Phagocytosis and Presentation of Amyloid β-Associated Antigens by Monocyte-Derived Macrophages in AD Pathology

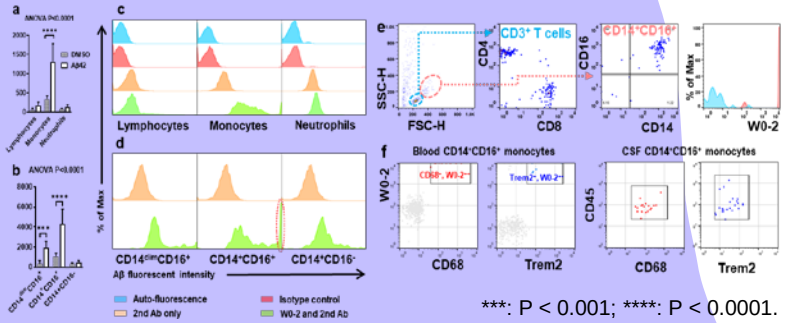
Xin Huang[1], Chris Fowler[1], Qiao-Xin Li[1,2], Jiaqi Sun[1], Keyla A. Perez[1], Céline Dubois[1], Yen Y. Lim[3], Candace Drysdale[1], Rebecca L. Rumble[1], Christopher C. Rowe[4], Ralph N. Martins[5], Paul Maruff[1,6], James D. Doecke[7], Yong Lin[8], Kevin J. Barnham[1], Colin L. Masters[1], Ben Gu[1,8†] and the AIBL Research Group[††]

Background:
Hydrophobic Aβ peptides require a carrier for transport through the interstitial fluid, CSF, lymphatic fluid and blood. Biomacromolecules, such as LRP1, RAGE and some of plasma proteins are considered as Aβ carriers and participate in different transport stages. However, the possibility of peripheral immune cells as carriers was simply ignored because they are too large to cross the blood-brain barrier. The current study revisits the puzzle of whether human monocyte-derived macrophages can remove Aβ from the brain and come to the periphery for antigen presentation.

Methods:
1) leukocytes capable of phagocytizing and transporting Aβ were identified by flow cytometry in vitro. Aβ-carrying monocyte-derived macrophages (MDM) in human blood circulation and cerebrospinal fluid (CSF) were identified and characterized.
2) The levels of Aβ carried by blood MDM were compared between AD patients and cognitively normal (CN) controls.
3) Intracerebroventricular injection of CFSE-labeled PBMC into AD mice was used to track whether there is an egress for CSF MDM to return to the peripheral blood and lymph.

Results:
1) Of the leukocytes tested, monocytes, especially $CD14^{+}CD16^{+}$ monocytes, were found to be the predominant cell type that phagocytizes Aβ peptides (**Fig. 1a-b**). Likewise, $CD14^{+}CD16^{+}$ monocytes were found to carry Aβ in human blood circulation (**Fig. 1c-d**) and CSF (**Fig. 1e**), and further immunophenotypes indicated that these cells were a type of MDM (**Fig. 1f**).

Figure 1. Identification of Aβ-carrying MDM in blood and CSF.



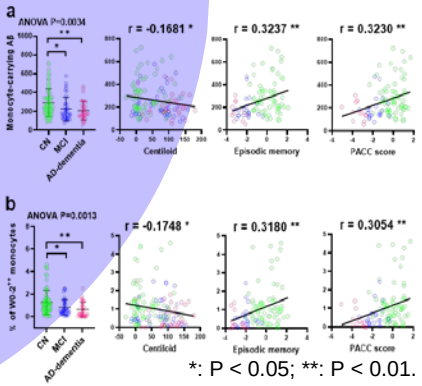
***: P < 0.001; ****: P < 0.0001.

