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Dementia-associated changes of immune cell composition within the cerebrospinal fluid

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ABSTRACT

Inflammation and alterations in essential protein structures in the brain might also change the cellular distribution in the cerebrospinal fluid (CSF).

Using flow cytometry, we analyzed cell populations of the innate and adaptive immune system associated with the most frequent forms of dementias. We included patients with mild cognitive impairment (MCI; N = 33), Alzheimer's disease (AD; N = 90), vascular dementia (VD; N = 35) and frontotemporal dementia (FTD; N = 17) at the time of diagnosis, before onset of treatment and 11 elderly non-demented individuals. Dependent on the form of dementia, an increased frequency of $CD14^+$ monocytes, NK cells and NKT cells was measured. Within the T cell population, a dementia-associated shift from central memory towards (late-stage) effector cells was detected. T cells and NKT cells were correlated with MMSE, NK and NKT cells were correlated with ptau, $CD14^+$ monocytes and NK cells were correlated with Amyloid-β 1–40.

Our data suggest that each investigated immune cell type is involved in dementia-associated alterations within the CSF, possibly having distinct functions in their pathogenesis.

1. Introduction

Demographic changes due to an ongoing aging population take place throughout the world. Europeans especially from Italy and Germany belong to the oldest populations in the world (Ferrucci et al., 2008). Aging has a profound impact on the immune system, affecting both the innate and the adaptive part and seems to contribute to a chronic low-grade inflammatory state. Consequently, the frequency of age-related disorders such as atherosclerosis, type II diabetes and Alzheimer's disease (AD) increases with age (Castelo-Branco and Soveral, 2014). AD was only recently added to the list of the leading causes of death and the proportion of people dying of it will probably grow substantially in the future (Ferrucci et al., 2008). Therefore, AD and dementia in general will be a major.

1.1. Health and socio-economic problem

Dementias are neurocognitive disorders caused by neurodegeneration and characterized by multiple cognitive impairments that result in the decline from previous function (LoGiudice and Watson, 2014). The main underlying causes of dementia are AD, Lewy Body dementia, frontotemporal dementia (FTD) and vascular dementia (VD) (Rizzi et al., 2014; Onyike and Diehl-Schmid, 2013; Sanford, 2018).

AD is the most common cause of dementia. This neurodegenerative disorder is indicated by senile plaques, neurofibrillary tangles or neuronal and synaptic loss. VD is caused e.g. by post-stroke, subcortical or multi-infarct dementia, diffuse white matter or strategic infarcts (Werring et al., 2010; Farooq and Gorelick, 2013). VD exhibits a comparatively abrupt onset and stepwise decline. AD and VD share several risk factors including age, major vascular risk factors (hypertension, smoking, diabetes, hypercholesterolemia, obesity), genetic variants, possibly also depression (Wuet al, 2016; Qiu et al., 2009) and inflammation. FTD is a heterogeneous group of hereditary and sporadic neurodegenerative disorders characterized by progressive deterioration of social and personal behaviour, executive functions and language, accompanied by impaired cognition (Bang et al., 2015). FTD substantially overlaps the symptoms of AD. Therefore, additionally to neurocognitive testing, such as the Mini-Mental State Examination (MMSE),

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brain imaging and analysis of the cerebrospinal fluid (CSF) are important tools for the diagnosis of dementia forms.

FTD predominantly develops frontal or temporal atrophy, whereas in AD, there could be detected a more generalized brain atrophy with the focus on the medial temporal lobe structures.

Mild cognitive impairment (MCI) represents a transitional stage between normal cognition in the process of healthy aging and dementia. MCI patients show a high rate of progression to dementia over a relatively short time (Roberts and Knopman, 2013).

In CSF it was shown that low Amyloid-β1-42 concentrations are a good biomarker for the presence of Amyloid deposition (Holtzman, 2011) and that total and phosphorylated tau indicate neurodegeneration (Goedert et al., 2017). In MCI, low Amyloid-β1-42 were determined in patients with a more advanced disease (Kennedy et al., 2012).

Due to epidemiologic, genetic and experimental evidence, immune mechanisms have come into focus recently and are now believed to contribute substantially to the risk, onset, and progression of dementia (Venegas and Heneka, 2019).

Monocytes, microglia, NK cells and NKT cells are part of the innate immune cells, B and T lymphocytes represent the adaptive immune system. It was shown that in aged persons, there is a shift of the immune system from immunoregulation towards inflammation. This chronic inflammation in the periphery, but also in the brain, might be important for the pathology of dementias (Franceschi et al., 2018; Minciulloet al, 2016). We have recently shown alterations in the frequency of monocytes, NK cells, B cells and naïve/memory T cells in peripheral blood obtained from AD, FTD and VD patients (Busseet al, 2017). Due to a cross talk between peripheral and central immunity, which might be mediated e.g. by the release of cytokines and chemokines, these cell populations might also play an important role in dementias.

Memory T cells might exhibit several roles in the brain, protective but also detrimental ones. They might protect against infections with neurotropic pathogens, but might also be involved in autoimmunity and other inflammatory disorders (Wuet al, 2018; Smolderset al, 2018). Microglia have an important function in the maintenance of brain homeostasis, which is lost in neurodegenerative disorders. Microglia, but also peripheral monocytes, contribute to the clearance cerebral Amyloid-β protein, a function that is deficient in dementias (Zuroff et al., 2017). The accumulation of Amyloid-β triggers neuroinflammation, leads to progressive synaptic loss, and finally results in cognitive decline.

NK(T) cells are important for host defense since they kill infected cells and secrete a broad range of cytokines and chemokines. Their role in neurodegenerative disorders remains to be clarified.

Standard CSF laboratory examinations included the number of monocytes, lymphocytes and granulocytes to exclude a bacterial or viral infection rather than having real input for pathology of dementia. Therefore, the aim of our study was to investigate the frequency of innate and adaptive immune cell populations, including monocytes, NK cells, NKT cells, B lymphocytes, T lymphocyte subpopulations in CSF from patients suffering from AD, FTD, VD and MCI. Besides, their activation state was determined. A group of non-demented, elderly persons served as control. Monocytes, NK cells, NKT cells, and T cells were correlated with MMSE and CSF standard values for Amyloid-β, tau and Q Albumin.

2. Materials and methods

2.1. Study cohort

The study was performed in accordance with German laws, the Declaration of Helsinki and the guidelines of the local institutional review board (reference number of the ethics committee 30/13). Written consent was obtained from all patients or patients' medical caregivers. 6 ml cerebrospinal fluid was collected additionally during routine CSF recovery without any further discomfort for the patients. The study included 33 MCI patients (22 female, 11 male; mean age 77.18 years), 90 AD patients (54 female, 36 male; mean age 80.01 years), 35 VD patients (55 female, 10 male; mean age 78.75 years) and 17 FTD patients (10 female, 7 male; mean age 74.94 years). AD patients were further subdivided according to the MMSE values into mild AD (38 patients; 25 female, 13 male; mean age 79.80 years), moderate AD (41 patients; 21 female, 20 male; mean age 80.24 years) severe AD (11 patients; 8 female, 3 male; mean age 79.83 years). 11 elderly non-demented patients served as control group (11 patients; 7 female, 4 male; mean age 71.18 years). These patients mainly suffered from depression and were admitted to our university hospital for a detailed diagnosis, but neither MMSE nor CSF values nor magnetic resonance imaging (MRI) measurements support a dementia diagnosis. The demographic data of the study cohort, including MMSE values, standard laboratory parameters obtained from CSF (ptau, htau, Amyloid-β1-40, Amyloid-β1-42, Amyloid-β ratio) and Q Albumin values, are shown in Table 1. Besides, routine blood analyses (including differential blood cell count, CRP level, glucose, lipids, liver enzymes, and thyroid hormones) were obtained in parallel. None of the study participants had to be excluded due to abnormal routine blood values or due to a history of immune diseases, immunomodulating treatment, cancer, chronic terminal disease, severe cardiovascular disorder, substance abuse, or severe trauma.

