Arginine Deprivation and Immune Suppression in AD

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Methods

* Quantitative RT-PCR
* Immunohistochemistry
* Flow Cytometry
* Fluorescent Activated Cell Sorting (FACS)
* Microarray analysis
* Brain Amino Acid Analysis
* Radial Arm Water Maze
* Difluoromethylornithine (DFMO) + putrescine
The CVN-AD Mouse Model

mNos2-/ - 

Nos2 = enzyme in activated macrophages that produces nitride oxide from arginine.

→ Lower nitride oxide level

CVN-AD

• Low nitride oxide levels
• Develops amyloid-β deposits
  → Disruption of Tau phosphorylation pathway
  → hyperphosphorylated Tau
  → neurofibrillary tangles
  → neuronal death
= Alzheimer’s phenotype

APPSwDI

Transgenic mouse with mutated human APP gene

→ Develops amyloid-β deposits
No Tau hyperphosphorylation
No neuronal death
Expression of immune-related genes

Gene X

Compared to WT
Expression of immune-related genes

Genes upregulated before week 36

Anti-inflammatory genes

Pro-inflammatory gene
Expression of immune-related genes

Genes upregulated after week 36

Pro-inflammatory

Anti-inflammatory

Anti-inflammatory

Anti-inflammatory
Presence of immune cells

* Immunostaining

* Antibodies for amyloid-β

* Antibodies for immune cells
  * Iba-1 → expressed on microglia in the CNS
  * CD45 → expressed in reactive microglia
  * CD11c → found in resting or activated microglia
Increase in both amyloid-β and the biomarkers of activated and reactive microglia.
Co-staining of CD11c and Iba-1

CD11c and Iba-1 co-localizes → glia cells express them both.
Increased expression of CD11c in CVN-AD model
The results so far suggest...

* The Alzheimer’s phenotype is mainly due to increased number of reactive microglia

* These microglia co-express CD45 and CD11
# Tissue-resident macrophages

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<table>
<thead>
<tr>
<th>Central nervous system</th>
<th>Microglia</th>
<th>Promote neuronal survival and are involved in frontline immune surveillance, removal of dead neurons and synaptic remodelling\textsuperscript{75, 118}, derived from yolk sac and maintained in adult and during inflammation independently of the bone marrow\textsuperscript{15, 25, 64}</th>
<th>F4/80\textsuperscript{+}, CD11b\textsuperscript{+}, CD45\textsuperscript{lo} (ref. 119)</th>
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<td>Perivascular macrophages</td>
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<td>F4/80\textsuperscript{+}, CD11b\textsuperscript{+}, CD163\textsuperscript{+}, CD45\textsuperscript{hi} (ref. 119)</td>
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<td>Meningeal macrophages</td>
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Figure 3. CD11c^+ cells from CVN-AD brains have a microglial phenotype. A, Representative flow cytometry plots from aged 48-week-old mNos2^−/− and CVN-AD brains after gating on CD45^+ cells. B, Quantification of total CD45^+ cells and individual cell types distinguished by FACS in 48-week-old mNos2^−/− and CVN-AD brains, including T lymphocytes (T), B lymphocytes (B), neutrophils (PMN), monocytes/macrophages/DCs (Mac/DC), and microglia (MG) from aged 48-week-old mNos2^−/− and CVN-AD brains. *p < 0.05; ****p < 0.0001. C, Representative flow plots of CD45 and CD11c expression from aged 48-week-old mNos2^−/− and CVN-AD brains after gating on all CD11b^+ cells. D, Representative histogram of CD11c expression on mNos2^−/− (closed gray) and CVN-AD (open black) CD11b^+ CD45^high cells, as well as quantitative summaries of the percentages and geometric mean fluorescence intensities of CD11c from mNos2^−/− and CVN-AD CD11b^+ CD45^high microglia. *p < 0.01, n = 4 mice per group.
CD11c+ microglia show an immunosuppressive phenotype through changes in gene expression

* Up-regulated genes
  * APoE4 – “sticky” Amyloid Beta **20.1 fold change**
  * Spp1 – enhances immune suppression, increased in CSF of AD patients **515 fold change**
  * Wfdc17 – overexpression of Arg-1 (arginase) **136 fold change**
  * PdCd1 – shifts microglia to M2 phenotype and involved in the apoptotic pathway **51 fold change**
  * Gp49a – inhibits LPS (mechanism of macrophage activation) **107 fold change**

* Down regulated genes
  * Apobec3 – promotes antiviral immunity **18 fold change**
  * Ifngr 1 – encodes LBD for IFN gamma (macrophage activation) **19 fold change**
  * Siglech – Dap12 signaling molecule (NK and Macrophage activation) **42 fold change**
  * Klf6 – negative regulator of immune suppression **12 fold change**
CVN-AD pathology is associated with increased arginine utilization and decreased brain arginine bioavailability.