2) In 154 individuals (Table 1), the levels of Aβ carried by blood monocytes were reduced by 26% in AD compared with control (**Fig. 2a**), and this change was correlated with the subject's brain PET Aβ score (Centiloid), episodic memory and PACC score. The percentage of $W0\text{-}2^{++}$ monocytes in total monocytes also decreased in MCI and AD-dementia compared with CN and correlated with Centiloid, EM and PACC score (**Fig. 2b**).

Table 1. Demographics of the study cohort.

Demographics	Control (<25CL)[a]	Case (>25CL)
Number of Participants (n)	60	94
Age (mean (SD))	73.05 (6.93)	74.93 (7.60)
Sex = Male (%)	24 (40.0)	47 (50.0)
Years of Education (mean (SD))	12.89 (3.08)	12.37 (3.03)
ApoE genotype (%)		
E2/E2	1 (1.7)	0 (0.0)
E3/E2	8 (13.6)	2 (2.1)
E3/E3	37 (62.7)	32 (34.0)
E4/E2	2 (3.4)	2 (2.1)
E4/E3	11 (18.6)	41 (43.6)
E4/E4	0 (0.0)	17 (18.1)
Clinical Classification (%)		
AD	3 (5.0)	32 (34.0)
CN	44 (73.3)	31 (33.0)
MCI	13 (21.7)	31 (33.0)
		91.08
Image PET Centiloid (mean (SD))	4.16 (9.89)	(33.81)
MMSE (mean (SD))	28.05 (2.38)	24.56 (5.24)
CDR (mean (SD))	0.18 (0.29)	0.64 (0.62)
Episodic Memory (mean (SD))	0.05 (1.10)	-1.26 (1.53)
Composite PACC score (mean (SD))	0.01 (0.96)	-1.38 (1.55)

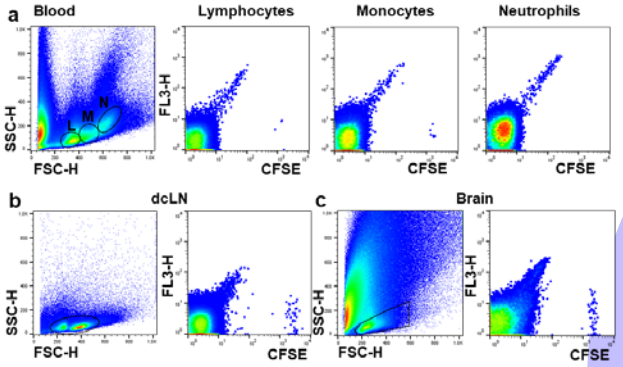
Figure 2. Blood monocyte-carried Aβ decreased in AD.



*: P < 0.05; **: P < 0.01.

3) The adoptive $CFSE^{+}$ PBMC were found in the peripheral blood and deep cervical lymph nodes (dcLN) of 85% AD mice two days after injection.

Fig. 3: Detection of adoptive $CFSE^{+}$ PBMC in blood and dcLN.



Conclusions:
Our study investigated the potential mechanism of MDM-based Aβ transport between CNS and periphery. The findings from our study shed light on how peripheral monocytes and phagocytosis contributes to AD pathology. Moreover, it may have important implications for understanding Aβ-associated antigen presentation and T- and B-cell activation in dcLN.

The Florey

Our Lab team

Author Affiliation: [1]The Florey Institute, the University of Melbourne; [2]National Dementia Diagnostics Laboratory, The University of Melbourne, VIC, Australia; [3]Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, Clayton, VIC Australia; [4]Department of Nuclear Medicine and Centre for PET, Austin Health, Heidelberg, Victoria, Australia; [5]Centre of Excellence for Alzheimer's Disease Research and Care, Edith Cowan University, Joondalup, Western Australia, Australia; [6]Cogstate Ltd., Melbourne, VIC, Australia; [7]CSIRO Health and Biosecurity, Australian E-Health Research Centre, Brisbane, Australia; [8]National Clinical Research Center for Aging and Medicine, Huashan Hospital, Fudan University, Shanghai, China.