2.2. Flow cytometry analysis

3 ml CSF per panel were centrifuged, incubated with the primary antibodies (Abs) at 4 °C for 20min. in the dark: FITC anti-human HLA-DR (G46-6) and PE anti-human CD3 (UCHT1) from BD Pharmingen (BD Biosciences; San Jose, CA, USA) and APC-eFluor 780 anti-human CD14 (61D3), APC-eFluor 450 anti-human CD19 (SJ25C1), APC anti-human CD56 (CMSSB), PE-Cy7 anti-human CD69 (FN5O); PE anti-human CD197 (CCR7; 3D12), APC anti-human CD45RA (HI100), FITC anti-human CD45 RO (UCHL1), eFluor 450 anti-human CD4 (SK3) and PE-Cy7 anti-human CD8 (SK1) from eBioscience, Frankfurt, Germany. After washing, data was collected on a flow cytometer (Fortessa, BD Biosciences, Mountain View, CA, USA) and analyzed using FACS DIVA software 6.1.3 (BD Biosciences, Mountain View, CA, USA) and FlowJo software (Treestar Inc., Ashland, OR, USA). The data were analyzed using biexponential transformation function for complete data visualization and the frequency of cells was shown.

2.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 software. Normality of distribution was determined by Shapiro-Wilk test. Differences between groups were analyzed by Kruskal-Wallis test, followed by Dunn's multiple comparisons test, thereby the mean rank of each group was compared with the mean rank of every other group. Differences in gender were analyzed by chi-square test. Correlation was analyzed using Spearman's correlation. Significance was defined as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ****$p < 0.0001$.

3. Results

Alterations in CSF innate immune cells in AD, FTD and VD compared to controls.

We have recently shown that dementia is associated with changes in the leukocyte populations in peripheral blood (Busseet al, 2017). The aim of the present study was to rule out whether an alteration in leukocyte composition is also detectable within the CSF. We used a flow cytometry approach to measure the leukocytes within the CSF; the gating strategy employed to identify the cell populations is shown in Suppl. Figure 1.

$CD14^+$ monocytes/macrophages were increased in AD compared to the level obtained in elderly non-demented controls ($p = 0.0035$; Fig. 1a). The activation state of $CD14^+$ cells, measured by the expression level of HLA-DR, was unchanged (Table 2a). According to the presence or absence of CD3 expression, $CD56^+$ cells can be divided into CD56 + $CD3^-$ NK cells and CD56 + $CD3^+$ NKT cells. Both cell types were raised in

Table 1
Demographic data of the study cohort. Shown are demographic data of the included patients, total number of individuals, age, gender and MMSE. In addition, the most important CSF parameters are presented, including ptau, htau, Amyloid-β1-40, Amyloid-β1-42, Amyloid-β ratio and Q Albumin as marker for the blood-brain-barrier integrity. Shown are the p values obtained from Kruskal-Wallis analysis or chi-square test (for gender analysis).

Characteristics	p	controls	MCI	AD				FTD	VD
				total	*mild*	*moderate*	*severe*		
total (n)		11	33	90	38	41	11	17	35
age (years; mean)	0.0085	71.18	77.18	80.01	79.80	80.24	79.83	74.94	78.75
gender (female/male)	0.6218	7/4	22/11	54/36	25/13	21/20	8/3	10/7	25/10
MMSE (mean)	<0.0001	28.09	26.85	20.75	23.11	14.71	5.727	19.24	18.21
ptau	<0.0001	47.50	49.16	84.52	90.28	83.39	65.36	46.94	43.66
htau	<0.0001	260.2	281.7	564.6	555.2	560.2	457.2	286.1	318.8
Amyloid-β 1-40	0.0423	10851	12231	12496	14303	11789	9612	11856	9899
Amyloid-β 1-42	<0.0001	1076	755.4	502.6	551.7	479.7	445.1	794.7	663.6
Amyloid-β ratio	<0.0001	1.080	0.838	0.459	0.450	0.466	0.458	0.788	0.806
Q Albumin	0.7001	6.890	8.683	9.505	8.480	10.20	10.60	8.244	9.472

