Elevated plasma levels of soluble CD40 in incipient Alzheimer´s disease

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Acknowledgements

Abstract

CD40 is a member of the tumor necrosis factor receptor super-family and has been suggested to play a role in the metabolism of β-amyloid (Aβ) in Alzheimer´s disease (AD). However, the role of CD40-signalling in incipient AD has not yet been studied. We investigated the plasma levels of soluble CD40 (sCD40) and the soluble CD40 ligand (sCD40L) at baseline in 136 subjects with mild cognitive impairment (MCI) and 30 age-matched controls. Sixty of the 136 MCI cases converted to AD (MCI-AD) during a clinical follow-up period of 4-7 years. The baseline levels of sCD40, but not sCD40L, were elevated in MCI-AD cases when compared to age-matched controls (Mann-Whitney U-test, $p = 0.02$). However, MCI patients who were cognitively stable or developed vascular dementia during follow-up did not have significantly increased levels of sCD40 or sCD40L when compared to controls. The levels of sCD40 correlated to decreased baseline performance on mini-mental state examination (MMSE) in both controls (r_s=-0.37, $p<0.05$) and MCI-AD cases (r_s=-0.29, $p<0.05$). Finally, the plasma levels of sCD40 correlated with the levels of soluble amyloid precursor protein-α (sAPP-α) (r_s=0.28, $p<0.01$) and sAPP-β (r_s=0.23, $p<0.05$) in cerebrospinal fluid. In conclusion, CD40-signalling might play a role in the pathogenesis of early AD.

Keywords: mild cognitive impairment; Alzheimer's disease; soluble CD40; CD40 ligand; beta-amyloid, CSF.

Introduction

Most cases of dementia are caused by Alzheimer's disease (AD), which is characterized by progressive accumulation of senile plaques, containing β-amyloid (Aβ), and neurofibrillary tangles, containing tau [4]. This process probably starts many years before the typical clinical symptoms of AD appear. The formation of Aβ results from the concerted cleavage of amyloid precursor protein (APP) by β- and γ-secretase and an imbalance between the production and clearance of Aβ is thought to represent the earliest event in the pathogenesis of AD [4].

Frequently, subjects with incipient AD exhibit mild deficits in episodic memory. In this stage, when they are not yet demented, the subjects may fulfil the criteria of mild cognitive impairment (MCI) [14]. However, as the MCI syndrome is heterogeneous, a sizable number of subjects with MCI eventually develop other forms of dementia or are cognitively stable over time [14].

A growing body of evidence indicates that inflammatory processes are involved in the pathogenesis of AD [21]. The trans-membrane receptor CD40 is a member of the tumor necrosis receptor super-family [6]. Interaction between CD40 and the CD40 ligand (CD40L) elicits activation, differentiation and proliferation of immune cells, as well as up-regulation of co-stimulatory molecules and cytokines [6]. CD40 is mainly expressed by immune cells, but also by e.g. endothelial cells [6]. Upon pro-inflammatory stimulation the levels of CD40 are markedly elevated [15]. Soluble forms of CD40 (sCD40) can be produced through alternative splicing of mRNA[20]. However, a shedding of the membrane CD40 through proteolytic cleavage cannot be ruled out. CD40L is mainly present on the surfaces of platelets and CD4^{+} T-cells [6]. Besides surface-expressed CD40L, there are also soluble, biologically active forms of CD40L (sCD40L) that are shed upon cell activation. Post-mortem studies of AD

patients have revealed up-regulated CD40 in the blood vessel walls [19], as well as CD40 and CD40L in senile plaques [5, 19], which might indicate that CD40-signalling plays a role in AD pathogenesis. Interestingly, $A\beta_{42}$-induced activation of microglia and phosphorylation of tau have been shown to be mediated by CD40-CD40L interaction both *in vitro* and *in vivo* [18]. Moreover, accumulation of $A\beta$-related pathology was dependent on CD40/CD40L-signalling in a transgenic mice model of AD [17]. Both sCD40 and sCD40L have been shown to be elevated in plasma of demented patients with AD [8, 13]. The present study was conducted on subjects with MCI and aimed at examining whether sCD40 and sCD40L were altered in plasma of patients with preclinical AD and whether CD40/CD40L signalling was associated with markers of $A\beta$ metabolism and cognitive function.

