Differential Expression of Apoptotic and Low-Grade Inflammatory Markers in Alzheimer Disease Compared to Diabetes Mellitus Type 1 and 2

Krystallenia I. Alexandraki,[1,2*] Nikolaos V. Apostolopoulos,[3] Christos Adamopoulos,[1] Evangelia Stamouli,[1] Georgia Dalagiorgou,[1] Theodoros G. Papaioannou,[4] Antonios Analitis,[5] Marianna Karamanou,[6] Konstantinos Makrilakis,[7] Antonios Politis,[8] and Christina Piperi[1]

Background: Neuroinflammation, impaired brain insulin signaling, and neuronal apoptosis may be interrelated in the pathophysiology of people with Alzheimer disease (AD) and diabetes, either type 1 or 2 diabetes (T1D or T2D, respectively).

Methods: We studied 116 patients: 41 with AD, 20 with T1D, 21 with T2D, and 34 healthy controls. The number (n) of cytokine-secreting peripheral blood mononuclear cells (PBMCs) before and after mitogenic stimulation was determined for interleukin 1β (IL1β), interleukin 6 (IL6), tumor necrosis factor (TNF) by the enzyme-linked-immunospot assay. Serum concentrations of C-reactive protein (CRP) and Fas ligand (FASLG) were determined by ELISA.

Results: The studied subgroups did not differ in sex but differed in age. Higher CRP concentrations were detected in the AD group than in the T1D group ($P = 0.02$) and lower in controls ($P < 0.001$). The nPBMCs was higher in AD patients after stimulation than in basal conditions: after stimulation in nTNF ($P < 0.001$ vs T2D; $P < 0.001$ vs T1D; $P = 0.001$ vs control), nIL6 ($P = 0.039$ vs T2D; $P < 0.001$ vs T1D; $P = 0.007$ vs control), and nIL1β ($P = 0.03$ vs control). The nPBMCs increased after stimulation with PMA in all the subgroups ($P < 0.001$). FASLG in the AD group displayed statistically higher concentrations than in all other subgroups ($P < 0.001$ vs T2D; $P < 0.001$ vs T1D; $P = 0.012$ vs control). The nPBMCs was positively correlated with plasma concentrations of FASLG in the AD subgroup.

Conclusions: Patients with AD display a low-grade systemic inflammation compared to people with diabetes. The FAS–FASLG pathway has a potential role because FASLG concentrations are positively correlated with the inflammatory response in AD. However, this positive correlation cannot be seen in people with diabetes, at least not with the apoptotic markers used in the present study.

IMPACT STATEMENT

Currently, there is intensive research around the relationship of Alzheimer disease and diabetes. Alzheimer disease has been characterized as insulin resistance in the brain, but the role of inflammation and apoptosis in this relationship is not clear. Alzheimer disease is characterized by inflammation that may normally react to increased demands, as opposed to the inflammation seen in diabetes that presents a defective response with increased demands. Apoptosis, as reflected by Fas ligand and tumor necrosis factor, seems to participate in the inflammatory process of Alzheimer disease but not of diabetes. The clarification of the molecular pathways involved in these chronic diseases may unravel novel druggable targets.

Alzheimer disease (AD)[9] is a neurodegenerative disorder characterized by formation and deposition of β amyloid, resulting in neuronal death mostly in vulnerable regions such as the neocortex and hippocampus (*1*). The neuroinflammation accompanying this neurodegenerative process is becoming more prominent both as a primary and/or secondary mediator. Moreover, the limited success of AD treatment indicates the implication of unknown pathogenic mechanisms. Recently, several studies have shed light on the pathogenic link of AD and either type 1 (T1D) or type 2 (T2D) diabetes, because both types are characterized by increased risk of cognitive dysfunction (*2*–*4*). The role of hyperglycemia or altered insulin signaling is under investigation because impaired brain insulin signaling leads to brain insulin resistance (*5*). Nevertheless, therapeutic trials with either insulin sensitizers (inositol) or glucagon-like peptide-1 receptor agonists have not been effective as yet (*6*, *7*). Another emerging molecular mechanism implicated in the pathophysiology of AD involves the activation of Fas receptor and the cognate Fas ligand (FASLG) signaling pathway that triggers apoptosis (*1*, *8*, *9*) but without consistent high production of FASLG in the AD brain (*10*).