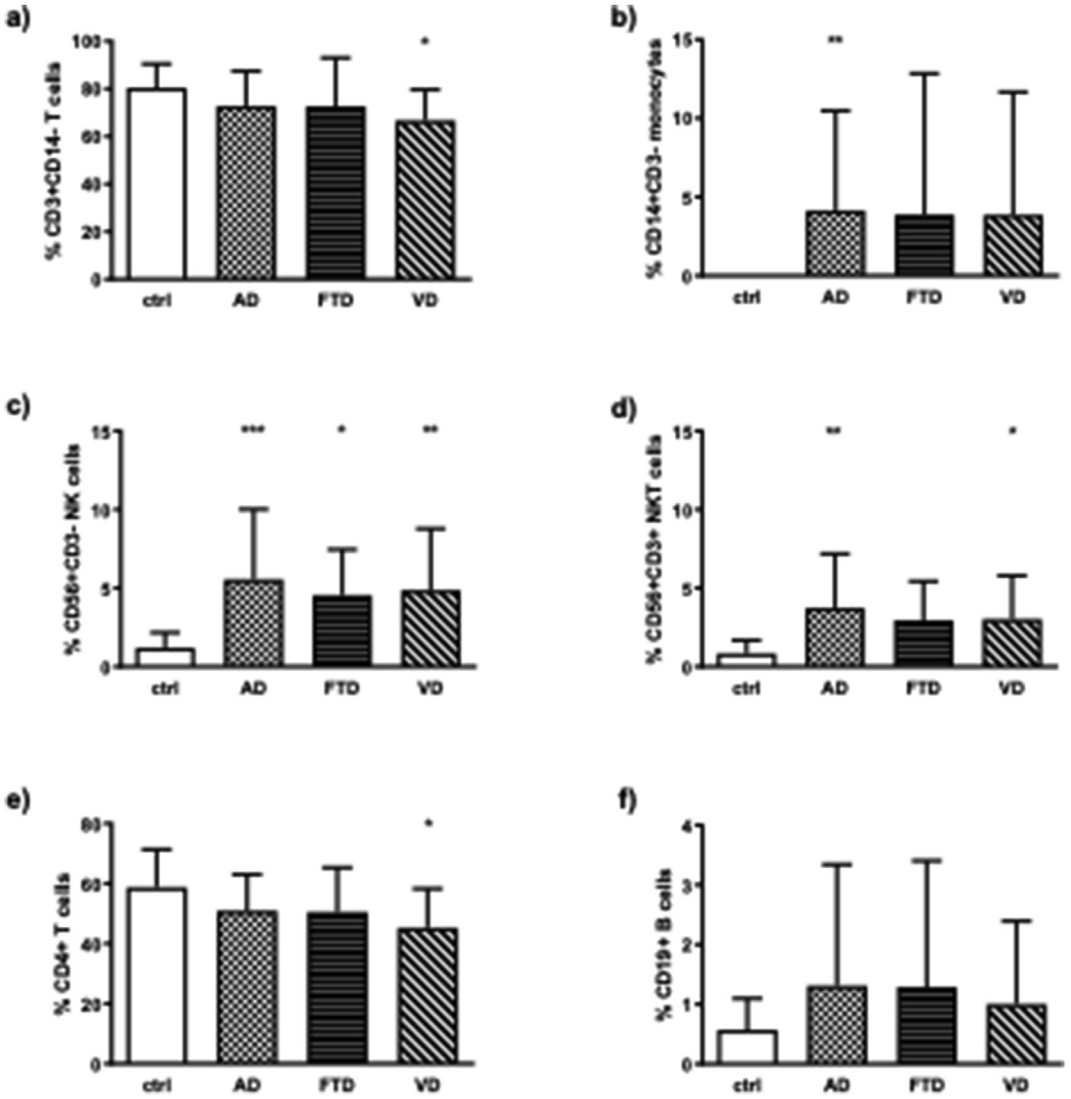

Fig. 1. Major changes in leukocyte frequencies in CSF from AD, FTD and VD patients. Shown are the main alterations in CSF leukocytes in patients with AD, FTD and VD compared to non-demented control individuals which include the percentages of CD14 + $CD3^-$monocytes (a), CD56 + $CD3^-$ NK cells (b), CD56 + $CD3^+$ NKT cells (c), $CD3^+CD14^-$ T cells (d), $CD4^+$ T cells (e) and $CD19^+$ B cells (f). Data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons test. Shown are the mean values with SD; *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to non-demented controls. No significant differences were obtained between AD, FTD and VD.

dementia, NK cells were enhanced in CSF of AD ($p = 0.0005$), FTD ($p = 0.0195$) and VD patients ($p = 0.0088$; Fig. 1b) while NKT cell levels were increased in AD ($p = 0.0016$) and VD patients ($p = 0.0289$; Fig. 1c). Furthermore, we detected a fraction of cells in CSF co-expressing CD3 and CD14, which were found in the controls as well as in demented patients (Table 2a). No differences were detected between the several

Table 2
Overview about major changes in innate and adaptive immune cell populations in dementia. Shown are the mean percentages (mean (%)) and the corresponding standard deviation (SD) values from the investigated innate and adaptive immune cell populations in AD, FTD and VD compared to non-demented controls (a) or in MCI, mild, moderate and severe AD compared to non-demented individuals (b). Data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons test. Since not significant differences were detected among the forms of dementia/MCI, the shown p values represent the difference between the form of dementia/MCI and the controls.

	controls		AD			FTD		p	VD		p
	mean (%)	SD	mean (%)	SD	p	mean (%)	SD		mean (%)	SD	
innate IS											
CD14 + CD3$^-$	0.056	0.104	4.146	6.341	0.0035	1.794	2.656	ns	3.873	7.794	0.0645
CD14 + CD3$^+$	15.30	10.11	13.46	10.58	ns	15.59	11.85	ns	18.54	12.33	ns
CD56$^+$	1.209	0.962	5.557	4.462	0.0005	4.553	2.903	0.0195	4.870	3.911	0.0088
CD56 + CD3$^+$	0.846	0.834	3.739	3.440	0.0016	2.953	2.478	ns	3.033	2.755	0.0289
HLA-DR + CD14$^+$	14.80	10.10	16.30	10.70	ns	17.45	15.95	ns	18.78	12.74	ns
adaptive IS											
CD3$^+$CD14$^-$	80.48	9.963	72.76	14.68	ns	72.74	20.30	ns	70.33	9.052	0.0443
CD4$^+$	58.75	12.59	50.98	12.03	ns	50.58	14.70	ns	45.31	12.99	0.0201
CD8$^+$	20.95	7.014	25.36	7.659	ns	23.04	9.024	ns	25.43	7.692	ns
CD19$^+$	0.573	0.526	1.310	2.031	ns	1.288	2.117	ns	1.006	1.387	ns
HLA-DR + CD3$^+$	3.480	2.488	5.606	5.920	ns	5.534	6.181	ns	14.08	8.106	ns
CD69 + CD3$^+$	22.53	14.92	27.22	13.12	ns	27.43	13.94	ns	31.22	15.29	ns

	controls		MCI			mild AD			moderate AD			severe AD		
	mean (%)	SD	mean (%)	SD	p	mean (%)	SD	p	mean (%)	SD	p	mean (%)	SD	p
innate IS														
CD14 + CD3$^-$	0.056	0.104	3.219	5.741	ns	4.373	5.932	0.0065	4.851	7.294	0.0037	1.033	2.019	ns
CD14 + CD3$^+$	15.30	10.11	11.36	9.625	ns	12.01	9.211	ns	13.50	10.48	ns	19.75	13.41	ns
CD56$^+$	1.209	0.962	4.441	3.228	0.0166	5.573	3.564	0.0005	5.756	5.478	0.0034	4.825	3.109	0.0307
CD56 + CD3$^+$	0.846	0.834	2.909	2.247	0.0403	3.495	2.779	0.0055	4.007	4.180	0.0078	3.575	2.536	0.0205
HLA-DR + CD14$^+$	14.80	10.10	12.78	9.471	ns	14.48	9.764	ns	17.04	10.92	ns	18.06	13.30	ns
adaptive IS														
CD3$^+$CD14$^-$	80.48	9.963	77.65	13.46	ns	69.64	22.35	ns	72.54	13.56	ns	71.10	17.61	ns
CD4$^+$	58.75	12.59	55.58	13.71	ns	53.87	10.10	ns	50.80	11.69	ns	41.65	15.34	0.0492
CD8$^+$	20.95	7.014	22.74	8.055	ns	24.64	6.892	ns	25.79	6.943	ns	26.25	12.24	ns
CD19$^+$	0.573	0.526	1.572	2.856	ns	1.395	1.656	ns	1.288	2.513	ns	1.125	1.179	ns
HLA-DR + CD3$^+$	3.480	2.488	4.783	6.607	ns	4.728	4.751	ns	5.906	6.812	ns	7.285	5.910	ns
CD69 + CD3$^+$	22.53	14.92	29.47	14.66	ns	24.06	12.58	ns	31.01	13.68	ns	23.97	9.809	ns

forms of dementia.

3.1. Alterations in CSF lymphocytes in AD, FTD and VD

The major cell population present in CSF is CD3$^+$ T cells, and despite a trend to be decreased in all forms of dementia, significance was only obtained in VD (p = 0.0443; Fig. 1d). We measured two T cell activation markers, CD69 and HLA-DR, none showed differences between the groups (Table 2a).

T lymphocytes can be further subdivided into CD4$^+$ and CD8$^+$ T cells. While the frequency of CD8$^+$ T cells was unchanged between the groups (Table 2a), the frequency of CD4$^+$ T cells were diminished in VD (p = 0.0201; Fig. 1e). The number of B lymphocytes was very low and unchanged between the patients' groups and controls (Fig. 1f). No differences were detected between the several forms of dementia.