Materials and methods

Subjects

The study sample was recruited at the memory clinic, Malmö University Hospital, Sweden. Physicians specialised in cognitive disorders performed a thorough clinical interview and a physical, neurological and psychiatric examination of each patient at baseline. Moreover, the subjects underwent computed tomography (CT) of the brain and cognitive tests, e.g. Mini-Mental State Examination (MMSE). The MCI criteria advocated by Petersen and colleagues were applied [14], including: 1) memory complaint, preferably corroborated by an informant; 2) objective memory impairment adjusted for age and education; 3) preservation of general cognitive functioning; 4) no or minimal impairment of daily life activities; 5) not fulfilling the DSM-IIIR criteria of dementia [3].

At baseline 136 MCI subjects were enrolled. After a clinical follow-up of at least four years (mean, 5.1 years; range, 4.0-6.8 years) 55 subjects (40%) were cognitively stable (MCI-stable). During the follow-up 60 MCI subjects (44%, annual incidence 12,4 %) developed Alzheimer's dementia according to the NINCDS-ADRDA criteria of probable AD [12] and DSM-IIIR requirements for dementia [3], while 15 (11%; annual incidence 3.1 %) subsequently progressed to vascular dementia (VaD) according to the criteria of probable VaD by NINDS-AIREN [16] and the DSM-IIIR requirements for dementia [3]. The residual six subjects developed other forms of dementia, e.g. dementia with Lewy bodies and frontotemporal dementia. Due to the small number of MCI subject in this group, they were excluded from further analyses. Thus, 130 subjects with MCI remained in the study. Baseline demographic variables are given in table 1.

The control population consisted of 30 healthy elderly volunteers, who were recruited in the city of Malmö, Sweden. They had no memory complaints or any other cognitive symptoms, preserved general cognitive functioning, as well as no active neurological or psychiatric disease. The controls were cognitively stable for at least three years.

At baseline, non-fasting plasma samples were collected in the morning by venipuncture in tubes containing EDTA as anticoagulant. After centrifugation, plasma samples were stored at -80ºC pending biochemical analyses. CSF samples were obtained at baseline by lumbar puncture in the L3/L4 or L4/L5 interspace. The CSF samples were gently mixed to avoid possible gradient effects, centrifuged, and stored at -80ºC.

The patients gave informed consent to participate in the study, which was conducted according to the provisions of the Helsinki Declaration and approved by the ethics committee of Lund University, Sweden.

Biochemical analyses

The levels of sCD40L and sCD40 were measured using commercially available immunoassays provided by "R&D System" (Minneapolis, USA) and "Bender MedSystems" (Vienna, Austria), respectively. The detection limit of the assay is 4.2 pg/ml for sCD40L and 6.9 pg/ml for sCD40 with an inter-assay variation of <10%.

The levels of $A\beta_{n-40}$ and $A\beta_{n-42}$ in CSF were quantified by commercially available ELISA kits (The Genetics Company) as previously described [9]. The CSF levels of total tau and tau phosphorylated at threonine 181 (P-tau) were quantified by Luminex xMAP technique as previously described [10]. Plasma concentrations of $A\beta_{n-40}$ and $A\beta_{n-42}$ were quantified by Luminex xMAP technique previously described [11]. Determinations of CSF concentrations

of α- and β-cleaved soluble amyloid precursor protein (sAPP-α and sAPP-β) and the β-site APP-cleaving enzyme 1 (BACE1) activity have also recently been described in detail [22]. The analyses were performed by experienced laboratory technicians, who were blinded to clinical diagnosis and other clinical information.

Statistical analyses

Mann-Whitney *U*-test was performed to compare continuous baseline data (and the annual MMSE change) between the diagnostic groups at follow-up, because the distribution of the data were non-parametric. Pearson´s x^2 test was used for dichotomous variables. Spearman´s correlation coefficient was used for correlation analyses. The statistical analyses were accomplished with SPSS for Windows, version 16.0.

