

Metabolic and Inflammatory Factors in Brain Ageing

Julian Trollor



Context

- Metabolic and inflammatory factors may be risk factors in cognitive ageing and dementias
- 2 large ageing cohorts (MAS & OATS)
- NHMRC DRG \$904,409 2008-2010
- Trollor / Campbell / Samaras / Baune / Brodaty / Wright / Martin / Wen / Baune / Sachdev / Schofield / Draper / Ames / Lee

Overall Aims

- Evaluate role of metabolic and inflammatory factors modulating effects of genetic susceptibility, physical health, lifestyle and nutrition on brain ageing.
- Discover factors that promote healthy ageing, and identify those at risk.
- Practical preventative strategies

Cohorts

- The Older Australian Twins Study (Sachdev, Martin, Ames, Schofield, Brodaty, Wright, Trollor, Wen, Lee)
- The Memory and Ageing Study (Brodaty, Sachdev, Draper, Broe and Trollor)

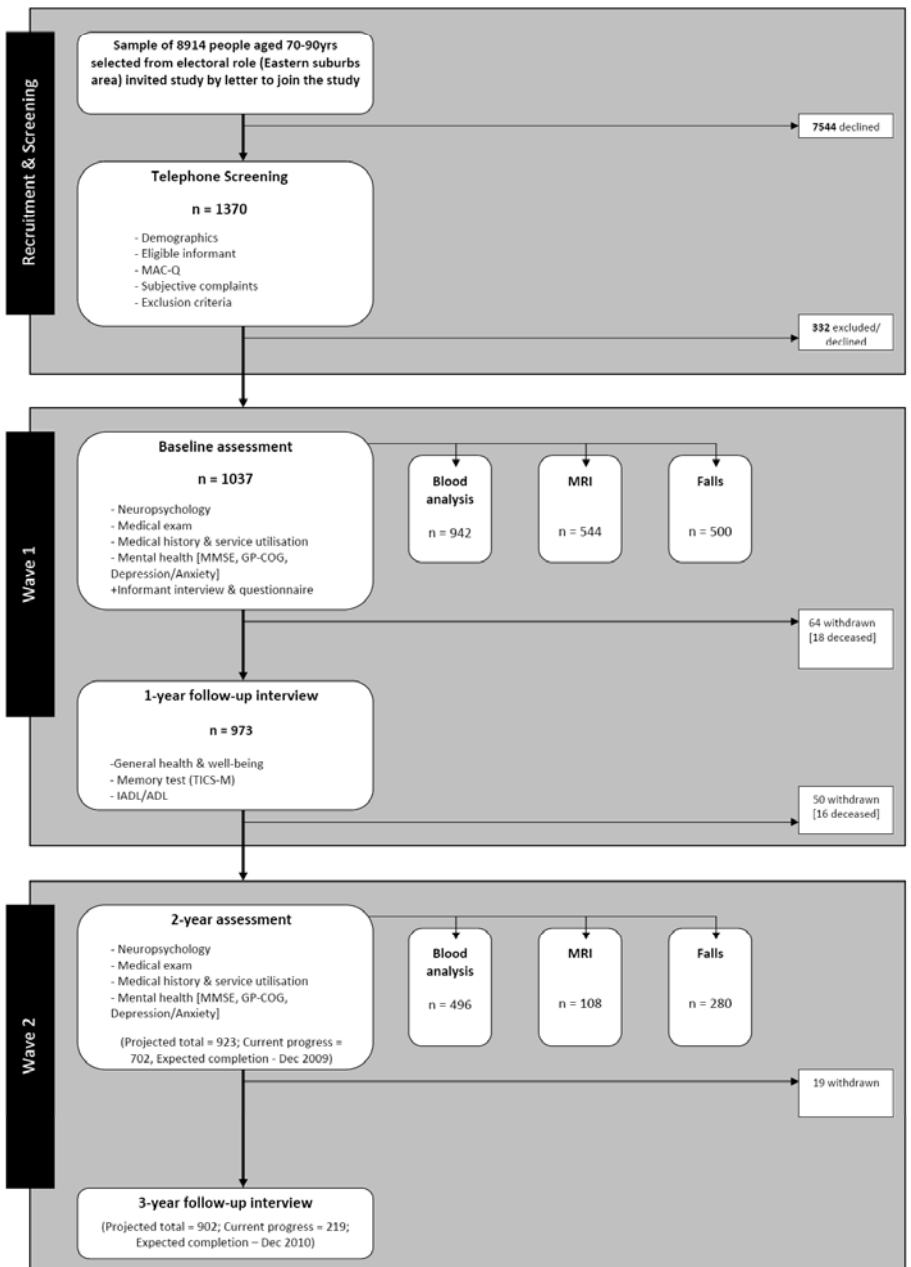
MAS

- Longitudinal Study, 2 year intervals
- Investigates predictors of cognitive decline in a community sample
1000 LGA residents aged 70-90
- NHMRC Program Grant 350833 for 2005-2009
- Wave 2 recruitment 75%; Wave 3 commencing Oct 09

- Serum from Wave 1
- Lab based assessments from Wave 2
- MRI scans of abdomen from Wave 3

