes encoding human mutated APP, PSEN1 and Tau
- significant impaired short-term memory in behavioral paradigms starts at 6 months of age
1. Capture and Rolling
   Mucins, selectins, integrins

2. Integrin activation
   GPCR-dependent signaling

3. Arrest
   Integrins

4. Transmigration

The multistep model of leukocyte transmigration in CNS venules
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1. Capture and Rolling
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   - Integrins

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Blood flow

Leukocyte

Endothelial cells

Endothelial basement membrane

Perivascular space

Perivascular macrophage

Glial basement membrane

Astrocytes
The multistep model of leukocyte transmigration in CNS venules

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Blood flow

Endothelial cells

Endothelial basement membrane

Perivascular space

Perivascular macrophage

Glial basement membrane

Oligodendrocyte

Neuron

Astrocytes

Microglia
Neutrophils promote Alzheimer’s disease–like pathology and cognitive decline via LFA-1 integrin

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Inflammation is a pathological hallmark of Alzheimer’s disease, and innate immune cells have been shown to contribute to disease pathogenesis. In two transgenic models of Alzheimer’s disease (5xFAD and 3xTg-AD mice), neutrophils extravasated and were present in areas with amyloid-β (Aβ) deposits, where they released neutrophil extracellular traps (NETs) and IL-17. Aβ42 peptide triggered the LFA-1 integrin high-affinity state and rapid neutrophil adhesion to integrin ligands. In vivo, LFA-1 integrin controlled neutrophil extravasation into the CNS and intraparenchymal motility. In transgenic Alzheimer’s disease models, neutrophil depletion or inhibition of neutrophil trafficking via LFA-1 blockade reduced Alzheimer’s disease–like neuropathology and improved memory in mice already showing cognitive dysfunction. Temporary depletion of neutrophils for 1 month at early stages of disease led to sustained improvements in memory. Transgenic Alzheimer’s disease model mice lacking LFA-1 were protected from cognitive decline and had reduced gliosis. In humans with Alzheimer’s disease, neutrophils adhered to and spread inside brain venules and were present in the parenchyma, along with NETs. Our results demonstrate that neutrophils contribute to Alzheimer’s disease pathogenesis and cognitive impairment and suggest that the inhibition of neutrophil trafficking may be beneficial in Alzheimer’s disease.

Alzheimer’s disease is the most common form of dementia. It affects more than 35 million people worldwide1. It is characterized by a progressive deterioration of cognitive function, and the neuropathological features include Aβ plaques, neurofibrillary tangles comprising aggregates of hyperphosphorylated tau, amyloid angiopathy, neuronal loss and synaptic dysfunction1,2. A large body of evidence suggests that Alzheimer’s disease is promoted by inflammation and innate immune mechanisms of the central nervous system (CNS)3–5. Chronic inflammatory disorders in humans, including atherosclerosis, obesity, diabetes and periodontitis, either represent risk factors for or are associated with late-onset Alzheimer’s disease6. Accordingly, epidemiological studies have suggested that nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the risk of Alzheimer’s disease, thus confirming its link with inflammation6,7. Even so, several prospective placebo-controlled trials to test the efficacy of NSAIDs against Alzheimer’s disease have failed, suggesting that specific inflammatory pathways need to be identified and targeted3,8,9.

It is widely accepted that microglia-mediated neuroinflammatory responses may promote neurodegeneration in Alzheimer’s disease10,11. Microglial activation precedes neuropil loss in patients with Alzheimer’s disease, and recent genome-wide association studies have revealed that microglial genes such as CD33, TREM2 and HLA-DR are associated with susceptibility to late-onset Alzheimer’s disease11–15. Activated microglia can induce highly detrimental neurotoxic effects, and hence the attenuation of the microglial response has been proposed as a potential therapeutic approach in Alzheimer’s disease10.

Vascular inflammation and a dysfunctional blood-brain-barrier (BBB) have been implicated in the pathogenesis of Alzheimer’s disease16,17. Blood-derived leukocyte subpopulations, including lymphocytes, monocytes and neutrophils, have been identified in the brains of patients with Alzheimer’s disease and in corresponding animal models16,18–22. Whereas blood monocytes have been associated with Aβ clearance23, the role of other circulating leukocytes in the induction of neuropathological changes and memory deficit associated with Alzheimer’s disease remains unclear.