Results

Plasma levels of sCD40 and sCD40L at baseline in the different diagnostic groups

The MCI subjects who subsequently developed AD during follow-up (MCI-AD) had significantly higher plasma levels of sCD40 already at baseline, when compared to the age-matched controls (Mann-Whitney *U*-test, p = 0.02, table 1, figure 1). However, the levels of sCD40 in the MCI subjects who subsequently developed VaD and the MCI subjects who were cognitively stable during follow-up did not differ significantly when compared to the age-matched controls (table 1). Furthermore, the plasma levels of sCD40L at baseline did not differ between the diagnostic subgroups (table 1). Neither the levels of sCD40, nor sCD40L, were associated with age, gender or *APOE* genotype in any of the studied groups (data not shown).

Correlations between baseline sCD40/sCD40L and MMSE and sAPP

The plasma levels of sCD40 at baseline correlated negatively with cognitive performance (MMSE score) in the entire study population (r_s=-0.19, p<0.05), in the MCI-AD cases (r_s=-0.29, p<0.05) and in the age-matched controls (r_s=-0.37, p<0.05). Moreover, there were positive correlations between the plasma levels of sCD40 and the CSF levels of sAPP-α (r_s=0.28, p<0.01) and sAPP-β (r_s=0.23, p<0.05) in the entire study population. In contrast, neither the plasma levels of sCD40, nor sCD40L, correlated to the levels of $A\beta_{n-40}$ and $A\beta_{n-42}$ in plasma and CSF or to tau, P-tau and BACE1 activity in CSF in the entire study population (data not shown).

Discussion

We show that plasma levels of sCD40 are significantly increased in subjects with preclinical AD compared to age-matched controls and the levels of sCD40 correlate with poor cognitive performance and elevated levels of sAPP-α and sAPP-β

The strengths of the present study are the relatively large number of included MCI cases and the long follow-up time. Extensive clinical follow-up prevents misclassification of incipient AD as stable MCI [14]. Another advantage with the present study is the analyses of APP metabolites both in CSF and plasma, as well as BACE1 activity in CSF, which enabled us to correlate these parameters to the levels of sCD40 and sCD40L. A limitation of the present study is that the control group is relative small, resulting in an increased risk of type II errors.

The soluble forms of CD40 lack ability to transduce the intracellular signals, necessary to elicit the known functions of CD40 [20]. The role of the soluble forms of CD40 has not yet been clarified, but soluble CD40 functions might regulate the CD40-CD40L signalling [20]. The soluble CD40 isoforms are expressed concomitantly as the trans-membrane form upon pro-inflammatory stimuli and activated cells that express large amounts of CD40 on their membranes also produce high levels of soluble CD40 [7, 20]. Therefore, measurement of sCD40 can be a valid assessment of the CD40 expression. The soluble form of CD40L is mainly released from activated platelets and remains biologically active [2].

In a previous report, Mocali and co-workers found significantly elevated plasma levels of sCD40 in AD subjects [13]. However, the levels of sCD40 were equally elevated through the disease stages [13], which might be explained by an early, but stable, up-regulation of CD40 in AD. The finding presented here that sCD40 levels are significantly increased already in

patients with preclinical AD (MCI-AD) supports this hypothesis. Moreover, we found a significant correlation between sCD40 and poor cognitive performance in elderly controls without cognitive symptoms, which might indicate that sCD40 is elevated in the very early stages of preclinical AD. However, longitudinal studies on healthy elderly controls are needed to resolve this issue. The increased levels of sCD40L in demented patients with AD, reported by Desideri and colleagues, were modest in early stages of AD, but increased substantially with disease progression [8]. These results, together with the findings in the present study, indicate that sCD40L is not substantially increased in the preclinical stages of AD but increase during the clinical course of AD.

Post-mortem studies of AD patients suggest that CD40-signalling plays a role in AD pathogenesis [5, 19]. Inhibition of CD40L results in decreased amyloid production and microglia activation in a transgenic animal model of AD [17]. Moreover, Aβ-induced microglia activation and tau phosphorylation are dependent on CD40/CD40L interaction [18]. In the present study we found that sCD40 correlates with sAPP-α and s-APP-β, which might suggest that CD40-signalling is associated with cleavage of APP by α- and β-secretase. However, we did not find any correlation between sCD40 and the activity of the major β-secretase, BACE1. The latter finding is supported by an earlier study showing that CD40/CD40L interaction in microglia up-regulates the putative α-secretases (ADAM10 and TACE), but not BACE1 [1].