Memory & Ageing Study – Longitudinal study flow chart

Date: 27.05.2009



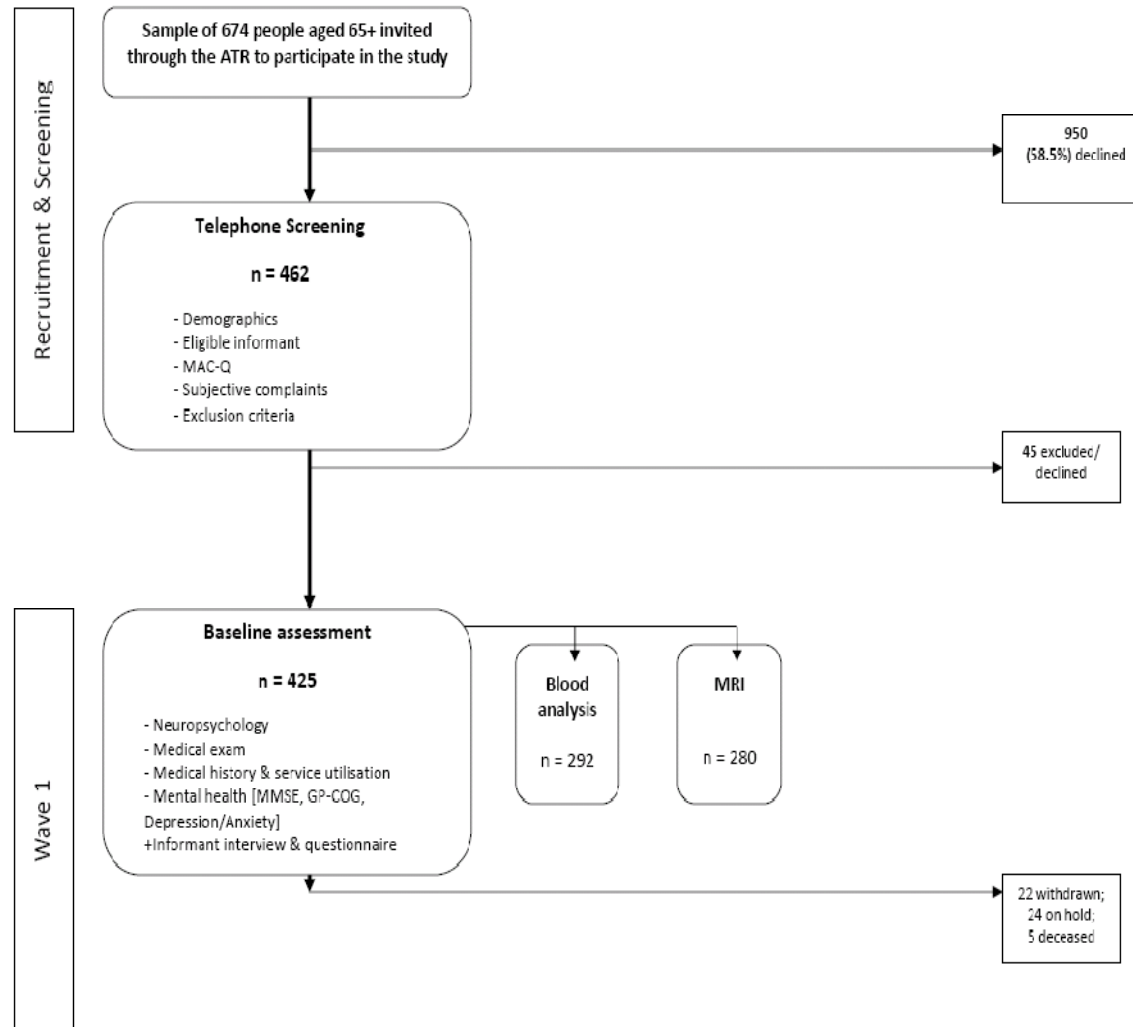
OATS

- Longitudinal Study, 2 year intervals
- Examines genetic and environmental effects and interactions on brain ageing
- 1000 elderly twins and their siblings
- 3 States (NSW, VIC QLD)
- twin-sib pair cohort aged >65 years
- NHMRC/ARC AWAP Grant 401162 from 2007-2011
- Status: Wave 1 recruitment 50%; wave 2 commencing
- Will start lab based assessments shortly

Older Australian Twins Study (OATS)

Longitudinal Flow Chart

18 June 2009



Primary Assessments

- detailed neurological, psychiatric and cognitive assessments
- structural MRI scans of the brain
- blood for biochemistry & DNA

Assessment

- Arterial Stiffness
- Blood Tests: Fasting glucose & insulin; lipids & lipid peroxidation products, total nitrates, nitric oxide synthase (NOS); inflammatory markers and adipokines: C-reactive protein (CRP), adiponectin, Tumour necrosis factor α (TNF- α), Interleukin assays (IL-1, IL6, IL-8, IL-10, IL-12p70), vascular cell adhesion molecule (VCAM); PAI 1 and serum amyloid A.
- Genetic Analyses
- MRI Scans: liver & Abdominal Fat
- Anthropometric Measures: Free fat and fat mass (bioelectrical impedance analysis)
- ECG
- Retinal Photography

Objectives

- To characterise the contribution of obesity, obesity-related proinflammatory cytokines and the metabolic syndrome to cognitive and imaging markers of ageing.
- To evaluate the interactive effect of biomarkers associated with both a 'proinflammatory state' and 'metabolic syndrome' and other risk factors for dementia.
- To identify the metabolic and genetic predictors of arterial stiffness and to examine the cognitive and brain imaging correlates of increased arterial stiffness.
- To identify new polymorphisms relating to inflammatory cytokines and examine their interactive effect with a range of vascular, lifestyle and other genetic risk factors for cognitive decline.

