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ORIGINAL ARTICLE

Decreased circulating CD34$^+$ stem cells in early Alzheimer's disease: evidence for a deficient hematopoietic brain support?

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Hematopoietic stem cells contribute to mammalian brain tissue regeneration by transdifferentiation processes. We found decreased counts of circulating CD34$^+$ cells in early Alzheimer's dementia (AD; $P=0.01$), which significantly correlated with age ($r=-0.661$; $P=0.001$), cerebrospinal fluid β-amyloid (Aβ)1–42 ($r=-0.467$; $P=0.025$) and most pronounced the Aβ42/40 ratio ($r=-0.688$; $P=0.005$). Our data suggest a deficient regenerative hematopoietic support for the central nervous system in early AD.
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Introduction

Alzheimer's disease (AD) is characterized by the deposition of β-amyloid (Aβ) peptides into neuritic plaques, the formation of neurofibrillary tangles, neuroinflammation and the degeneration of synapses and neurons in brain regions critical for learning and memory.[1] In the mammalian brain, neurogenesis originating from neuronal stem cells persists in adulthood, but it decreases with age.[2] Neurogenesis responds to environmental demands such as brain injury or mental activity.[3,4] Neogenesis of neuronal and glial cells also occurs by transdifferentiation of hematopoietic stem cells (HSC) from the peripheral blood, suggesting that a regenerative interface exists between the nervous and hematopoietic organ systems.[5–7] This view is strengthened by findings showing that stress related to ischemic stroke triggers the mobilization of HSC into peripheral blood.[8] Recently, it was shown that intracerebral HSC implantation induced neuroplasticity and improved neurological function in rats after chronic cerebral ischemia.[9] Therefore, central nervous system regeneration by transdifferentiation of HSC might be relevant for diagnosis and therapy of neurodegenerative diseases. This study was undertaken to evaluate the frequency of HSC in the blood of patients with early AD.

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Materials and methods

The study protocol was approved by the Ethics Committee of the University of Erlangen-Nuremberg and all participants provided written informed consent. The early AD group fulfilled the NINCDS-ADRDA criteria for probable AD and the diagnosis was supported by cerebrospinal fluid (CSF)-based neurochemical dementia diagnostics using commercially available enzyme-linked immunosorbent assay kits to assess Aβ1–40, Aβ1–42, tau and phospho-tau proteins (The Genetics Company, Zurich, Switzerland; Innogenetics, Ghent, Belgium).[10,11] Spouses or caregivers served as non-demented age, sex and environmentally matched controls.

Monoclonal antibodies (mAbs) directed against CD45R0-fluorescein isothiocyanate (FITC), CD34-phycoerythrin (PE) and isotype controls were purchased from Becton Dickinson (Heidelberg, Germany). One hundred microlitres of ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood were incubated with the respective mAb at a concentration determined in pretitrating for 30 min at 4°C. Erythrocytes were removed with the Multi-Q-Prep/ImmunoPrep systems (Beckman Coulter, Fullerton, CA, USA). Cells were washed, fixed with phosphate-buffered saline/1% paraformaldehyde (pH 7.4, 4°C) and analyzed by flow cytofluorometry (EPICSXL, Beckmann Coulter, USA). HSCs were identified as CD34$^+$ and by low levels of CD45R0 expression (CD45R0^{low}) to exclude irrelevant populations of mature blood cells.[12]

Statistical calculations were performed using the SPSS 12 software (SPSS Inc., Chicago, IL, USA). All statistical tests were two-sided and the significance

level was set at $\alpha = 0.05$. Statistical significance was assessed using *t*-tests for unpaired samples. The normal distribution of the data was confirmed with the Kolmogorov–Smirnov test with Lilliefors correction, variance homogeneity was assessed according to Levene and correlation coefficients were determined using the method of Pearson.

Results

We performed a pilot study analyzing several cellular markers in the blood of patients with early AD and controls. Neither percentages of monocytes, lymphocytes or granulocytes nor counts of $CD4^+$, $CD8^+$, $CD3^+/CD56^+$, $CD3^+/CD25^+$, $CD4^+/CD25^+$, $CD4^+/CD28^+$, $CD8^+/CD25^+$ and $CD45^+/CD133^+$ cells did significantly differ between early AD patients ($n = 12$) and control persons ($n = 10$). However, $CD45R0^{low}/CD34^+$ cell counts were significantly decreased in early AD ($T = -2.82$; $P = 0.017$). We assessed the same patients/control persons again enlarging the groups to $n = 23$ (early AD) and $n = 25$ (controls). The number of circulating $CD45R0^{low}/CD34^+$ stem cells was again significantly decreased as compared with controls ($T = -2.68$; $P = 0.01$; Figure 1), confirming the data from the first cohorts. Also the new 11 patients and 15 controls in the enlarged sample differed significantly ($T = -2.77$; $P = 0.01$).