3.2. Alterations in CSF leukocytes in AD stages

According to the degree of cognitive decline, AD can be divided into a mild, moderate and severe form. Although MCI patients have only mild cognitive deficits, many of them develop dementia within the following years and therefore MCI might be seen as pre-stage dementia. Comparing the different stages of AD might give an impression of how the immune system reacts towards the disorder-specific alterations in the brain during the progression of AD. Compared to non-demented controls, the frequency of CD14$^+$ monocytes was increased in mild (p = 0.0065) and moderate AD (p = 0.0037; Fig. 2a) with a sharp decline in severe AD. Altered numbers of NK cells and NKT cells were detected in MCI and all AD stages. An enhanced frequency of NK cells was present in MCI (p = 0.0166), mild (p = 0.0005), moderate (p = 0.0034) and severe AD (p = 0.0307; Fig. 2b), a higher frequency of NKT cells as compared to non-demented controls were found in MCI (p = 0.0403), mild (p = 0.0055), moderate (p = 0.0078) and severe AD (p = 0.0205; Fig. 2c).

The frequency of CD3$^+$ T cells was not changed in MCI or AD stages compared to non-demented controls (Fig. 2d). The number of CD4$^+$ T cells constantly declined with disease progression and reached significance in severe AD (p = 0.0492; Fig. 2e). MCI or AD stages did not lead to changes in the frequency of CD8$^+$ T cells (Table 2b) or CD19$^+$ B lymphocytes Fig. 2f) compared to the control group.

3.3. Dementia-associated changes in naive and memory T cells

CD45 can be expressed in several isoforms, allowing the discrimination between naive and memory cells: CD45RA is expressed on naive T cells and T effector. Central and effector memory T cells express CD45RO. The chemokine receptor CCR7 is found on naive and central memory cells and absent on effector memory and effector cells.

In non-demented controls, only few T cells were naïve (CD45RA + CD45RO-; Fig. 3a and e), the huge majority of T cells were memory cells (CD45RA-CD45RO+), CD4$^+$ T cells (mean 94.56; Table 3a) as well as CD8$^+$ T cells (mean 83.35). In contrast to that, these memory T cells were severely reduced in dementia. CD45RA-CD45RO + memory CD4$^+$ T cells were decreased in AD (p = 0.0016), FTD (p = 0.0508) and VD (p = 0.0068; Fig. 3b). The most frequent memory T cell population in non-demented controls were central memory cells, and these cells were reduced in demented patients. The population of CCR7+CD45RO + central memory CD4$^+$ T cells declined in AD (p = 0.0003), FTD (p = 0.0036) and VD (p = 0.0323; Fig. 3c). That was accompanied by the enhanced frequency of late-stage effector cells (CCR7-CD45RA + CD4$^+$) in AD (p = 0.0268; Fig. 3d) and effector memory CD4$^+$ T cells in AD (p = 0.0061) and FTD (p = 0.0008; Table 3a).

An enhanced frequency of CD45RA + CD45RO- CD8$^+$ naive T cells

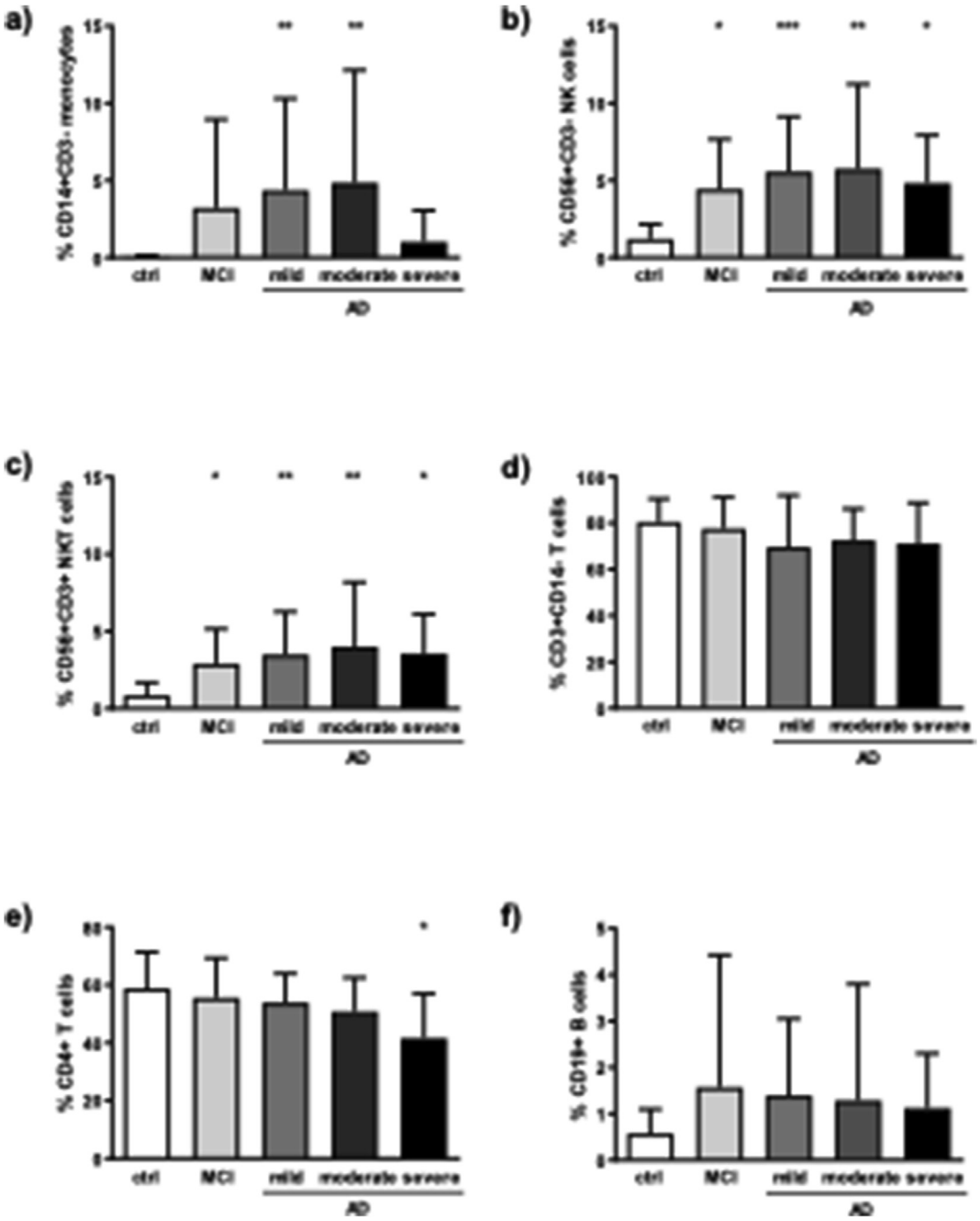

Fig. 2. Major changes in leukocyte frequencies in CSF in MCI and stages of AD. Shown are the main alterations in CSF leukocytes of patients with MCI, mild, moderate and severe AD compared to non-demented control individuals. Presented are the frequencies of CD14 + $CD3^-$monocytes (a), CD56 + $CD3^-$ NK cells (b), CD56 + $CD3^+$ NKT cells (c), $CD3^+CD14^-$ T cells (d), $CD4^+$ T cells (e) and $CD19^+$ B cells (f). Data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons test. Shown are the mean values with SD; *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to non-demented controls. No significant differences were obtained between MCI, mild, moderate and severe AD.

was found in CSF in AD ($p = 0.0047$) and VD patients ($p = 0.0382$; Fig. 3e and Table 3a). CD45RA-CD45RO + memory $CD8^+$ T cells were diminished in AD ($p = 0.0003$) and VD ($p = 0.0017$; Fig. 3f). The CCR7+CD45RO + $CD8^+$ central memory $CD8^+$ T cells were reduced in AD ($p = 0.0001$), FTD ($p = 0.0008$) and VD ($p = 0.0009$; Fig. 3g). Within the $CD8^+$ T lymphocytes, the central memory cells population shifted towards late-stage effector (CCR7-CD45RA + $CD8^+$) in AD ($p = 0.0008$), FTD ($p = 0.0507$) and VD ($p = 0.0151$; Fig. 3h), and effector memory cells in AD ($p = 0.0059$), FTD ($p = 0.0011$) and VD ($p = 0.0323$; Table 3a).