Hypotheses -1

- A proinflammatory state predicts the volume of white matter hyperintensity (WMH) volume on T2-weighted MRI at baseline and progression in WMH over time.
- Measures of oxidative stress predict the rate of cognitive decline in the elderly.
- Obesity and insulin resistance:
 - are associated with greater volume of WMH and poorer cognitive function, independent of genetic factors.
 - interact with genetic factors and/or susceptibility to greater volume of WMH and significantly poorer measures of cognitive function.

Hypotheses -2

- Arterial stiffness is associated with poorer cognitive function, greater volume of WMH and predicts subsequent cognitive decline over a 2 year interval.
- The inflammatory and vascular phenotypes associated with Metabolic Syndrome in genetically predisposed individuals:
 - are associated with greater volume of WMH and poorer cognitive function,
 - are predictive of future WMH changes and cognitive decline over a 2 year interval.

Broad Strategy

- Metabolic Factors
- Inflammatory Factors
- Measures of Body Composition
- Measures of Arterial Stiffness

Key Questions: Inflammation & Cognition

Is inflammation a 'driving force' of AD pathology, or just an epiphenomenon?

Inflammation: Primary or Secondary?

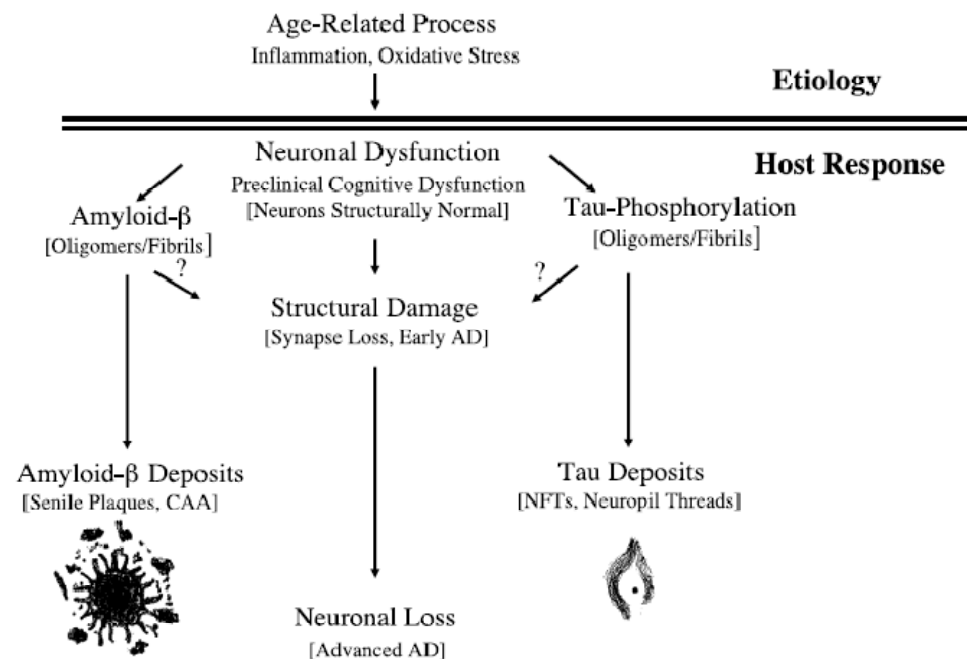


FIGURE 2. Host response hypothesis. Age-related etiologic factors lead to multiple host responses, among which are the lesions of AD.

What is the Relationship between CNS Inflammation and Systemic Measures?