Intriguingly, only within the AD group $CD45R0^{low}/CD34^+$ stem cell counts were highly correlated with age ($n = 23$; $r = -0.661$; $P = 0.001$; Figure 2a). Furthermore, within the AD group $CD45R0^{low}/CD34^+$ cell counts were significantly correlated with CSF Aβ1–42 ($n = 23$; $r = -0.467$; $P = 0.025$) and even closer with the Aβ ratio 42/40 ($n = 15$; $r = -0.688$; $P = 0.005$; Figure 2b). The correlations between $CD45R0^{low}/CD34^+$ counts and total tau or phospho-tau protein were not significant.

Discussion

This study shows for the first time that counts of circulating HSC are decreased and significantly correlated with age in early AD. As stem cell dysfunction/decrease with increasing age was found also in other stem cell lineages, decreased HSC counts may reflect an accelerated aging process and a premature exhaustion of the stem cell pool in AD.[2]

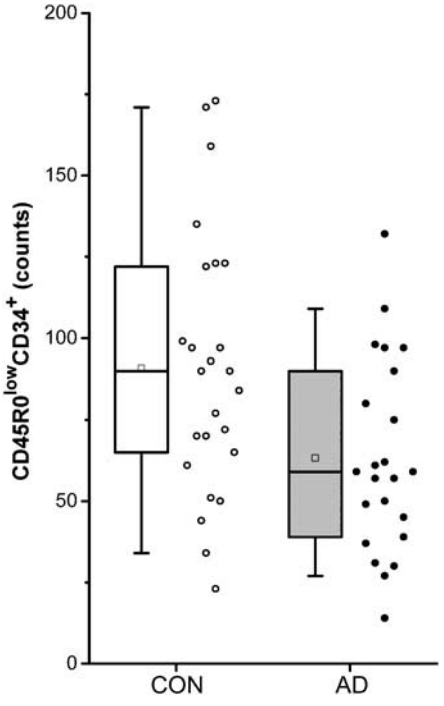

Figure 1 $CD45R0^{low}/CD34^+$ stem cell frequency in early AD patients (●) and controls (○). EDTA-anticoagulated whole-blood samples were labeled with anti-CD45R0-FITC and anti-CD34-PE simultaneously, fixed and analyzed with an EPICSXL flow cytometer. The counts refer to a whole-blood volume of 60 μl. The mean is indicated with (□), solid lines within the box mark the median, upper/lower boundaries show the 75th/25th percentiles and whiskers indicate the 95th and 5th percentiles, respectively. The data showed a normal distribution; hence, statistical significance was calculated with the *t*-tests for unpaired samples (AD, $n = 23$; CON, $n = 25$; $T = -2.68$; $P = 0.01$).

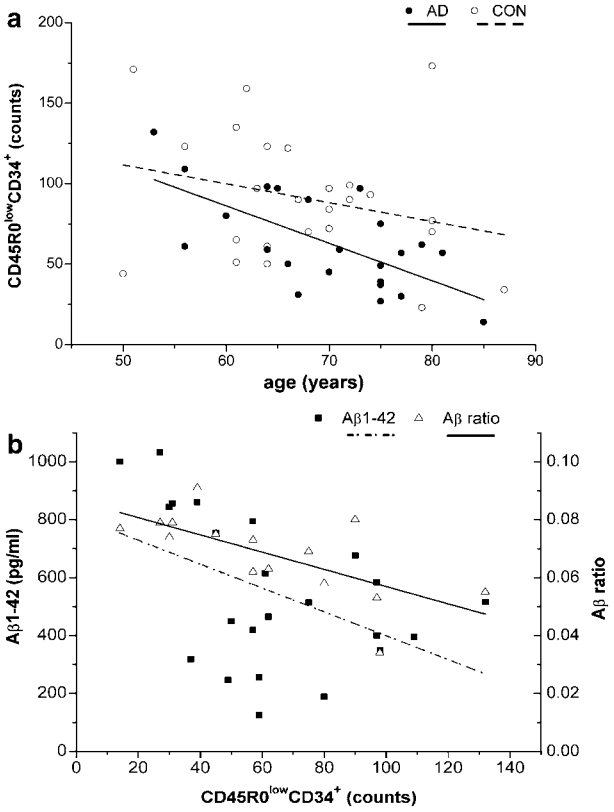

Figure 2 Correlation between HSC frequency and age, Aβ1–42 and Aβ ratio 42/40. In (**a**) $CD45R0^{low}/CD34^+$ cell counts are plotted against age in the early AD group (●) and control subjects (○). (**b**) Relationship of $CD45R0^{low}/CD34^+$ cell counts and CSF Aβ1–42 (■, $n = 23$) or Aβ ratio 42/40 (Δ, $n = 15$) within the AD group. The lines through the data represent the least-squares linear regression.