Inversely, the presence of low-grade systemic inflammation in people with long-standing diabetes, either T1D or T2D, independent of glycemic control and obesity has been documented. A higher degree of inflammation was observed in people with T2D than in those with T1D at basal conditions, associated with impaired elevation of cytokine-secreting peripheral blood mononuclear cells (PBMCs) number after mitogenic stimulation, implying an immune defect with high demands (*11*). Because the mediator of the aforementioned common pathway has not been clarified and the FAS–FASLG signaling pathway has already been involved in the regulation of insulin secretion (*12*, *13*), it can be speculated as a common pathogenic molecular event of both T1D and T2D (*14*). Several studies have also demonstrated that FAS–FASLG signaling might protect organs from excessive inflammation (*15*, *16*).

The aim of the present study was to investigate the links of both inflammation and apoptosis as common underlying causative mechanisms of diabetes and AD. We assessed C-reactive protein (CRP) and FASLG concentrations in AD and long-standing T1D and T2D patients along with their capacity to produce the proinflammatory cytokines interleukin 1β (IL1β), interleukin 6 (IL6), and tumor necrosis factor (TNF) from PBMCs in a basal state and poststimulation.

PARTICIPANTS AND METHODS

Patients

The study consisted of 116 patients, 21 patients with T2D, 20 T1D, 41 with AD, and 34 healthy controls recruited from previous published studies (*11*, *17*). Patients' inclusion criteria included AD diagnosis by National Institute of Neurological and

[1]Department of Biological Chemistry, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [2]Endocrine Unit, First Department of Propaedeutic Medicine, Laiko University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece; [3]Iaso Maternity Hospital, Marousi, Greece; [4]First Department of Cardiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece; [5]Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece; [6]Biomedical Research Foundation of the Academy of Athens, Athens, Greece; [7]Diabetologic Center, First Department of Propedeutic Medicine, Laiko University Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [8]First Department of Psychiatry, Eginitio Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece.
***Address correspondence to this author at:** School of Medicine, National & Kapodistrian University of Athens, 132nd Kifisias Avenue, 15125 Amarousion, Athens, Greece. Fax 0030-2108024440; e-mail alexandrakik@gmail.com.
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[9] **Nonstandard abbreviations:** AD, Alzheimer disease; T1D, type 1 diabetes; T2D, type 2 diabetes; FASLG, Fas ligand; PBMCs, peripheral blood mononuclear cells; CRP, C-reactive protein; hsCRP, high-sensitivity C-reactive protein; IL1β, interleukin 1β; IL6, interleukin 6; TNF, tumor necrosis factor; PMA, phorbol 12-myristate 13-acetate; R, percent rise.

Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association Mini–Mental State Examination score of ≥10, stable medical history, and stable general health. Patients were excluded from the study for the following reasons: patient had a lifetime diagnosis of schizophrenia, bipolar disorder, or pre-AD anxiety disorder; patient had a current substance use disorder, diabetes, or hypertension; patient who was acutely suicidal or requiring inpatient psychiatric hospitalization. None of the participants were on any medication including aspirin, immunosuppressive medications, antibiotics, or antiinflammatory drugs or other agents known to affect cytokine production (*11*, *17*).

All participants gave written informed consent. The study protocol complies with the Declaration of Helsinki, and it was approved by the University of Athens Medical School Bioethics Committee.

Materials and methods

Blood samples (20 mL) were collected with standard venipuncture technique between 9:00 and 11:00 AM. Serum samples were separated immediately after centrifugation at 4 °C with 2000 g for 15 min and stored at −20°C until analysis. The analysis was performed within 2 months from storage day.