Sources of Cytokines

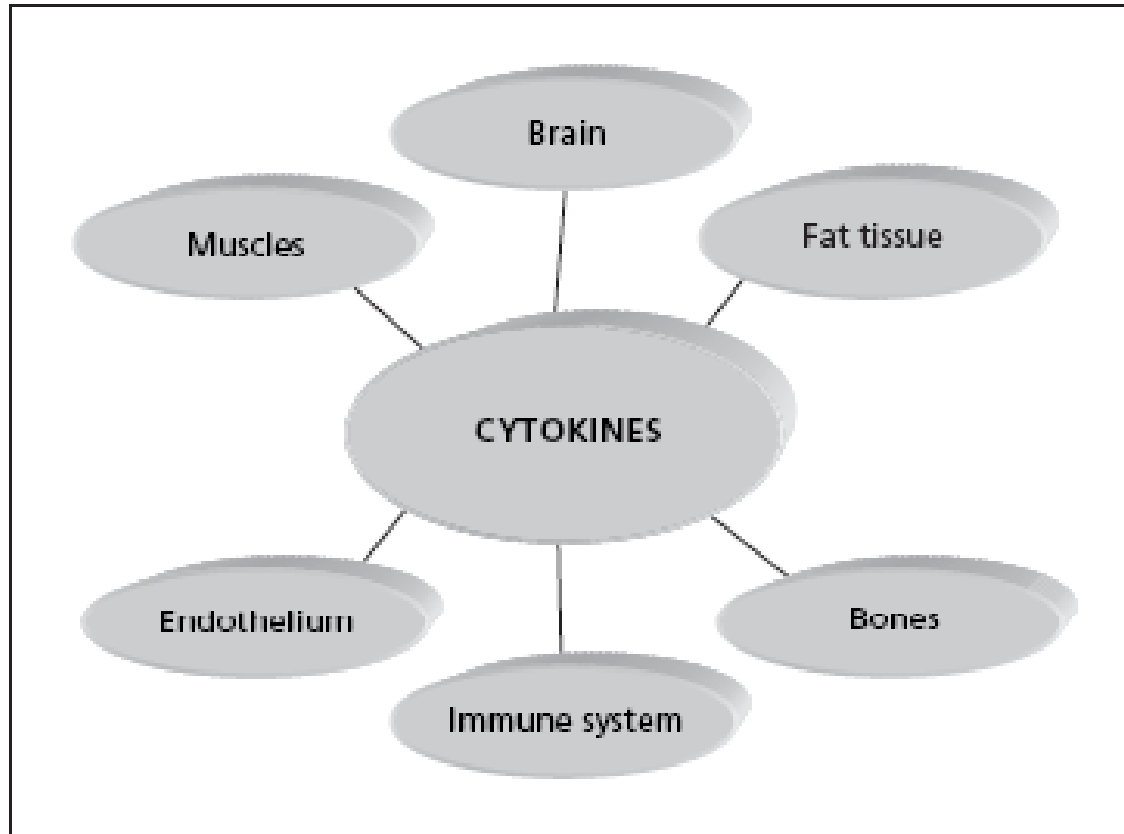


Figure 2. Sources of cytokines. A wide range of organs and different cell types contributes to the production of cytokines such as TNF- α and IL-6, which have local as well as endocrine activities and act as important regulators of the metabolism and immune functions.

How to deal with multiple interacting variables

Inflammatory Burden

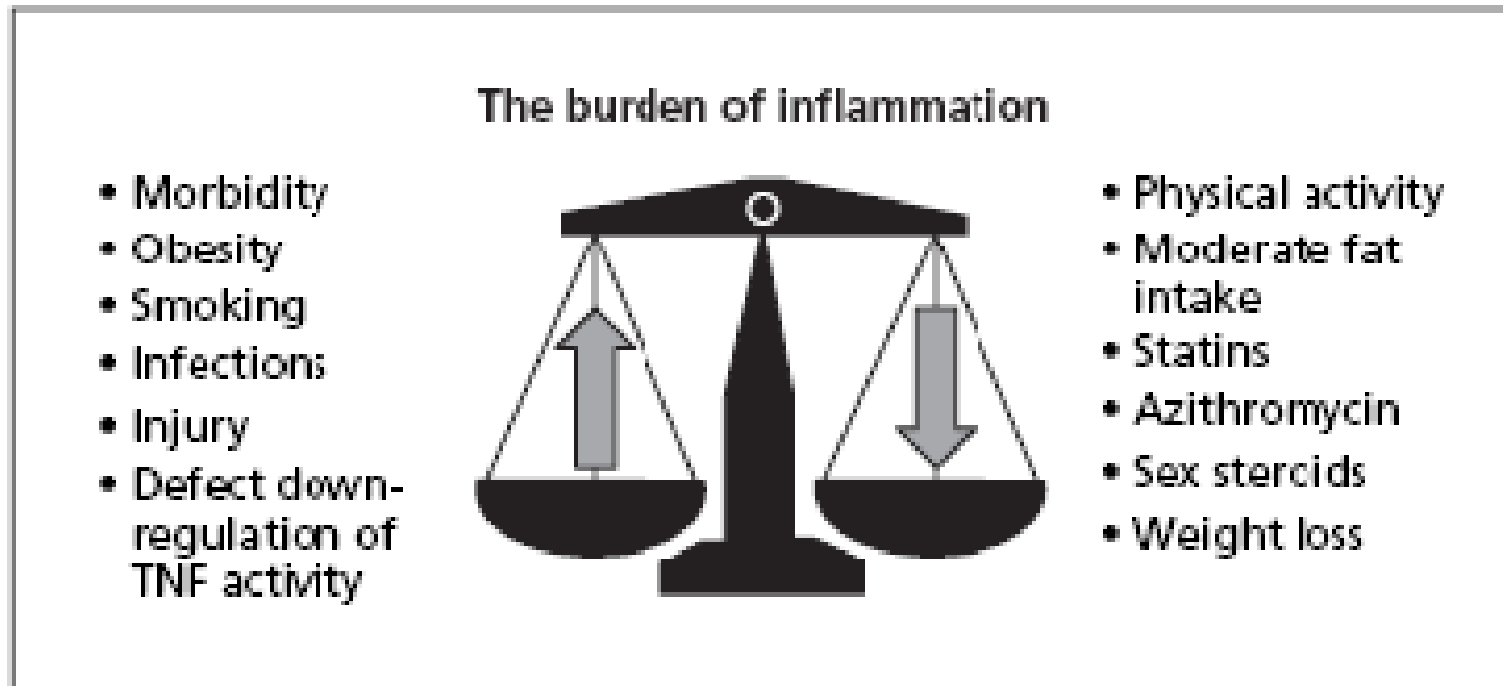


Figure 17. The origin of the inflammatory burden in elderly populations. A wide range of factors contributes to systemic low-level inflammation in the elderly. Counteracting factors are only at the beginning to be understood.