RESULTS
Vascular adhesion molecules are expressed in disease models
We performed confocal microscopy experiments on brain sections of 5xFAD mice, which overexpress mutant human APP (995) with the Swedish (K670N, M671L), Florida (I716V) and London (V717I) familial Alzheimer’s disease (FAD) mutations and human P51 harboring the two FAD mutations M146L and L286V. These mice accumulate Aβ deposits by 2 months of age and exhibit memory impairment starting at 4 months of age23. We found that the expression of E-selectin,
Brain vessels express adhesion molecules in mice with memory loss during early disease.
Brain vessels express adhesion molecules in mice with memory loss during early disease.

WT control

4 month-old 5xFAD (meninges)

WT control

6 month-old 3xTg-AD (hippocampus)
Brain vessels express vascular adhesion molecules in proximity to Aβ deposits in 5xFAD mice
Soluble oligomeric Aβ1-42 peptide upregulates the expression of endothelial adhesion molecules

Zenaro et al., Nat Med 2015
Neutrophils (Ly6G+ cells) infiltrate AD-like brain during early disease

Zenaro et al., Nat Med 2015
Neutrophils (naphtol AS-D chloroacetate esterase + cells) accumulate in the brain during early disease.

Zenaro et al., Nat Med 2015
Neutrophils accumulate in the brain during all disease phases

Zenaro et al., Nat Med 2015
Neutrophils:
- Phagocytosis
- Release cytokines
- Degranulate and release many types of enzymes (are called granulocytes!)
- Produce reactive oxygen species (ROS)
- Release neutrophil extracellular traps (NETs)

The power of neutrophils

Neutrophils play a role in both acute and chronic inflammatory diseases
Neutrophils generate neutrophil extracellular traps (NETs) in the brain of Alzheimer’s disease mice.
Neutrophils produce IL-17 in the brain of Alzheimer’s disease mice.
In vivo imaging of neutrophil trafficking in the cortex of mice with Alzheimer's like disease

4 month-old 5xFAD mouse

Download Video

Neutrophils
Blood vessels
Neutrophils adhere in blood vessels with Aβ deposits.
Neutrophils migrate inside the parenchyma in areas with Aβ deposits.
Soluble oligomeric Aβ triggers rapid integrin-dependent adhesion in vitro

**Human neutrophils**

- **Adherent PMN on fibrinogen / 2 min**
  - Ctrl, fMLP, 1, 5, 10, 20 μM

- **Adherent PMN on ICAM-1 / 2 min**
  - Ctrl, fMLP, 1, 5, 10, 20 μM

**Murine neutrophils**

- **Adherent PMN on fibrinogen / 2 min**
  - Ctrl, fMLP, 1, 5, 10, 20 μM

- **Adherent PMN on ICAM-1 / 2 min**
  - Ctrl, fMLP, 1, 5, 10, 20 μM
Soluble oligomeric Aβ triggers LFA-1 integrin high-affinity state

LFA-1 affinity measurement

Zenaro et al., Nat Med 2015
LFA-1 integrin controls neutrophil migration into the brain of mice with Alzheimer’s-like disease.
Inhibition of neutrophil function in mice with cognitive deficit during early disease

% NEUTROPHILS

Anti-Gr-1
Anti-Ly6G
Anti-LFA-1

Cognitive decline

Behavioral tests and neuropathological assessment

Months of age
Depletion of Ly6G+ cells restores cognitive functions in 3xTg-AD mice

**CFC**

**Contextual fear conditioning test**
associative learning task

* P<0.05
Depletion of Ly6G+ cells restores cognitive functions in 3xTg-AD mice

Contextual fear conditioning test
associative learning task

Y-maze
spatial working memory

* P<0.05
**P<0.005

Zenaro et al., Nat Med 2015
Depletion of Gr1+ cells restores cognitive functions in 3xTg-AD mice

CFC

** Contextual fear conditioning test

** Associative learning task

Y-Maze

** Y-maze

** Spatial working memory

* P<0.05

** P<0.005

Zenaro et al., Nat Med 2015
Depletion of Gr1+ or Ly6G+ cells restores cognitive functions in 5xFAD mice

Supplementary Figure 15

Supplementary Figure 15. Neutrophil depletion restores cognitive function in 5xFAD mice (*P < 0.05; ** P < 0.005 and *** P < 0.0005). Statistical analysis was carried out using the Mann-Whitney test.