3.4. Dementia-associated changes in naive and memory T cells in stages of AD

Due to the alterations in CSF cell distribution in AD, we addressed the question whether these changes also exist in MCI and how the leukocyte frequency changes during the progression of AD.

While the population of naïve CD45RA + CD45RO- $CD4^+$ T cells was not significantly altered in MCI or the different stages of AD (Fig. 4a), the reduction in CD45RA-CD45RO + central memory $CD4^+$ T cells was detected in MCI ($p = 0.0016$), mild ($p = 0.0003$), moderate ($p = 0.0012$)

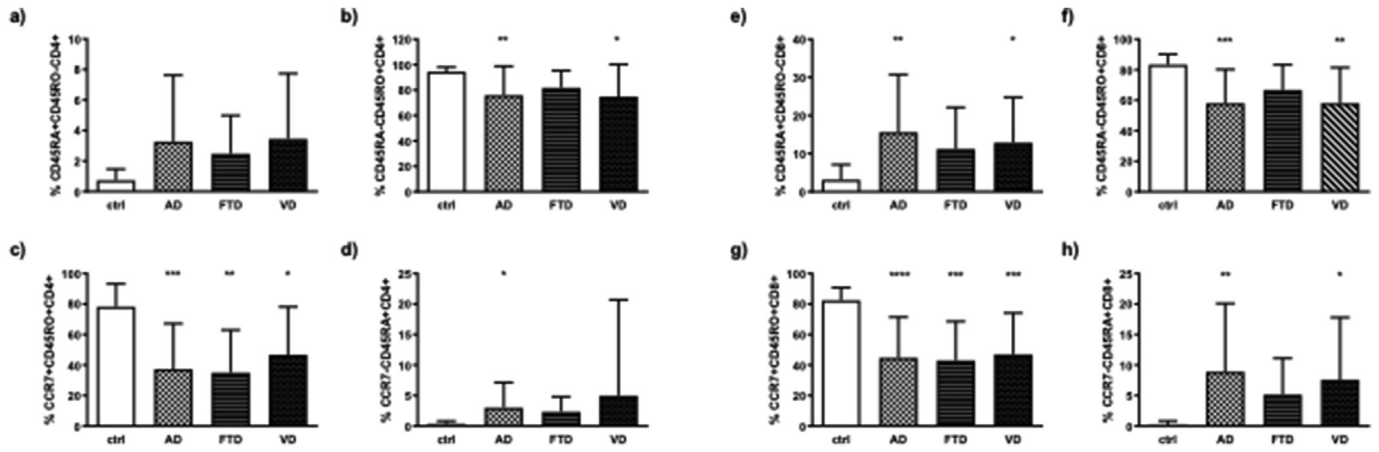

Fig. 3. Alterations in CD4 and CD8 T cell populations in CSF from AD, FTD and VD patients. The main changes in CD4 (a–d) and CD8 (e–h) cell populations in CSF from patients with AD, FTD and VD compared to non-demented control individuals were determined by flow cytometry. Shown are the frequencies of naïve (a), memory (b), central memory (c) and late-stage effector (d) $CD4^+$ T cells and naïve (e), memory (f), central memory (g) and late-stage effector (h) $CD8^+$ T cells. Data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons test. Shown are the mean values with SD; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$ compared to non-demented controls. No significant differences were obtained between AD, FTD and VD.

Table 3
Overview about alterations in CD4 and CD8 T cell populations in dementia. Shown are the mean percentages (mean (%)) and the corresponding standard deviation (SD) values from the analyzed CD4 and CD8 T cell populations according to their expression of CD45RA and CD45RO (a) or based on CD45RA, CD45RO and CCR7 expression in AD, FTD and VD compared to non-demented controls. (b). The presented data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons test. Since not significant differences were detected between the forms of dementia, the shown p values represent the difference between the form of dementia and the controls.

Marker	controls			AD			FVD			VD		
	T cell subpopulation	mean (%)	SD	mean (%)	SD	p	mean (%)	SD	p	mean (%)	SD	p
CD45RA-CD45RO-$CD4^+$	double negative $CD4^+$ T cells	4.136	2.993	18.91	20.18	0.0151	12.25	12.88	ns	17.46	23.47	ns
CD45RA + CD45RO-$CD4^+$	naive $CD4^+$ T cells	0.736	0.737	3.268	4.363	ns	2.476	2.511	ns	3.451	4.283	ns
CD45RA-CD45RO + $CD4^+$	memory $CD4^+$ T cells	94.58	3.490	75.91	22.83	0.0016	81.88	13.47	0.0508	74.88	25.39	0.0068
CD45RA + CD45RO + $CD4^+$	early activated $CD4^+$ T cells	0.536	0.762	1.918	2.046	ns	2.218	2.189	ns	4.223	9.777	ns
CD45RA-CD45RO-$CD8^+$	double negative $CD8^+$ T cells	11.61	4.548	20.89	15.51	ns	16.74	9.150	ns	21.87	17.78	ns
CD45RA + CD45RO-$CD8^+$	naive $CD8^+$ T cells	3.145	3.972	15.62	15.13	0.0047	11.22	10.90	ns	12.87	11.91	0.0382
CD45RA-CD45RO + $CD8^+$	memory $CD8^+$ T cells	83.35	6.892	58.04	22.20	0.0003	66.76	16.52	ns	58.06	23.38	0.0017
CD45RA + CD45RO + $CD8^+$	early activated $CD8^+$ T cells	1.909	1.872	5.446	5.200	ns	5.276	4.638	ns	7.194	10.67	ns
CCR7+CD45RA + $CD4^+$	naive $CD4^+$ T cells	0.855	0.844	1.803	1.782	ns	2.382	2.021	ns	5.077	10.47	0.0688
CCR7+CD45RO + $CD4^+$	central memory $CD4^+$ T cells	78.27	15.01	37.29	29.92	0.0003	35.05	27.87	0.0036	46.70	31.50	0.0323
CCR7-CD45RA + $CD4^+$	late-stage effector $CD4^+$ T cells	0.327	0.478	3.003	4.122	0.0268	2.341	2.454	ns	4.997	15.71	ns
CCR7-CD45RO + $CD4^+$	effector memory $CD4^+$ T cells	16.94	13.88	41.23	24.34	0.0061	51.36	23.66	0.0008	32.64	22.00	ns
CCR7+CD45RA + $CD8^+$	naive $CD8^+$ T cells	4782	5.144	11.73	9.820	ns	10.87	11.41	ns	12.40	14.65	ns
CCR7+CD45RO + $CD8^+$	central memory $CD8^+$ T cells	82.43	8.399	44.78	26.70	<0.0001	43.01	25.61	0.0008	47.06	27.07	0.0009
CCR7-CD45RA + $CD8^+$	late-stage effector $CD8^+$ T cells	0.246	0.568	8.903	11.19	0.0008	5.159	5.953	0.0507	7.577	10.23	0.0151
CCR7-CD45RO + $CD8^+$	effector memory $CD8^+$ T cells	2736	3220	18.57	16.04	0.0059	29.93	25.22	0.0011	18.19	18.15	0.0323

and severe AD ($p = 0.0114$; Fig. 4b and Table 4a). The population of CCR7+CD45RO + central memory $CD4^+$ T cells decreased in MCI ($p = 0.0058$), mild ($p < 0.0001$), moderate ($p = 0.0004$) and severe AD ($p = 0.0255$; Fig. 4c and Table 4b) compared to the control group. However, in frequency of CCR7-CD45RA + -stage effector cells $CD4^+$ T cells was higher MCI ($p = 0.0122$), mild ($p = 0.0011$) and moderate AD ($p = 0.0127$; Fig. 4d and Table 4b).