All reagents were purchased from Sigma-Aldrich, unless otherwise described. Ficoll–Hypaque solution was obtained from Amersham Biosciences. ELISPOT reagents and plates were purchased from Diaclone (Diaclone ELISPOT, Diaclone SAS). Percent concentration of HbA1c (%) was measured in vitro in whole blood by an immunoassay (turbidimetric inhibition immunoassay) in an automatic analyzer of clinical chemistry (Hitachi 912, Roche). All measurements were performed at the Chemwell analyzer (Awareness), as previously described (*11*, *17*). FASLG ELISA kit (FASLG, pg/mL) serum concentrations and high-sensitivity CRP (hsCRP, mg/L, hsCRP enzyme immunoassay test kit, LI7500, Linear Chemicals) were determined by ELISA. The intra- and interassay coefficients of variance for hsCRP were 7.5% and 4.1% for low concentrations and 2.3% and 2.5% for high concentrations, respectively, while the minimum detectable dose of FASLG was <12 pg/mL.

PBMCs Isolation. Peripheral blood samples (15 mL) from all participants were collected in heparin-containing tubes and immediately processed for lymphocyte separation by use of the Ficoll procedure. Briefly, 7 mL of blood was layered on top of 7 mL of Ficoll–Hypaque solution, according to manufacturers' instructions. After centrifugation, erythrocytes and granulocytes sedimented at the tube bottom while PBMCs (lymphocytes, monocytes) concentrated at the interface. By use of aseptic techniques, PBMCs were collected in separate tubes and resuspended in 1.5 mL of solution containing 80% fetal bovine serum and 20% dimethyl sulfoxide (DMSO). Cell viability as measured by trypan blue exclusion method exceeded 95%. PBMCs (1×10^6 cells/mL) containing cryovials were stored in liquid nitrogen until analysis.

ELISPOT Assay. For peripheral cytokines (IL1β, TNF, IL6) measurement, the enzyme-linked immunospot (ELISPOT) method was performed with commercially available kits.

Briefly, 100 μL of cell suspensions (ranging from 5×10^5 to 2×10^6 cells/mL) were added into ELISPOT nitrocellulose membrane-bottomed microwell plates precoated with the desired anticytokine capture antibody. Each cell suspension was tested in duplicate with and without the well-established mitogen phorbol 12-myristate 13-acetate (PMA, 300 ng/mL) that induces activation of T lymphocytes to secrete cytokines and proliferate. Cell suspensions were incubated at 37 °C in a 5% CO_2-humidified incubator for 24 h. The next day, cell suspensions were aspirated, and wells were washed twice with deionized water and 3 times with 200 μL of wash buffer (PBS with 0.1% Tween-20). One hundred microliters of the detection antibody solution (toward the cytokines IL1β,

Table 1. Serum Fas ligand concentrations measured in the 4 studied groups.[a]

Parameter studied	Controls (N = 34)	T1D (N = 20)	T2D (N = 21)	AD (N = 41)
Age, years	52.88 ± 14.69[b]	30.90 ± 8.65[b,c,d]	58.67 ± 13.79[b]	70.39 ± 8.62
Sex, F, M	17, 17	7, 13	11, 10	19, 22
FASLG, pg/mL	515.56 ± 233.30[b]	345.87 ± 160.33[b,c,d]	172.26 ± 120.22[b,c]	1122.41 ± 710.34
hsCRP, mg/L	1.67 ± 2.37[b]	4.37 ± 3.12[b,c,d]	7.77 ± 4.80[c]	9.07 ± 6.22

[a] The 4 study groups are control group, people with T1D, people with T2D, and people with AD. Data are mean ± SD.
[b] $P < 0.05$ vs AD.
[c] $P < 0.05$ vs controls.
[d] $P < 0.05$ vs T2D.

TNF, IL6) was added to each well, and the plate was incubated for 2 h at room temperature. After washing, streptavidin–horse radish peroxidase solution was added at 100 µL per well. The plate was incubated for 1 h at room temperature, and after extensive washing, 100 µL aminoethylcarbazole substrate solution was added to each well. "Spot" development was monitored from 5 to 60 min. The reaction was stopped by washing the wells with deionized water and allowing the plate to dry at room temperature. Spots were enumerated automatically with an ImmunoSpot Analyzer (Aid). Optimization of the ELISPOT assay regarding sensitivity and minimization of background signal was performed before evaluation of the experimental samples (*11*, *17*).