TNF & Metabolic Syndrome

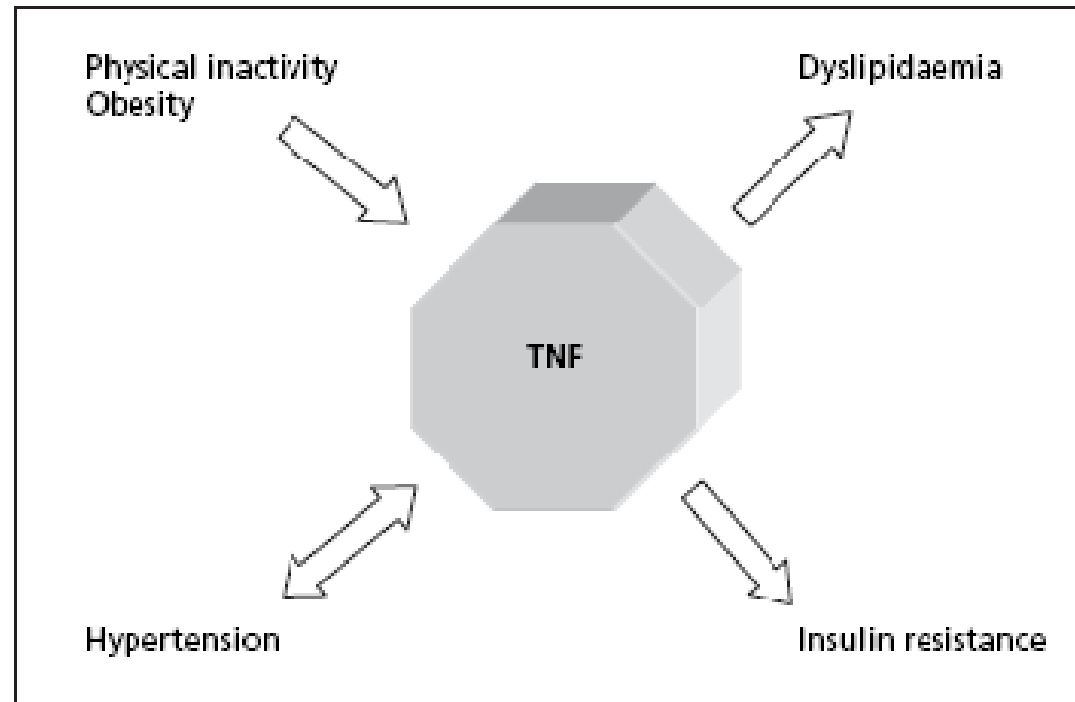


Figure 10. TNF- α as a driver in the metabolic syndrome. Fat tissue produces TNF- α and circulating levels of TNF- α is correlated with the fat mass. Muscle contractions inhibit TNF- α production in vivo and physical inactivity is associated with high circulating levels of TNF- α . Hypertension may induce increased production of TNF- α and vice versa. TNF- α induces insulin resistance. See text for further details.

How Do Inflammatory Factors Exert Their Cognitive Effects

Inflammation and Cognitive Function: Mechanisms

- Neuronal damage mediated by activation of microglia, astrocytes and the complement system.
- Increased levels of IL-1 α have been found to interfere with long-term potentiation in hippocampus and are associated with the development of cognitive deficits.
- Transgenic mice expressing high brain IL-6 levels show defects in learning suggestive of reduced synaptic plasticity [23-25].