Aβ1–42 is abundant in brain tissue but decreased in CSF in AD.[13] Decreased Aβ1–42 and even more the lowered Aβ ratio 42/40 in CSF can be regarded as trait markers of AD, which are already altered preclinically in patients with mild cognitive impairment.[10] By contrast, total tau and phospho-tau in CSF are increased in AD.[10] Aβ1–42 and even closer the Aβ ratio 42/40 in CSF were highly correlated with $CD45R0^{low}/CD34^{+}$ stem cells, which was not observed for total tau and phospho-tau 181. Accordingly, Aβ peptides or other metabolites of the Aβ precursor protein might modulate the number of circulating hematopoietic $CD45R0^{low}/CD34^{+}$ stem cells, thus providing further evidence for a crosstalk between brain and bone marrow.[14,15] Circulating $CD34^{+}$ HSCs were also found to be decreased in coronary artery disease and to correlate with the number of atherosclerotic risk factors.[16] Interestingly, some risk factors such as hypertension, hypercholesterolemia and diabetes are also relevant for AD.[17] However, in this study, these cardiovascular risk factors were evenly distributed among the AD and control group.

The CD34 surface antigen characterizes a heterogenous population of cells including subsets of hematopoietic or endothelial progenitor cells, mature endothelial cells and tissue committed stem cells.[18] Identifying the most affected sub-populations and understanding the mechanism of HSC consumption and trafficking in AD may help to develop novel diagnostic tools and therapeutic approaches to alleviating the course of the disease.

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References

1 DeKosky ST, Scheff SW, Styren SD. Structural correlates of cognition in dementia: quantification and assessment of synapse change. *Neurodegeneration* 1996; **5**: 417–421.
2 Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* 1996; **16**: 2027–2033.
3 Rice AC, Khaldi A, Harvey HB, Salman NJ, White F, Fillmore H *et al.* Proliferation and neuronal differentiation of mitotically active cells following traumatic brain injury. *Exp Neurol* 2003; **183**: 406–417.
4 Kempermann G, Brandon EP, Gage FH. Environmental stimulation of 129/SvJ mice causes increased cell proliferation and neurogenesis in the adult dentate gyrus. *Curr Biol* 1998; **8**: 939–942.
5 Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 2000; **290**: 1779–1782.
6 Cogle CR, Yachnis AT, Laywell ED, Zander DS, Wingard JR, Steindler DA *et al.* Bone marrow transdifferentiation in brain after transplantation: a retrospective study. *Lancet* 2004; **363**: 1432–1437.
7 Sigurjonsson OE, Perreault MC, Egeland T, Glover JC. Adult human hematopoietic stem cells produce neurons efficiently in the regenerating chicken embryo spinal cord. *Proc Natl Acad Sci USA* 2005; **102**: 5227–5232.
8 Paczkowska E, Larysz B, Rzeuski R, Karbicka A, Jalowinski R, Kornacewicz-Jach Z *et al.* Human hematopoietic stem/progenitor-enriched CD34(+) cells are mobilized into peripheral blood during stress related to ischemic stroke or acute myocardial infarction. *Eur J Haematol* 2005; **75**: 461–467.
9 Shyu WC, Lin SZ, Chiang MF, Su CY, Li H. Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. *J Neurosci* 2006; **26**: 3444–3453.
10 Wiltfang J, Lewczuk P, Riederer P, Grunblatt E, Hock C, Scheltens P *et al.* Consensus paper of the WFSBP Task Force on Biological Markers of Dementia: the role of CSF and blood analysis in the early and differential diagnosis of dementia. *World J Biol Psychiatry* 2005; **6**: 69–84.
11 Lewczuk P, Esselmann H, Otto M, Maler JM, Henkel AW, Henkel MK *et al.* Neurochemical diagnosis of Alzheimer's dementia by CSF Abeta42, Abeta42/Abeta40 ratio and total tau. *Neurobiol Aging* 2004; **25**: 273–281.
12 Fritsch G, Buchinger P, Printz D, Fink FM, Mann G, Peters C *et al.* Rapid discrimination of early CD34+ myeloid progenitors using CD45-RA analysis. *Blood* 1993; **81**: 2301–2309.
13 Kuo YM, Emmerling MR, Vigo-Pelfrey C, Kasunic TC, Kirkpatrick JB, Murdoch GH *et al.* Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. *J Biol Chem* 1996; **271**: 4077–4081.
14 Simard AR, Soulet D, Gowing G, Julien JP, Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* 2006; **49**: 489–502.
15 Chen CW, Boiteau RM, Lai WF, Barger SW, Cataldo AM. sAPPalpha enhances the transdifferentiation of adult bone marrow progenitor cells to neuronal phenotypes. *Curr Alzheimer Res* 2006; **3**: 63–70.
16 Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H *et al.* Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001; **89**: E1–E7.
17 Sjogren M, Blennow K. The link between cholesterol and Alzheimer's disease. *World J Biol Psychiatry* 2005; **6**: 85–97.
18 Kucia M, Ratajczak J, Reca R, Janowska-Wieczorek A, Ratajczak MZ. Tissue-specific muscle, neural and liver stem/progenitor cells reside in the bone marrow, respond to an SDF-1 gradient and are mobilized into peripheral blood during stress and tissue injury. *Blood Cells Mol Dis* 2004; **32**: 52–57.