Statistical Analysis. Values are presented as mean value ± SD. Statistical significance was accepted at a P value <0.05. Normal distribution of continuous variables was assessed graphically by histograms and statistically by the nonparametric Kolmogorov–Smirnov test. Comparisons between groups were made by the Mann–Whitney *U*-test. Correlations were performed with Spearman ρ calculation. Partial correlation was used to correct the parameters studied with age in the total population studied. Correlations between categorical variables were estimated by the χ^2 test. Statistical analysis was performed with IBM SPSS Statistics for Windows (Version 22.0, IBM Corp).

RESULTS

The studied subgroups did not differ in the female-to-male ratios (AD, 19:22; T1D, 7:13; T2D, 10:11; controls, 17:17; $P = 0.672$). The AD subgroup significantly differed in age from all the other subgroups ($P < 0.001$ vs controls; $P < 0.001$ vs T1D; $P = 0.001$ vs T2D). The T1D subgroup also differed in age from the controls ($P < 0.001$) and T2D ($P < 0.001$), but the T1D group did not differ in age from controls (Table 1).

Assessment of basal and poststimulation inflammatory response

There was a continuum decrease in hsCRP concentrations from patients with AD to T2D, to T1D to control group, but hsCRP concentrations were statistically higher in the AD patient group than in controls ($P < 0.001$) or T1D ($P = 0.02$), in the T2D group than in controls ($P < 0.001$) or the T1D group ($P = 0.18$), and in T1D than in controls ($P < 0.001$) (Table 1).

The number (n) of cytokine-secreting PBMCs (per 10^5 cells) in basal conditions and after stimulation in the studied groups is summarized in Fig. 1. In basal conditions, nTNF was higher in the AD group than in controls ($P < 0.001$) or T1D ($P = 0.020$), nIL6 was higher in the AD group than in controls ($P < 0.001$), and nIL1β was higher in the AD group than in controls ($P = 0.032$). After stimulation with PMA, nTNF was higher in the AD group than in controls ($P = 0.001$), T1D ($P < 0.001$), or T2D ($P < 0.001$), nIL6 was