Zenaro et al., Nat Med 2015

*P<0.05, **P<0.005, ***P<0.0005
Depletion of Gr-1+ cells reduces microglial activation in 3xTg-AD mice

Zenaro et al., Nat Med 2015

*P < 0.05; **P < 0.005
Depletion of Gr-1+ cells reduces Aβ deposition in 3xTg-AD mice

Amyloid Beta

*P<0.05

Zenaro et al., Nat Med 2015
Depletion of Gr-1+ cells reduces tau phosphorylation in 3xTg-AD mice

*P<0.05

Zenaro et al., Nat Med 2015
Depletion of Gr-1+ cells restores synaptic protein expression in 3xTg-AD mice

Zenaro et al., Nat Med 2015
Does interference with neutrophil function during early disease have an impact on late disease?

Zenaro et al., Nat Med 2015
Transient depletion of Gr-1+ cells during early disease has long term beneficial effect

Zenaro et al., Nat Med 2015
Treatment with an anti-LFA-1 antibody restores cognitive function in 3xTg-AD mice

Efalizumab target: LFA-1 integrin

** P<0.005   ** P<0.005

Improvement in psoriasis plaques

Zenaro et al., Nat Med 2015
3xTg-AD/Itgal\(^{-/-}\) lacking LFA-1 integrin show memory improvement and lower microglia activation

Y-Maze

* \(P<0.05\)  ***\(P<0.0005\)

Zenaro et al., Nat Med 2015
Neutrophils adhere in human brain vessels and migrate in the parenchyma in subjects with AD

Zenaro et al., Nat Med 2015
MPO+ cells accumulate in proximity of amyloid plaques in subjects with AD

Zenaro et al., Nat Med 2015
Neutrophils accumulate in the brain in patients with Alzheimer’s disease

**P < 0.005; ***P < 0.0005

Zenaro et al., Nat Med 2015
Neutrophils accumulate in the brain of subjects with Alzheimer’s disease

Zenaro et al., Nat Med 2015
Neutrophil extracellular traps (NETs) are produced in the brain of subjects with Alzheimer’s disease.
Pericyte
Endothelial cell
Astrocyte
Tight junction
Chemoattractant
Mucin
Selectin
Integrin
Integrin-ligand
Perivascular macrophage
Neuron
Blood vessel
Endothelial basement membrane
Perivascular basement membrane
Brain parenchyma
Perivascular macrophage
Neuron
Integrin
Integrin-ligand
Chemoattractant
Aβ plaque
Pericyte
Endothelial cell
Astrocyte
Tight junction
Microglia
Neuron
Integrin
Mucin
Selectin
GPCR
Aβ plaque
Blood vessel

Endothelial basement membrane

Perivascular basement membrane

Brain parenchyma

Pericyte

Endothelial cell

Astrocyte

Tight junction

Neuron

Microglia

Integrin

Mucin

Selectin

GPCR

Aβ plaque

Perivascular macrophage

Integrin-ligand

Chemoattractant

Aβ plaque

Rolling

Integrin-activation and arrest
Blood vessel

Endothelial basement membrane

Perivascular basement membrane

Brain parenchyma

Pericyte
Endothelial cell
Astrocyte
Tight junction

Rolling

Integrin-activation and arrest

Transmigration

Cytokines, ROS, Enzymes

Perivascular macrophage

Microglia

Neuron

Integrin
Integrin-ligand
Chemoattractant
Mucin
Selectin
GPCR
Aβ plaque

Cytokines, ROS, Enzymes
Future studies on the role of peripheral leukocytes

1. Characterize the mechanisms of neutrophil-dependent damage

2. Characterize leukocyte trafficking mechanisms in AD

3. Establish the role of leukocyte subsets

4. Study the interplay between leukocyte subsets

5. Clarify the disease phase in which leukocyte subsets have a role
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