23. Bellinger F.P., Masamba S., Siggins G.R., et al. (1993) *Brain Res* 628:27-234

24. Gilbertini M., Newton C., Freidman J., et al. (1995) *Brain Behav Immun* 9; 113-128

25. Heyser C.J., Masliah E., Samimi A., et al. (1997) *Proc Nat Acad Sci, USA* 94:1500-1505

IL-1: Complex Association with AD

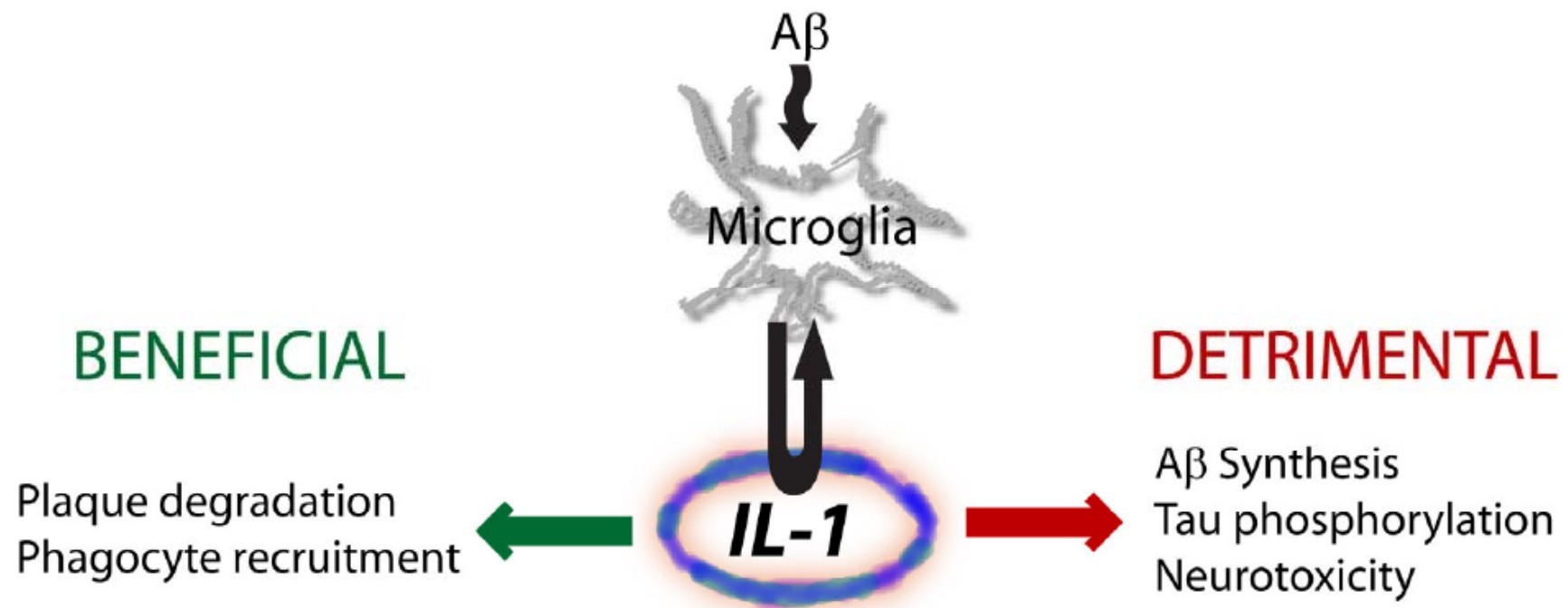
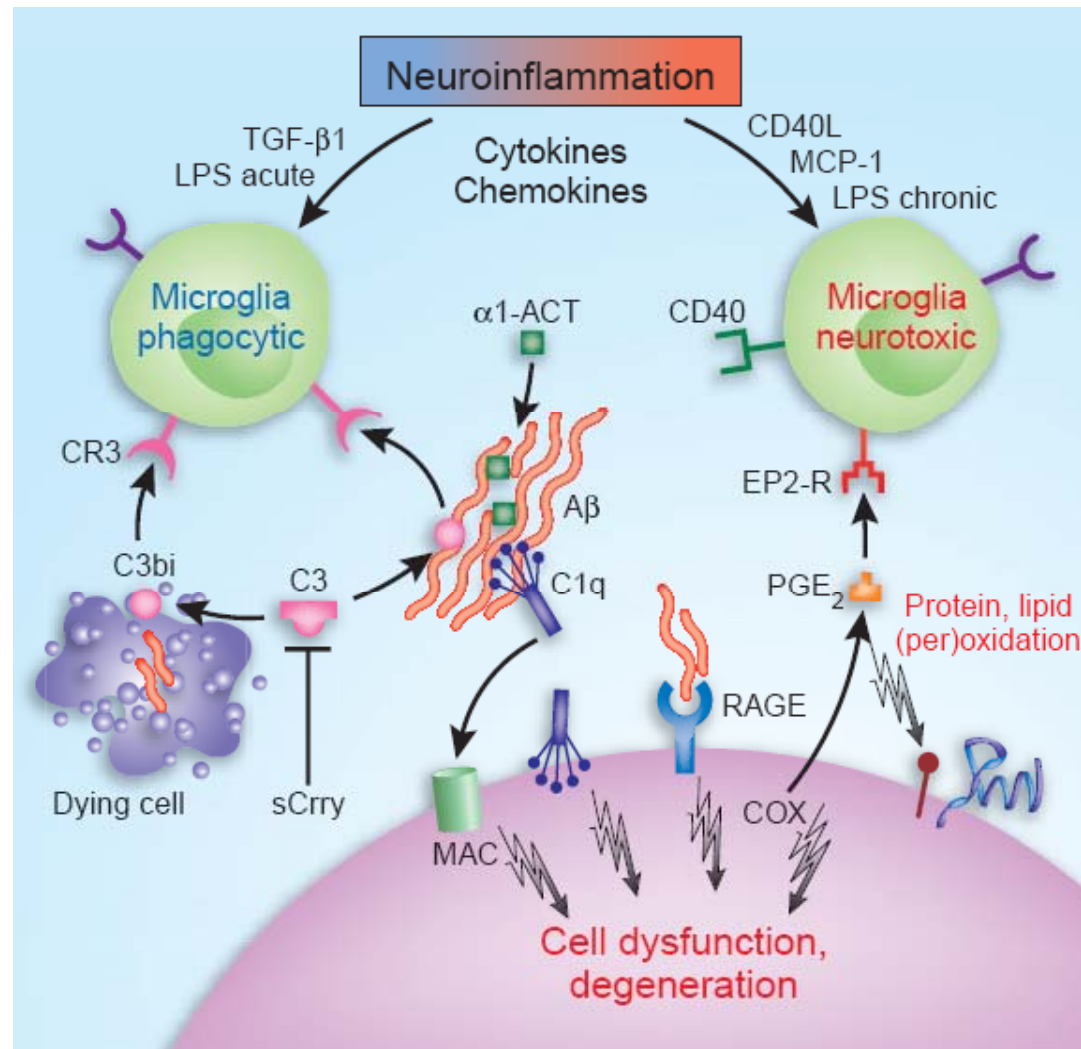


Figure 1
A schematic depiction of potential roles of IL-1 in AD.