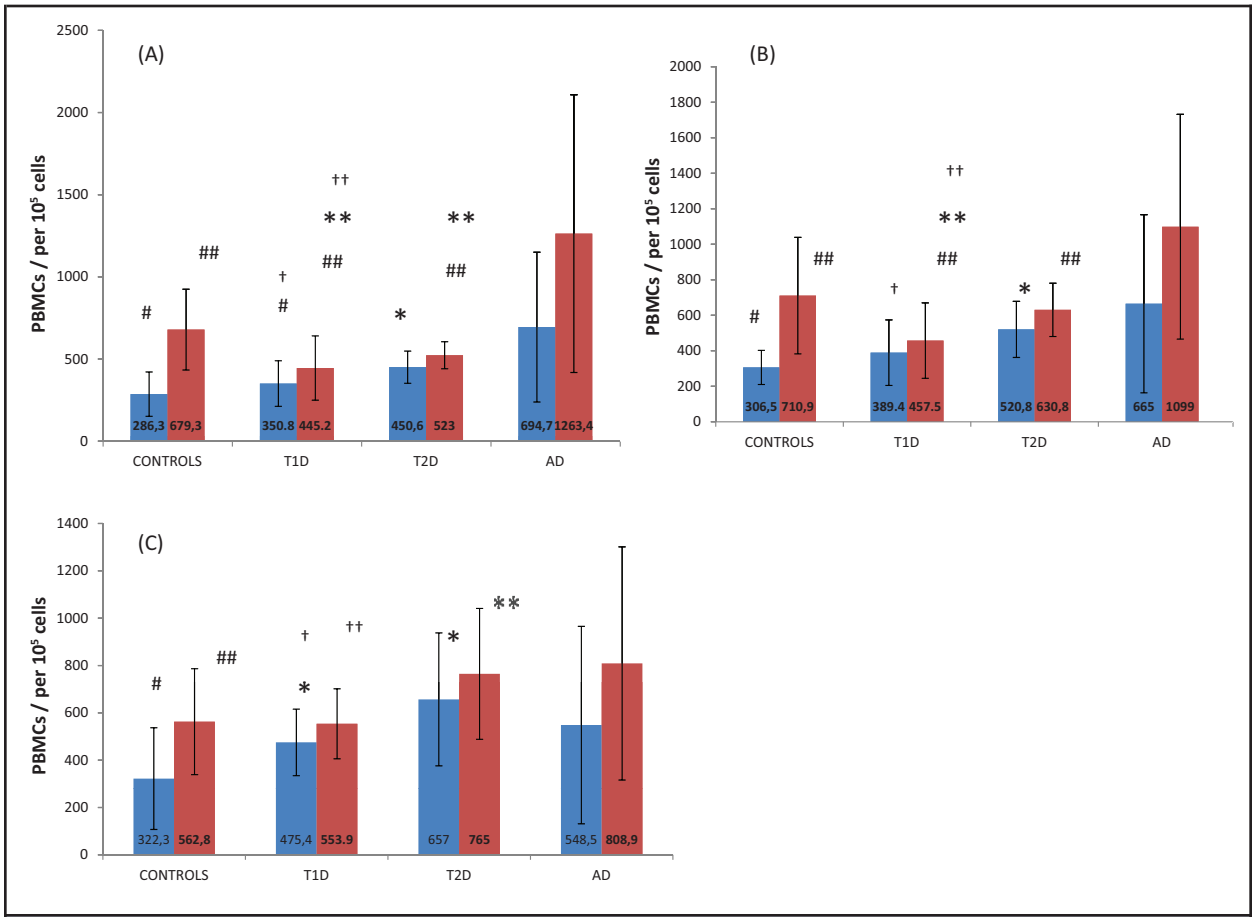

Fig. 1. The number of PBMCs secreting cytokines (per 10^5 cells) in the studied groups, in basal conditions and after stimulation.

Four groups were studied: control group, people with T1D, people with T2D, and patients with AD. The number of PBMCs secreting TNF (nTNF; per 10^5 cells) in the studied groups in basal conditions (blue column) and after stimulation (red column) (A). The number of PBMCs secreting IL6 (nIL6; per 10^5 cells) in the studied groups in basal conditions (blue column) and after stimulation (red column) (B). The number of PBMCs secreting IL1β (nIL1β; per 10^5 cells) in the studied groups in basal conditions (blue column) and after stimulation (red column) (C). Data are means ±SD. * $P < 0.05$ vs controls basal conditions; # $P < 0.05$ vs AD basal conditions; † $P < 0.05$ vs T2D basal conditions; ** $P < 0.05$ vs controls after stimulation; ## $P < 0.05$ vs AD after stimulation; †† $P < 0.05$ vs T2D after stimulation.

higher in the AD group than in controls ($P = 0.007$), T1D ($P < 0.001$), or T2D ($P = 0.039$), and nIL1β was higher in the AD group than in controls ($P = 0.030$) only.

Assessment of the magnitude of increase of the inflammatory response

The elevation of cytokine-secreting PBMC numbers (delta, d) after stimulation with PMA in the studied groups is summarized in Table 2. The nPBMCs increased after stimulation with PMA in all the subgroups ($P < 0.001$). The dnTNF, dnIL6, and dnIL1β did not differ between the AD group and the controls, but the percent rise (R) of nTNF was higher in the control group ($P = 0.016$). On the contrary, dnTNF, dnIL6, dnIL1β, RnTNF, RnIL6, and RnIL1β all differed between AD and T1D ($P < 0.001$; $P < 0.001$; $P = 0.001$; $P < 0.001$; $P < 0.001$; $P = 0.006$,

Table 2. Delta value of the number of PBMCs secreting cytokines (per 10^5cells) was their value after stimulation minus their value at basal conditions in the 4 studied groups.[a]