In conclusion, CD40-signalling might play a role in the pathogenesis of early AD. However, further studies of subjects with preclinical AD and different stages of established dementia are needed to elucidate the precise role of the CD40/CD40L system and its association to Aβ-metabolism.

Disclosure statement

The authors have no conflicts of interest.

References
[1]   G. Ait-Ghezala, V.S. Mathura, V. Laporte, A. Quadros, D. Paris, N. Patel, C.H. Volmar, D. Kolippakkam, F. Crawford, M. Mullan, Genomic regulation after CD40 stimulation in microglia: relevance to Alzheimer's disease, Brain Res Mol Brain Res 140 (2005) 73-85.
[2]   P. Andre, L. Nannizzi-Alaimo, S.K. Prasad, D.R. Phillips, Platelet-derived CD40L: the switch-hitting player of cardiovascular disease, Circulation 106 (2002) 896-899.
[3]   A.P. association, Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, Arlington, VA, USA, 1987.
[4]   K. Blennow, M.J. de Leon, H. Zetterberg, Alzheimer's disease, Lancet 368 (2006) 387-403.
[5]   N.Y. Calingasan, H.A. Erdely, C.A. Altar, Identification of CD40 ligand in Alzheimer's disease and in animal models of Alzheimer's disease and brain injury, Neurobiol Aging 23 (2002) 31-39.
[6]   K. Chen, J. Huang, W. Gong, L. Zhang, P. Yu, J.M. Wang, CD40/CD40L dyad in the inflammatory and immune responses in the central nervous system, Cell Mol Immunol 3 (2006) 163-169.

[7]   P. De Paoli, M. Cozzi, R. Tedeschi, A. Gloghini, A.M. Cilia, C. van Kooten, G. Gaidano, A. Carbone, High CD40 membrane expression in AIDS-related lymphoma B cell lines is associated with the CD45RA+, CD45RO+, CD95+ phenotype and high levels of its soluble form in culture supernatants, Cytometry 30 (1997) 33-38.

[8]   G. Desideri, F. Cipollone, S. Necozione, C. Marini, M.C. Lechiara, G. Taglieri, G. Zuliani, R. Fellin, A. Mezzetti, F. di Orio, C. Ferri, Enhanced soluble CD40 ligand and Alzheimer's disease: evidence of a possible pathogenetic role, Neurobiol Aging 29 (2008) 348-356.

[9]   O. Hansson, H. Zetterberg, P. Buchhave, U. Andreasson, E. Londos, L. Minthon, K. Blennow, Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment, Dement Geriatr Cogn Disord 23 (2007) 316-320.

[10]   O. Hansson, H. Zetterberg, P. Buchhave, E. Londos, K. Blennow, L. Minthon, Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study, Lancet Neurol 5 (2006) 228-234.

[11]   O. Hansson, H. Zetterberg, E. Vanmechelen, H. Vanderstichele, U. Andreasson, E. Londos, A. Wallin, L. Minthon, K. Blennow, Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment, Neurobiol Aging (2008) doi:10.1016/j.neurobiolaging.2008.1003.1027.

[12]   G. McKhann, D. Drachman, M. Folstein, R. Katzman, D. Price, E.M. Stadlan, Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease, Neurology 34 (1984) 939-944.

[13]   A. Mocali, S. Cedrola, N. Della Malva, M. Bontempelli, V.A. Mitidieri, A. Bavazzano, R. Comolli, F. Paoletti, C.A. La Porta, Increased plasma levels of soluble CD40, together with the decrease of TGF beta 1, as possible differential markers of Alzheimer disease, Exp Gerontol 39 (2004) 1555-1561.

[14]   R.C. Petersen, Mild cognitive impairment as a diagnostic entity, J Intern Med 256 (2004) 183-194.