Shaftel et al J Neuroinflammation 2008;5:7

Beneficial v Detrimental Effects of Immune Activation



Wyss-Coray Nature Medicine 2006; 12(9):1005-1015

Are there other biomarkers apart from cognition which inform the inflammatory hypothesis: eg MRI?

Aims of Today's Workshop

- Understanding the data set
- Reviewing available literature
- Planning analysis
- Planning Future aspects of the study



SUMMARY OF ANALYSES TO DATE

Relationships between Inflammatory Markers and MCI classifications

- Comparisons of normals v's MCI (DVs = normalized values of inflammatory markers)
 - Mann-Whitney test
 - T-test
- Differences in normalised inflammatory markers across 5 groups (normals, 4 MCI groups) and 3 groups (normals, All amnestic MCI and all nonamnestic MCI)
 - Kruskal Wallis
 - One-way ANOVA
 - Multiple comparisons for normals and MCI subtypes
- ANCOVA
 - Factor with 2 levels = normals versus all MCIs
 - Factor with 3 levels = normals, all amnestic and all non amnestic MCI
 - Factor with 5 levels = normals plus 4 MCI subtypes
 - Multiple comparisons between pair of levels of above 5-level factor

Relationships between blood levels and (continuous) neuropsych measures

- Raw correlations inflammatory markers and neuropsych (individual tests, component and cognitive domain scores)
 - ESBs only
 - Spearman & pearson
- Regression of neuropsych tests & cognitive domains on inflammatory markers

Inflammatory Markers and MRI Measures

- Correlations of Inflammatory markers with:
- WB measures
- Regional Volumes
- Regional WMH Volumes

RESULTS SUMMARY

Inflammatory Markers and MCI classifications

Normals v's all MCI

- Mann-Whitney
 - PAI1 ($p = .02$)
 - NB for SAA, $p = .07$.
- T-test
 - PAI1 ($p=0.028$)
 - SAA ($p=0.042$)

3 Groups (Normals, all amnestic and all non amnestic MCI subtypes)

- Kruskal Wallis
 - PAI1, $p = 0.042$
 - SAA, $p = 0.08$
- ANOVA
 - PAI1, $p=0.042$
 - SAA, $p = 0.064$
- Multiple comparisons: non-significant after correction for multiple comparisons
 - PAI1 normals > amnestic MCI subtypes ($p = 0.012$)
 - SAA nonamnestic MCIs > amnestic MCIs ($p=0.022$)

5 Groups (normals, aMCI, na MCI, amdMCI, namdMCI)

- Kruskal Wallis
 - PAI1 ($p = .035$)
 - NB for SAA, $p = .07$.
- ANOVA
 - PAI1 ($p=0.036$)
 - Multiple comparisons: non-significant after correction for multiple comparisons
 - PAI1 normals > amdMCI; namdMCI > amdMCI ($p = .02$)

ANCOVA

- **Normalised inflammatory markers (DVs) and MCI classifications (treated as fixed effects factors)**
 - Control variables (covariates): sex, age, years of education, smoking, BMI, diabetes, glucose level, CVA, TIA and angina.
- **Significant effect of factor (MCI/Normal) on inflammatory markers.**
 - SAA ($p = .007$)
 - TFN α ($p = .038$)
- **Significant effect of MCI classification on inflammatory markers,**
 - 3 Groups (normals, all amnesic MCI, all non amnesic MCI)
 - SAA ($p = 0.019$)
 - TNF α ($p = 0.041$)
 - 5 Groups Normals, aMCI, naMCI, amdMCI, namdMCI)
 - SAA ($p = .008$)
 - TFN α ($p = .030$)

Multiple comparisons without Bonferroni correction

- sVCAM1 3 > 5 (p = .047); 4 > 5 (p = .045)
- PAI1 1 > 4 (p = .018); 5 > 4 (p = .019)
- SAA 5 > 1 (p = .001); 5 > 2 (p = .015); 5 > 3 (p = .015);
5 > 4 (p = .024)
- IL12 5 > 1 (p = .005); 5 > 2 (p = .005); 5 > 3 (p = .010);
5 > 4 (p = .011)
- TNF α 5 > 1 (p = .003); 5 > 2 (p = .013); 5 > 3 (p = .041);
5 > 4 (p = .026)
- IL1b 5 > 1 (p = .018); 5 > 2 (p = .009); 5 > 3 (p = .027);
5 > 4 (p = .045)

- 1 = Normals; 2 = aMCI; 3 = naMCI; 4 = amdMCI; 5 = namdMCI.