Parameter studied	Controls (N = 34)	T1D (N = 20)	T2D (N = 21)	AD (N = 41)
dnTNF, %[b]	393.03 ± 164.84 (165.79 ± 104.89)[c]	94.40 ± 100.59[c,d] (28.33 ± 27.86)[c,d]	72.42 ± 59.01[c,d] (18.51 ± 18.63)[c,d]	568.70 ± 513.66 (119.56 ± 106.45)
dnIL6, %	380.65 ± 291.98 (138.70 ± 98.99)	68.10 ± 64.49[c,d] (19.26 ± 17.50)[c,d]	110.00 ± 109.31[c,d] (31.35 ± 60.24)[c,d]	434.00 ± 392.03 (107.26 ± 104.10)
dnIL1β, %	240.50 ± 184.50 (107.86 ± 82.14)	78.55 ± 53.56[c,d] (18.21 ± 14.82)[c,d]	108.00 ± 89.83[c,d] (20.96 ± 20.19)[c,d]	260.38 ± 278.21 (85.74 ± 82.96)

[a] The 4 groups studied were control group, people with T1D, people with T2D, and people with AD. Data are mean ±SD.
[b] dnIL1β, delta value of the number of PBMCs secreting interleukin 1β; dnTNF, delta value of the number of PBMCs secreting tumor necrosis factor; dnIL6, delta value of the number of PBMCs secreting interleukin 6.
[c] $P < 0.05$ vs AD.
[d] $P < 0.05$ vs controls.

respectively). Similarly, dnTNF, dnIL6, dnIL1β, RnTNF, RnIL6, and RnIL1β all differed between AD and T2D ($P < 0.001$; $P < 0.001$; $P = 0.005$; $P < 0.001$; $P = 0.001$; $P = 0.005$, respectively).

Assessment of the FASLG

The AD subgroup displayed statistically higher FASLG concentrations than all other subgroups (vs controls, $P = 0.012$, and vs T1D and T2D, $P < 0.001$), whereas the T2D and T1D subgroups exhibited statistically lower concentrations than controls ($P < 0.001$ and $P = 0.035$, respectively), and the T2D subgroup showed statistically lower FASLG concentrations than the T1D subgroup ($P < 0.001$) (Table 1).

Correlations of parameters studied

In the whole population studied, the nPBMCs of all 3 cytokines before and after stimulation with PMA was positively correlated with plasma FASLG concentrations after correction for age (Table 3). The same correlations were seen in the AD subgroup. Regarding the delta values, dnTNF was positively associated with FASLG concentrations in the whole population studied after correction for age, but in the AD subgroup, dnIL1β and the percentage rise of the number of all 3 cytokines secreted from PBMCs after stimulation with PMA (RnTNF, RnIL6, RnIL1β) was negatively correlated with plasma FASLG concentrations. Moreover, in the T1D subgroup, FASLG concentrations were negatively correlated with only age; in controls, FASLG concentrations were positively related with only dnTNF.

DISCUSSION

In the present study, a higher inflammatory response was observed post stimulation in patients with AD than with either T1D or T2D. The FAS–FASLG pathway may play a role in this process because it is extrapolated by the higher

Table 3. FASLG concentration correlations with the parameters studied corrected for age in the whole population (partial correlation for age) and in the subgroups of patients (Spearman correlation).[a]

	r	*P* value
Partial correlation for age for the total population		
FASLG		
nTNF basal	0.605	<0.001
nTNF post-PMA	0.615	<0.001
dnTNF	0.526	<0.001
nIL6 basal	0.510	<0.001
nIL6 post-PMA	0.414	0.002
nIL1β basal	0.327	0.015
nIL1β post-PMA	0.350	0.009
Spearman correlation for AD subgroup		
Age	0.572	<0.001
nTNF basal	0.653	<0.001
nTNF post-PMA	0.529	0.001
RnTNF	−0.566	<0.001
nIL6 basal	0.615	<0.001
nIL6 post-PMA	0.435	0.007
RnIL6	−0.599	<0.001
nIL1β basal	0.578	0.001
nIL1β post-PMA	0.438	0.017
dnIL1β	−0.405	0.029
RnIL1β	−0.445	0.016
Spearman correlation for T1D subgroup		
Age	−0.495	0.026
Spearman correlation for control subgroup		
dnTNF	0.733	0.025