[15]   H. Qin, C.A. Wilson, S.J. Lee, X. Zhao, E.N. Benveniste, LPS induces CD40 gene expression through the activation of NF-kappaB and STAT-1alpha in macrophages and microglia, Blood 106 (2005) 3114-3122.

[16]   G.C. Roman, T.K. Tatemichi, T. Erkinjuntti, J.L. Cummings, J.C. Masdeu, J.H. Garcia, L. Amaducci, J.M. Orgogozo, A. Brun, A. Hofman, et al., Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop, Neurology 43 (1993) 250-260.

[17]   J. Tan, T. Town, F. Crawford, T. Mori, A. DelleDonne, R. Crescentini, D. Obregon, R.A. Flavell, M.J. Mullan, Role of CD40 ligand in amyloidosis in transgenic Alzheimer's mice, Nat Neurosci 5 (2002) 1288-1293.

[18]   J. Tan, T. Town, D. Paris, T. Mori, Z. Suo, F. Crawford, M.P. Mattson, R.A. Flavell, M. Mullan, Microglial activation resulting from CD40-CD40L interaction after beta-amyloid stimulation, Science 286 (1999) 2352-2355.

[19]   T. Togo, H. Akiyama, H. Kondo, K. Ikeda, M. Kato, E. Iseki, K. Kosaka, Expression of CD40 in the brain of Alzheimer's disease and other neurological diseases, Brain Res 885 (2000) 117-121.

[20]   M. Tone, Y. Tone, P.J. Fairchild, M. Wykes, H. Waldmann, Regulation of CD40 function by its isoforms generated through alternative splicing, Proc Natl Acad Sci U S A 98 (2001) 1751-1756.

[21] T. Wyss-Coray, Inflammation in Alzheimer disease: driving force, bystander or beneficial response?, Nat Med 12 (2006) 1005-1015.

[22] H. Zetterberg, U. Andreasson, O. Hansson, G. Wu, S. Sankaranarayanan, M.E. Andersson, P. Buchhave, E. Londos, R.M. Umek, L. Minthon, A.J. Simon, K. Blennow, Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease, Arch Neurol 65 (2008) 1102-1107.

Tables

Table 1. Baseline data and annual change of MMSE in the patients with mild cognitive impairment (MCI) and the controls.

	Controls	MCI-AD	MCI-VaD	MCI-stable
Age, years	72.3±8.4	74.3±5.8[c]	75.3±6.6[c]	64.2±9.0[a]
Sex, M/F	13/17	16/44[d, f]	10/5	29/26
APOE ε4 carrier, %	23	75[a, d, e]	27	49[b]
MMSE at baseline (0-30 p)	29.3±1.0	26.8±1.4[a]	26.8±1.6[a]	27.3±1.8[a]
Annual change of MMSE during follow-up, points/year	+0.01±0.3	-3.0±2.3[a, c]	-2.5±2.4[a, c]	+0.2±0.4[b]
Plasma sCD40, pg/ml	40.5 (46.5)	64.5 (33.3)[b]	67.5 (34.0)	56.0 (37.5)
Plasma sCD40L, pg/ml	364 (6529)	468 (3371)	106 (2263)	538 (1346)

Values are means ±SD, except the values of sCD40 and sCD40L [median values (interquartile range)] and when noted otherwise.

[a] $p <0.001$ vs Controls, [b] $p <0.05$ vs Controls, [c] $p < 0.001$ vs MCI- stable, [d] $p <0.01$ vs MCI- stable, [e] $p < 0.001$ vs MCI- VaD, [f] $p <0.01$ vs MCI- VaD.

Abbreviations: Controls, healthy controls, with unimpaired cognition during 3 years of follow-up; MCI-AD, MCI patients that developed Alzheimer´s disease during follow-up; MCI-VaD, MCI patients that developed vascular dementia during follow-up; MCI-stable, MCI patients with stable cognitive functions during a follow-up period of 4.0-6.8 years; MMSE, Mini-Mental State Examination; *APOE* ε4 carrier, at least one apolipoprotein E ε4 allele; sCD40, soluble CD40; sCD40L, soluble CD40 ligand.