Compared to non-demented control persons, the population of naïve CD45RA + CD45RO- $CD8^+$ T cells was enhanced in MCI ($p = 0.0040$), mild ($p = 0.0005$) and moderate AD ($p = 0.0037$; Fig. 4e), while the $CD8^+$ memory cells (CD45RA-CD45RO+) declined in MCI ($p = 0.0012$), mild ($p < 0.0001$), moderate ($p = 0.0005$) and severe AD ($p = 0.0031$; Fig. 4f and Table 4a). Within the $CD8^+$ memory cells in CSF, we detected a decrease in central memory cells in MCI ($p = 0.0078$), mild ($p < 0.0001$), moderate ($p < 0.0001$) and severe AD ($p = 0.0039$; Fig. 4g and Table 4b), combined with enhanced CCR7-CD45RA + $CD8^+$ late-stage

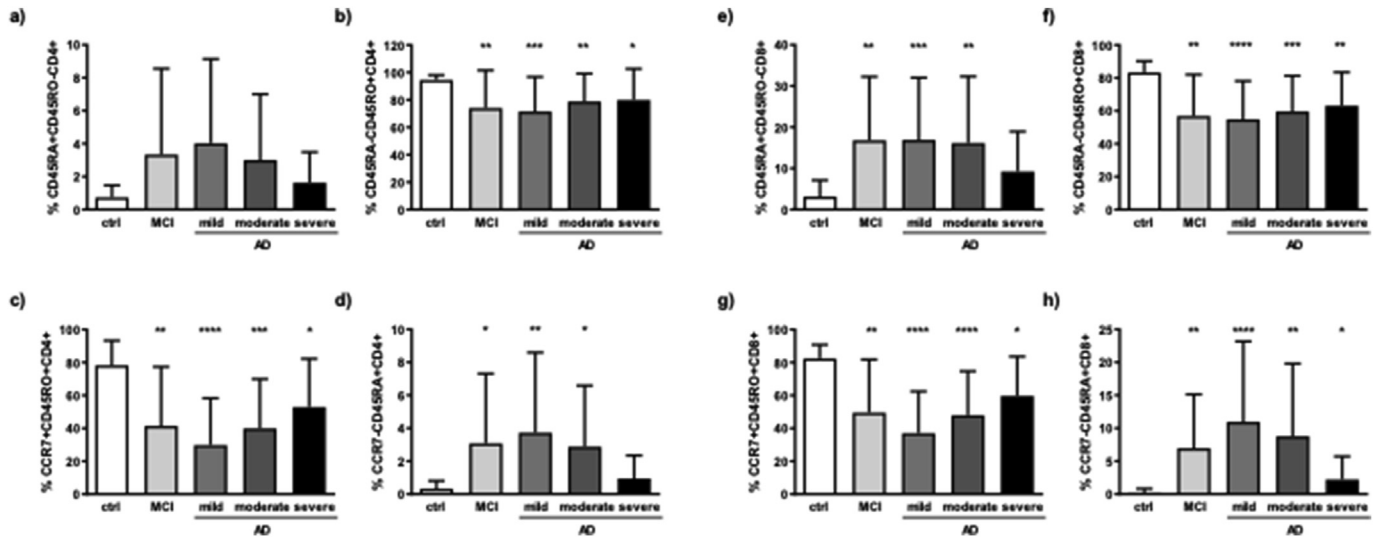

Fig. 4. Alterations in CD4 and CD8 T cell populations in CSF from AD, FTD and VD patients. The most important alterations in CD4 and CD8 CSF cell populations from patients with MCI, mild, moderate and severe AD compared to non-demented controls include the percentages of naïve (a), memory (b), central memory (c) and late-stage effector (d) $CD4^+$ T cells and naïve (e), memory (f), central memory (g) and late-stage effector (h) $CD8^+$ T cells. Data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons test. Shown are the mean values with SD; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$ compared to non-demented controls. No significant differences were obtained between MCI, mild, moderate and severe AD.

effector cells in MCI ($p = 0.0051$), mild ($p < 0.0001$), moderate ($p = 0.0014$) and severe AD ($p = 0.0326$; Fig. 4h) as well as effector memory $CD8^+$ T cells (Table 4b).

3.5. Correlation analyses

CSF markers obtained by standard laboratory analysis (ptau, Amyloid-β1-40, Amyloid-β ratio and Q Albumin) and the cognitive state of the patient, obtained by MMSE, were correlated with the CSF cell populations. CD56 + $CD3^+$ NKT cells ($p = 0.0217$) were negatively correlated with the MMSE, indicating an enhanced frequency of these cells with decreasing MMSE values (Table 5), while $CD3^+$ T cells were positively correlated with MMSE ($p = 0.0322$). $CD56^+$ NK cells and NKT cells were positively associated with ptau (NK cells: $p = 0.0058$; NKT cells: $p = 0.0300$), a marker increased in AD at all stages with the highest concentration at mild AD (Table 1) (see Table 6).

$CD14^+$ monocytes ($p < 0.0001$) and $CD56^+$ NK cells ($p = 0.0128$) were positively correlated with Amyloid-β1-40. Moreover, $CD14^+$ monocytes ($p < 0.0001$), $CD56^+$ NK cells ($p = 0.0004$) and CD56 + $CD3^+$ NKT cells ($p = 0.0074$) were negatively correlated with the Amyloid-β ratio ($p < 0.0001$). No cell population was correlated with Q Albumin.

4. Discussion

In our recent study, we have shown that neurodegenerative disorders are associated with alterations in the cellular contribution of immune cells within the CSF.

In the cohort of elderly patients without neurodegenerative disease we found a CSF cell composition that was described by others in younger volunteers: While the frequency of B lymphocytes, NK cells and NKT cells was rather low, $CD3^+$ T lymphocytes were the vast majority of CSF lymphocytes and most of them $CD4^+$ T cells (Kivisakket al, 2003). Most T cells exhibited a central memory cell phenotype, both within the $CD4^+$ and $CD8^+$ subpopulations. It was proposed that these CD45RO + T cells patrol the brain boundaries for pathogens (Engelhardt and Ransohoff, 2005) while retaining their ability to return to the lymph nodes, as suggested by the expression of CCR7 (Kivisakket al, 2004). Others found that HLA-DR was not upregulated on CSF T cells (Svenningssonet al, 1993; Svenningsson et al., 1995). It is hypothesized that activated memory T cells enter CSF directly from the systemic circulation and monitor the subarachnoid space. They retain the capacity to either initiate local immune reactions or return to secondary lymphoid organs. It was shown that T cells circulate through the CSF for about 6 h before returning to the circulation (Engelhardt and Ransohoff, 2005), which is compared to peripheral lymphocyte recirculation a low trafficking rate (Young, 1999). T cells have been proposed to exhibit a neuroprotective effect, by the production of neurotrophins (Moalemet al, 1999; Kerschensteineret al, 1999), the modulation of glutamate release by astrocytes and microglia (Garg et al., 2008) and by regulating innate immunity at the site of injury (Shechteret al, 2009).