[a] nTNF, number PBMCs secreting TNF; nIL6, number PBMCs secreting IL6; nIL1β, number PBMCs secreting IL1β; dnTNF, delta value of nPBMCs secreting TNF after stimulation minus before stimulation with phorbol–myristate acetate (PMA); dnIL6, delta value of nPBMCs secreting IL6 after stimulation minus before stimulation with PMA; dnIL1β, delta value of nPBMCs secreting IL1β after stimulation minus before stimulation with PMA; RnTNFa, percentage increase of nPBMCs secreting TNF after stimulation with PMA; RnIL6, percentage increase of nPBMCs secreting IL6 after stimulation with PMA; RnIL1β, percentage increase of nPBMCs secreting IL1β after stimulation with PMA.

concentrations of FASLG in AD patients than with people with diabetes who displayed the lowest FASLG concentrations. Furthermore, in AD patients, FASLG concentrations were positively correlated with the magnitude of inflammation, as assessed by the number of PBMCs secreting cytokines at baseline conditions and with high demands.

FASLG is a 40-kDa type II membrane protein, which upon cleavage is released in a soluble form. FASLG exhibits cytotoxic activity against FAS-producing cells as a potent activator of the FAS-mediated apoptotic cell death pathway such as TNF–FAS–FASLG, and β amyloid was suggested to exert its effects on neuronal death through a FAS-like pathway (*9*, *18*–*20*). FASLG has been detected in apoptotic areas of postischemic brain (*21*) and in glia cells of patients with multiple sclerosis (*22*). However, the presence of inflammation, both basal and induced after stimulation, has been confirmed in AD patients (*8*, *17*). Disease progression in AD seems to parallel an increase in inflammation, as was shown by the positive association of FASLG concentrations and the increased number of inflammatory cells. However, the negative correlation of FASLG concentrations with the percent increase of cytokine secretion response upon demands suggests some defect of inflammatory machinery (Table 3). Moreover, cell loss in AD may be attributed to decreased cell production, increased destruction, or a combination of the 2 mechanisms, as in the case of the aforementioned cell death pathway. Apoptosis occurs during normal development by the same pathway. The increased production of another apoptotic molecule, TNF, has been reported to induce apoptosis (*8*, *23*, *24*) as observed in age-related neurodegenerative diseases (*9*). Some factors related to the observed immune dysfunction may induce production of TNF receptors, thereby rendering cells more susceptible to TNF. In our study the number of cells secreting TNF had the highest correlation with FASLG concentration, in accordance with a previous study (*8*) that suggested TNF as the main cytokine-mediator of apoptosis along with the highest concentration of FASLG. Therefore, our data provide evidence that patients with AD have a state of immune activation that leads to apoptosis

along with a generalized immune dysfunction. The positive correlation of FASLG concentration with the number of PBMCs producing all cytokines studied even in basal conditions and under increased demands confirms the immune activation. However, the magnitude of inflammatory response seems defective, as mentioned above. Hence, as the apoptotic process progresses, it may become defective in terms of impaired inflammatory response; this speculation may explain the reduced number of PBMCs secreting TNF compared to healthy participants (Table 2). There are no data to support a defective mechanism of apoptosis to explain the impairment in inflammatory response; instead there have been reports that both intrinsic and extrinsic apoptotic pathways are activated by β amyloid and τ protein (*25*, *26*). Possibly, as previously seen in diabetes (*11*), the inflammatory machinery is functioning at a higher concentration, but it cannot be as protective as it can in healthy participants to act toward an inflammatory stimulus, rendering patients with AD vulnerable to stimuli, such as infections or cancer. On the other hand, cytokines play an important role in the pathogenesis of central nervous system diseases altering growth and differentiation and modulating neuronal and neuroendocrine activities contributing to neurodegeneration (*8*). This evidence reflecting a low-grade inflammation in AD patients is further confirmed by the statistically higher absolute number of PBMCs secreting all 3 cytokines studied in either basal or poststimulation state than the respective number of healthy controls (Fig. 1).