“The immunosuppressive phenotype observed in CVN-AD mice brain suggested a potential causative role for this immune process in disease progression. In particular, Arg1, a critical anti-inflammatory gene that codes for the enzyme arginase-1, was expressed predominantly in young mice in brain areas associated with AB deposits but before neuronal loss in this model of AD.”
Figure 4. CVN-AD pathology is associated with arginase-1. 

A. Representative sagittal sections from CVN-AD mice at 6, 12, 24, and 52 weeks of age stained for arginase-1 in sister sections from the same mice as in Figure 1. Scale bar, 500 μm. 

B. Magnified view of arginase immunoreactivity in the subiculum. Representative sagittal sections from 24-week-old mNos2−/− and CVN-AD stained for arginase-1. Scale bars: top, 500 μm; bottom, 50 μm. 

C. Sister coronal sections from the same 52-week-old CVN-AD brain stained for Aβ, CD11c, Iba-1, and arginase-1 to show regional associations.
Increase Arg-1 → Decreased bioavailability of arginine → Compensation by up regulating arginine receptors
Figure 5. CVN-AD brains have decreased total -arginine bioavailability and increased expression of arginine transporters. A, Global arginine bioavailability (arginine/ornithine + citrulline) for CVN-AD, mNos2<sup>−/−</sup>, WT, and APPSwDI mice. Average values (± SEM) per genotype were calculated for individual mice (n = 3–12 mice per group). Amino acid levels were measured using HILIC LC-MS/MS. **p < 0.01 (one-way ANOVA), B–E, Relative gene expression (mean ± SEM) was measured in total brain homogenates from CVN-AD, mNos2<sup>−/−</sup>, WT, and APPSwDI mice for the neutral arginine transporters Slc7a5 (LAT1) and Slc7a8 (LAT2) (B, C) and for the cationic amino acid transporters Slc7a1 (CAT1) and Slc7a2 (CAT2) (D, E). Samples were analyzed by two-way ANOVA and post hoc multiple comparison test with Bonferroni’s correction. *Comparisons between CVN-AD or APPSwDI and WT. **Comparisons between CVN-AD and APPSwDI mice. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. †p < 0.05. ‡p < 0.01. §§p < 0.001. §§§p < 0.0001. n = 4–8 mice per group.
“Amino acid starvation leads to GCN2 kinase-mediated phosphorylation of eIF2α, leading to cell autophagy or apoptosis” (Young et al., 2009; Altman and Rathmell, 2012)
* GADD34 = Growth Arrest and DNA Damage – Inducible Protein
  * Associated with the apoptotic pathway

* CHOP – transcription factor for the transcription of genes that code for apoptotic proteins and autophagy related protein
Dual role for CHOP in the crosstalk between autophagy and apoptosis to determine cell fate in response to amino acid deprivation

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Gadd34 functional domains involved in growth suppression and apoptosis

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Received 19 June 2002; Revised 25 February 2003; Accepted 5 March 2003.
Treatment

* Difluoromethylornithine (DMFO) + Putrescine
  * Shown to decrease Arg1 activity to ameliorate AD pathology
Conclusion

* Cd11c+ microglia accumulate in the same location as beta amyloid (hypothalamus)
* Cd11c+ microglia have an immunosuppressive phenotype
* Extracellular arginase accumulates in the same spaces as beta amyloid and Cd11+ microglia, suggesting that the microglia are synthesizing and secreting the arginase
* Arginine deficiency creates stress that eventually leads into autophagic and apoptotic pathways
* Arginase blocking treatment can ameliorate AD pathology and decrease level of Cd11c+ microglia
So what?

* “One current view proposes that extracellular AB peptide activates CNS immune cells, including microglia and perivascular macrophages, to produce inflammatory cytokines, such as TNFa, IL-1, and reactive nitrogen or oxygen species that initiate neuronal death”

.......... But this happens later in the progression of the disease and they are counteracted by the immune-suppressing proteins that were discussed in this paper