In AD, FTD and VD the frequency of $CD4^+$ and $CD8^+$ central memory cells was severely reduced, instead an increase of CCR7-negative (late-stage) effector T cells was detected. These cells lost their lymph node homing capacity and could therefore not communicate efficiently in the periphery. On the other hand, the otherwise small fraction of naive $CD4^+$ T cells increased in FTD and VD, and naive $CD8^+$ T cells in AD and VD. The infiltration of naive T cells into the brain might be caused by neuroinflammation (Henekaet al, 2015) and/or a decreased function of the blood–CSF–barrier (Marques et al., 2013). Both indicators are discussed to have an important role in AD pathology (Henekaet al, 2015). Interestingly, these changes in cell populations in CSF were already detected in MCI patients. This might be associated with the observation that MCI could be seen as the preclinical stage of AD and elevated CNS inflammation. Besides, lower Amyloid-β was detected in MCI patients (Melahet al, 2016; Monsonet al, 2014).

No correlation was obtained between Q Albumin and any CSF cell population. This might be explainable with the fact that we detected also no significance in Q Albumin values between non-demented controls and demented patients. However, AD patients (except mild AD) and VD patients had an increased Q Albumin <9.1, which indicated a disturbed blood–CSF–barrier function (Marques et al., 2013; Busseet al, 2018).

The frequency of B lymphocytes in CSF was not altered in demented patients in our study. In peripheral blood, it was detected that their frequency and their capacity to produce antibodies declines with age (Frasca and Blomberg, 2009). In plasma of AD patients, anti-Aβ antibodies were detected as well as an enhanced number of plasma cells producing antibodies toward Aβ42 protofibrils (Sollvanderet al, 2015; Agrawal et al., 2018). Moreover, B cells might contribute to cognitive impairment following stroke (Doyleet al, 2015) and could be therefore important for VD. However, since their frequency in CSF was low and

Table 4

Overview about alterations in CD4 and CD8 T cell populations in MCI and AD stages. The CD4 and CD8 T cell populations were analyzed based on their expression of CD45RA and CD45RO (a) or based on their CD45RA, CD45RO and CCR7 expression (b) in CSF obtained from patients with MCI, mild, moderate and severe AD, compared to non-demented controls. Shown are the mean percentages (mean (%)) and the corresponding standard deviation (SD) values. The data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons test. No significant differences were detected between MCI and the separated stages of AD. Thereby, the shown p values represent the difference MCI/the stage of AD and the control group.

marker	T cell subpopulation	controls		MCI			mild AD			moderate AD			severe AD		
		mean (%)	SD	mean (%)	SD	p	mean (%)	SD	p	mean (%)	SD	p	mean (%)	SD	p
CD45RA-CD45RO-$CD4^+$	double negative $CD4^+$ T cells	4.136	2.993	21.15	25.52	0.0109	22.55	22.16	0.0032	16.22	17.69	0.0059	16.37	21.62	0.0385
CD45RA + CD45RO-$CD4^+$	naive $CD4^+$ T cells	0.736	0.737	3.335	5.218	ns	4.024	5.112	ns	3.007	3.996	ns	1.627	1.862	ns
CD45RA-CD45RO + $CD4^+$	memory $CD4^+$ T cells	94.58	3.490	74.03	27.47	0.0016	71.55	25.20	0.0003	78.86	20.33	0.0012	79.97	22.63	0.0114
CD45RA + CD45RO + $CD4^+$	early activated $CD4^+$ T cells	0.536	0.762	1.481	1.785	ns	1.863	1.895	ns	1.944	2.291	ns	2.009	1.724	ns
CD45RA-CD45RO-$CD8^+$	double negative $CD8^+$ T cells	11.61	4.548	20.56	20.64	ns	22.53	16.02	ns	19.05	14.51	ns	22.12	17.95	ns
CD45RA + CD45RO-$CD8^+$	naive $CD8^+$ T cells	3.145	3.972	16.81	15.42	0.0040	16.91	15.01	0.0005	16.14	16.20	0.0037	9.264	9.696	ns
CD45RA-CD45RO + $CD8^+$	memory $CD8^+$ T cells	83.35	6.892	56.85	25.16	0.0012	54.84	23.31	<0.0001	59.63	21.69	0.0005	63.15	20.34	0.0031
CD45RA + CD45RO + $CD8^+$	early activated $CD8^+$ T cells	1.909	1.872	6.065	6.988	ns	5.734	5.086	ns	5.173	5.305	ns	5.464	5.631	ns
CCR7+CD45RA + $CD4^+$	naive $CD4^+$ T cells	0.855	0.844	15.72	17.68	ns	12.01	9.707	ns	12.04	10.10	ns	9.618	9.818	ns
CCR7+CD45RO + $CD4^+$	central memory $CD4^+$ T cells	78.27	15.01	41.47	35.87	0.0058	29.82	28.48	<0.0001	40.00	29.99	0.0004	52.99	29.26	0.0255
CCR7-CD45RA + $CD4^+$	late-stage effector $CD4^+$ T cells	0.327	0.478	3.077	4.238	0.0122	3.732	4.866	0.0011	2.880	3.705	0.0127	0.946	1.400	ns
CCR7-CD45RO + $CD4^+$	Effector memory $CD4^+$ T cells	16.94	13.88	41.23	24.34	0.0256	51.36	23.66	0.0011	41.03	41.29	0.0015	32.64	22.00	ns
CCR7+CD45RA + $CD8^+$	naive $CD8^+$ T cells	4782	5.144	15.72	17.68	ns	12.01	9.707	ns	12.04	10.10	ns	9.618	9.818	ns
CCR7+CD45RO + $CD8^+$	central memory $CD8^+$ T cells	82.43	8.399	49.55	32.21	0.0078	37.00	25.43	<0.0001	47.99	26.76	<0.0001	59.71	23.89	0.0039
CCR7-CD45RA + $CD8^+$	late-stage effector $CD8^+$ T cells	0.246	0.568	3.077	4.238	0.0051	3.732	4.866	<0.0001	2.880	3.705	0.0014	0.946	1.400	0.0326
CCR7-CD45RO + $CD8^+$	effector memory $CD8^+$ T cells	2736	3220	34.71	25.88	ns	44.45	25.03	<0.0001	41.76	24.72	0.0047	28.15	16.78	ns

Table 5
Correlation between cognitive parameter and indicated immune cell populations. Correlation analysis was performed between MMSE, ptau, htau, Q Albumin, Amyloid-β1-40, Amyloid-β1-42 and the Amyloid-β ratio and the frequency of CD14 + $CD3^-$monocytes, $CD3^+CD14^-$ T cells, CD56 + $CD3^+$ NKT cells and CD56 + $CD3^-$ NK cells. Shown are the Spearman r value, the p value (two-tailed) and the p value summary. $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$.