Diabetes, mostly T2D, has been correlated with dementia due to vascular disease. The relationship with AD is less clear. Epidemiological data suggest a connection with vascular dementia independently of other vascular diseases and marginally with AD in this population (*2*, *3*, *27*, *28*). Studies in animal models of diabetes support these findings (*29*, *30*). Glucose deregulation may be linked to alterations in amyloid metabolism through changes in insulin and its receptor in the brain, resulting in brain insulin resistance, a known risk factor in metabolic states. Through the formation of toxic factors such as advanced glycation end products (*31*), inflammation may arise or not to an earlier activation of the apoptotic pathway (*25*). In humans, aging alone is associated with insulin resistance, reflecting the decreased metabolic turnover, decreased glucose use, reduced insulin, and reduced IGF1 signal transduction due to receptor desensitization (*32*, *33*). Another reason for the low concentrations of FASLG in diabetes patients may be the fact that naive islets produce low concentrations of FAS, because they are intrinsically resistant to apoptosis triggered by its receptor (*34*). However, islets become gradually sensitive to apoptosis during the course of inflammation (*35*) owing to increased FAS production in β cells (*36*, *37*) and concomitant sensitization to FAS-mediated apoptosis (*38*, *39*). A number of proinflammatory cytokines induce FAS transcription and TNF that synergize with FAS as effector mechanisms of β-cell destruction (*39*). Cytokines secreted by the islets themselves paradoxically enhance immune activation and islet injury under inflammatory conditions (*40*, *41*). It is difficult to define the exact time of interaction of inflammatory factors with the apoptotic pathway, because most cytokines induce FAS production (*34*, *42*). Nonetheless, whether inflammation is an early phenomenon or a late one must be further investigated.

Amyloidogenic peptides such as amylin, an islet amyloid polypeptide, may drive neuronal cell death, involving the activation of the FAS-associated protein with the death domain pathway (*9*, *18*). Even though increased production and activation of FAS and FASLG have been shown in β-cells dysfunction (*14*), in the present study a decreased concentration of FASLG was detected in both T1D and T2D patients compared to controls. A possible explanation arises from the evidence that amylin misfolding could generate aggregates that upregulate FAS and lead to the formation of amylin

oligomer–FAS receptor complexes that elicit apoptosis (*14*).

Despite the fact that the aim of the present study was to underline the link between diabetes and AD in the context of common apoptotic and inflammatory pathways, there is evidence for the individual role of each one of the proinflammatory cytokines. TNF has been discussed already, because it participates in the extrinsic apoptotic cell death pathway. IL1β is the prototypical inflammatory cytokine, and as a critical early mediator of inflammation (*43*), it was shown to be secreted by a similar absolute number in all 3 chronic diseases under study; however, the magnitude of increase was more higher in AD than in either type of diabetes, confirming that IL1β is a crucial cytokine in AD and directly related to plaque formation and progression (*44*). Finally, the IL6 secretory profile is explained by its pleiotropic effects. It exhibits a dual role because it is involved in both the amplification of and protection against inflammation or in transcriptional activities of genes encoding mainly proapoptotic but also some antiapoptotic molecules; additionally, it synergizes with other cytokines such as TNF or IL1β (*43*).

There are several limitations in the present study, such as the limited number of patients studied and the difference in age between the examined subgroups, but this difference is expected because of the different natural courses and age-range onset of the studied diseases.

In conclusion, the data from this study indicate a state of low-grade inflammation underlying AD that parallels the activation of apoptosis. However, this positive correlation cannot be seen in people with diabetes, at least with the apoptotic markers used in the present study. Previous studies have also suggested interplay between diabetes, impaired cognitive function, and molecular and structural AD-like changes; however, whether inflammatory cytokines and the FAS–FASLG system are a part of these links remains to be confirmed in further studies.

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