Multiple comparisons after Bonferroni correction

- SAA
 - namdMCI > normals (p = .01)
- TNF α
 - namdMCI > normals (p = .03)
- IL12
 - namdMCI > normals (trend p= 0.05), namdMCI > aMCI (trend p= 0.05)
- IL1 β
 - namdMCI > aMCI (trend p= 0.09)
- VCAM1. PAI1, ns

**Relationships between blood levels and
(continuous) neuropsych measures**

Correlations Inflammatory Markers and Cognition ESBs ONLY

		VCAM	PAI1	SAA	IL12	TNFa	IL10	IL6	IL1b	IL8
Psychomotor	Correlation Coefficient	-.119	.061	-.104	-.044	-.015	-.046	-.095	-.016	-.071
	Sig. (2-tailed)	.001	.095	.004	.229	.681	.212	.009	.661	.050
ProcSpeed	Correlation Coefficient	-.090	.066	-.100	-.042	-.018	-.010	-.069	-.032	-.057
	Sig. (2-tailed)	.013	.068	.005	.245	.621	.786	.056	.374	.116
FineMotor	Correlation Coefficient	-.157	.018	-.098	-.032	-.010	-.084	-.119	.005	-.095
	Sig. (2-tailed)	.000	.627	.007	.376	.781	.021	.001	.901	.009
Memory	Correlation Coefficient	-.083	.085	-.005	-.042	-.045	-.060	-.041	.003	-.057
	Sig. (2-tailed)	.021	.019	.897	.249	.210	.098	.257	.924	.116
Language	Correlation Coefficient	.001	.081	-.066	-.017	-.051	-.029	-.023	-.030	-.038
	Sig. (2-tailed)	.981	.024	.066	.638	.154	.412	.529	.404	.292
Spatial	Correlation Coefficient	-.064	.024	-.115	-.044	-.016	-.040	-.051	-.052	-.092
	Sig. (2-tailed)	.075	.504	.001	.216	.654	.266	.157	.146	.011
Executive	Correlation Coefficient	-.043	.010	-.047	-.052	-.055	-.044	-.065	-.075	-.091
	Sig. (2-tailed)	.239	.789	.202	.153	.133	.233	.074	.040	.013

N= 715-781 Significance (2-tailed): $p \leq 0.05$ $p \leq 0.01$

REGRESSION OF NORMALISED TEST SCORES ON NORMALISED MARKERS, ESBs ONLY

Tests (raw scores)	sVCAM1	Amyloid	NIL12p70	NIL10	NIL6	NIL8
NART						
GPegtest						
DSym						
TMTA		.019	.037			
LM_immed					.040	
LM_delay		.025		.042	.007	
RVLTtot						
RVLT6						
RVLT7						
BVRT			.042		.003	
BNT	.041	.010				
Animals						
Blok		.023			.044	
FAS						
TMTB						
Interference					.001	.016

No significant results for PAI1, TNF2 nor IL1

REGRESSION OF TEST COGNITIVE DOMAIN SCORES ON BLOOD LEVELS ESBs ONLY

(Numbers in table are p values for the statistical significant of regression coefficients.)

<u>Cogn Domains</u>	<u>SAA</u>	<u>NIL6</u>	<u>IL12</u>	<u>Component Tests</u>
Psychomotor	.050			Gpegtest, Digit Symbol, Trail Making test A
Processing Speed	.051		.049	Digit Symbol, Trail Making Test A
Fine Motor				Gpegtest
Memory				Logical Memory (im & delay, RVLTS (1-5) 6 &7, BVRT
Language				Animals, BNT
Spatial	.023	.044		Block Design
Executive				Trail Making Test A, FAS, Stroop Interference

No significant results for VCAM, PAI1, IL12, TNF2, IL10, IL1 nor IL8

Notes: The first three domains are not independent. (2 was created to reduce the motor component.)

Domains 3 and 6 were represented in the battery by only one test each.

Domain scores were formed as the sum of the Z-scores of component tests.

REGRESSION OF TEST COMPONENTS ON BLOOD LEVELS (NORMALISED) ESBs ONLY

(Numbers in table are p values for the statistical significant of regression coefficients)

	SAA	IL12	IL6	IL10
Components				
F1: Non verbal	.017	.043	.004	
F2: RVLt tests				
F3: Verbal				
F4: Logical memory	.049		.011	0.039

	Component			
	1	2	3	4
TMTA	.784			
GPegtest	.749		-.164	
TMTB	.745			
DSym	.697		.171	
Blok	.678	-.118		.120
Interference	.514	-.135		.108
BVRT	.505		.103	
RVLT7		.936		
RVLT6		.919		
RVLTtot		.817	.134	
FAS		.129	.848	-.159
NART		-.103	.811	.109
BNT	.210		.577	
Animals	.241	.126	.419	.133
LM_immediate				.943
LM_delayed		.145		.906

No significant results for VCAM, PAI1, TNF2, IL10, IL1 nor IL8

Imaging Data

- Few significant correlations between inflammatory markers and white matter hyperintensity volume
- Correlations between regional volumes and some markers (VCAM, SAA, IIL6)



Output Nodes

- Inflammation and Cognition
 - Inflammation and MRI
 - Inflammation, genetics & Cognition/MRI
 - Nutrition, lifestyle and inflammation
 - SEM & inflammation
- Metabolic Syndrome, Insulin Resistance and Body Composition
- Arterial Stiffness
- ECG parameters
- Retinal Photography

Study Issues

- Repeated Measures
 - Bloods
 - Lab based assessments
- MRI issues
- Collaborative Efforts