MMSE	Parameter	CD14 + $CD3^-$	$CD3^+CD14^-$	CD56 + $CD3^+$	$CD56^+$
	Spearman r	−0.09711	0.1616	−0.173	−0.1353
	P value (two-tailed)	0.1998	0.0322	0.0217	0.0734
	P value summary	ns	*	*	ns
Ptau	Parameter	CD14 + $CD3^-$	$CD3^+CD14^-$	CD56 + $CD3^+$	$CD56^+$
	Spearman r	0.06088	0.03382	0.1637	0.2071
	P value (two-tailed)	0.4221	0.6558	0.0300	0.0058
	P value summary	ns	ns	*	**
Htau	Parameter	CD14 + $CD3^-$	$CD3^+CD14^-$	CD56 + $CD3^+$	$CD56^+$
	Spearman r	0.1014	−0.02426	0.08755	0.1306
	P value (two-tailed)	0.1807	0.7492	0.2479	0.084
	P value summary	ns	ns	ns	ns
Q Albumin	Parameter	CD14 + $CD3^-$	$CD3^+CD14^-$	CD56 + $CD3^+$	$CD56^+$
	Spearman r	0.02002	−0.1514	−0.0239	−0.02668
	P value (two-tailed)	0.7974	0.0508	0.7592	0.7322
	P value summary	ns	ns	ns	ns
Amyloid-β 1-40	Parameter	CD14 + $CD3^-$	$CD3^+CD14^-$	CD56 + $CD3^+$	$CD56^+$
	Spearman r	0.3486	−0.1456	0.1223	0.1878
	P value (two-tailed)	<0.0001	0.0545	0.1068	0.0128
	P value summary	****	ns	ns	*
Amyloid-β 1-42	Parameter	CD14 + $CD3^-$	$CD3^+CD14^-$	CD56 + $CD3^+$	$CD56^+$
	Spearman r	−0.09623	−0.04183	−0.08646	−0.09193
	P value (two-tailed)	0.2039	0.5815	0.2539	0.225
	P value summary	ns	ns	ns	ns
Amyloid-β ratio	Parameter	CD14 + $CD3^-$	$CD3^+CD14^-$	CD56 + $CD3^+$	$CD56^+$
	Spearman r	−0.4292	0.1073	−0.2006	−0.2625
	P value (two-tailed)	<0.0001	0.1550	0.0074	0.0004
	P value summary	****	ns	**	***

Table 6
Schematic presentation of alterations in CSF immune cells in dementia. Shown are changes in monocytes, NK(T) cells and subsets of CD4 and CD8 T cells in AD, FTD, and VD compared to non-demented elderly individuals. ↑ = increased, ↓ = decreased, ↔ not changed.

Cell subpopulation		AD	FTD	VD
$CD14^+$ monocytes		↑	↔	↔
$CD56^+$ NK cells		↑	↑	↑
CD56 + $CD3^+$ NKT cells		↑	↔	↑
$CD4^+$ T cells	Naive	↔	↔	↔
	central memory	↓	↓	↓
	late-stage effector	↑	↔	↔
	effector memory	↑	↑	↔
$CD8^+$ T cells	Naive	↔	↔	↔
	central memory	↓	↓	↓
	late-stage effector	↑	↑	↑
	effector memory	↑	↑	↑

unaltered between demented and non-demented controls, these observations raise the question about the relevance of Aβ-specific B cells for brain pathology. Since anti-Aβ antibodies were also detected in healthy individuals (Maetzleret al, 2011; Mafteiet al, 2012) their function remains to be further investigated in the same way as their level compared to healthy elderly people and patients with other neurodegenerative disorders (Mafteiet al, 2013; Quet al, 2014).

$CD14^+$ monocytes were increased in CSF of AD patients, in particular in mild and moderate AD. These cells were positively correlated with Amyloid-β1-40 and negatively correlated with the Amyloid-β ratio. Several authors provided data showing that activated inflammatory cells, in particular brain-resident microglia and infiltrated blood-borne monocyte-derived macrophages, are important for the physiological clearance of Aβ (Koronyo-Hamaouiet al, 2009; El Khouryet al, 2007; Simard et al., 2006). CD14-positive microglia might act as a receptor for phagocyting Amyloid-β in AD (Liuet al, 2005). Following phagocytosis, monocytes/macrophages might present Amyloid-β peptides to specific T cells. This results in the activation and differentiation of T cells. In addition, it was shown that ptau was correlated with microglial markers which indicated activation corresponding the number of T cells (Zotovaet al, 2013). Microglia were shown to accumulate in Amyloid-β plaques in brains of AD patients and release pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and CRP (Akiyamaet al, 2000; Swardfageret al, 2010; Yasojima et al., 2000). These immune responses in the brain may have beneficial or detrimental roles (Rezai-Zadeh et al., 2009). The recruitment of peripheral $CD14^+$ monocytes/macrophages could contrast the formation and extension of Aβ plaques (Town et al., 2005a; Hawkes and McLaurin, 2009; Townsendet al, 2005; Town et al., 2005b; Tanet al, 1999; Town et al., 2001). Investigations upon the integrity of the BBB/B-CSF-B in AD showed inconsistent results, with either an elevated CSF/serum albumin ratio in AD patients or no differences compared to controls. Nevertheless, it is known that cytokines such as IL-6, IL-1 or TNF-α influence the entry of immune cells in the brain (Elovaara et al., 1985; Wada, 1998; Mecocciet al, 1991). Moreover, increased $CD3^+$ T cell numbers were detected in post mortem AD brain. These T cells accumulated in the hippocampus, the area with the most prominent neuronal damage (Togoet al, 2002). Nevertheless, it remains to be clarified to what extent antigen-loaded monocytes get into the periphery via CSF and activate T cells there.

In our study, NK and NKT cells were increased in CSF in AD (all stages including MCI), VD and FTD. NK cells express TLR2 and TLR4 which recognize Amyloid-β (Liuet al, 2012; Udan et al., 2008). In peripheral blood, enhanced activated NK cells were described in MCI, but unaltered in AD, but also enhanced or decreased NK cell activity were shown (Araga et al., 1991; Solerte et al., 2000; Le Pageet al, 2015). However, there is a lack of studies investigating NK and NKT cells in CSF. Solana et al. proposed a pathway, that NK cells might be recruited into the brain by microglia, which phagocytosed Amyloid-β and secreted proinflammatory cytokines. Consequently, NK cells become activated and secrete IFN-γ and TNF-α. The inflammatory milieu might result in neuronal damage and death and thereby neurodegeneration (Solana et al., 2018). It needs to be investigated whether the enhanced NK(T) cell numbers in CSF in our study are associated with an altered function.

We determined a negative correlation between NK(T) cells in CSF and MMSE and a positive correlation with ptau. Solerte at al., found that peripheral NK cell activity in AD patients was inversely correlated with

the MMSE score (Solerteet al, 1998). In our study cohort, the level of ptau was increased in AD, but not in MCI, with decreasing values from mild to severe AD. This might be due to the fact that ptau is released after neuronal destruction in AD. The number of neurons that could release ptau is constantly decreasing. NKT cells might be involved in ptau-induced immunity.

Taken together, we found alterations in innate and adaptive immune cell populations, which were dependent on the type of dementia. Further analysis will gain further insight into the functional consequences of these changes.

5. Conclusions

Dementia is associated with specific changes in the immune system which could be detected in CSF. Our study determined alterations of innate and adaptive immune cells in CSF in AD, FTD and VD.

$CD14^+$ monocytes in CSF were increased in mild and moderate AD. Moreover, NK and NKT cells were enhanced in AD, FTD and VD. Whereas the number of $CD19^+$ B cells and total $CD3^+$ T cells was unchanged in CSF, the T cell subpopulations were affected in demented patients. Central memory cells were reduced in all forms of dementia, while effector memory cells were enhanced in patients with AD and FTD compared to elderly controls.

Our finding might help to improve our understanding of the dementia-associated immune responses within the brain.

Declaration of competing interest

All authors declare that they have no conflict of interest.

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