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Telomere shortening in T cells correlates with Alzheimer's disease status

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Abstract

Telomeres, the repeated sequences that cap chromosome ends, undergo shortening with each cell division, and therefore serve as markers of a cell's replicative history. In vivo, clonal expansion of T cells during immune responses to both foreign and autoantigens is associated with telomere shortening. To investigate possible immune alterations in Alzheimer's disease (AD) that might impact current vaccine-based therapeutic strategies, we analyzed telomere lengths in immune cell populations from AD patients. Our data show a significant telomere shortening in PBMC from AD versus controls ($P = 0.04$). Importantly, telomere length of T cells, but not of B cells or monocytes, correlated with AD disease status, measured by Mini Mental Status Exam (MMSE) scores ($P = 0.025$). T cell telomere length also inversely correlated with serum levels of the proinflammatory cytokine TNFα (a clinical marker of disease status), with the proportion of CD8+ T cells lacking expression of the CD28 costimulatory molecule, and with apoptosis. These findings suggest an immune involvement in AD pathogenesis.

Keywords: Alzheimer's disease; Telomeres; T cells; Immune system

1. Introduction

Telomeres are repetitive DNA structures at the ends of linear chromosomes that prevent end-to-end joining and other cytogenetic abnormalities [5]. Due to the nature of DNA replication machinery, the 3′ end of each DNA strand is incompletely copied during each round of cell division, leading to the attrition of the telomere sequences. Cell culture studies on a variety of cell types have shown that telomeres shorten at a rate of 50–100 base pairs per cell division [3]. Telomere shortening has also been documented in vivo and has provided novel insights into disease pathogenesis. For example, vascular endothelial cells in areas of hemodynamic stress have telomeres that are shorter than comparable cells from undiseased areas [11]. Similarly, epithelial cells isolated from affected but not normal joints in rheumatoid arthritis patients show evidence of telomere shortening, indicative of high cell turnover associated with inflammation [41]. Arguably, the most dramatic example of telomere shortening in vivo occurs in the CD8 T cell subset of persons with chronic HIV infection [17]. Longitudinal studies on individual patients has shown that the increase in the proportion of CD8 T cells with shortened telomeres parallels the loss of control over the infection and progression to AIDS. Thus, in a variety of contexts, telomere length measurement has proven to be a powerful tool in the analysis of disease processes.

Manipulation of the immune system using vaccine-based strategies is an active area of Alzheimer's disease (AD) research. A recent flurry of studies using a murine model of human AD have suggested that inoculation with the amyloid beta peptide results in diminished plaque formation and improved mental function, with no evidence of toxicity [31,43]. Nonetheless, vaccines aimed at treating human AD must take into account the possibility that the immune system may itself be involved in or altered by the ongoing disease process. Indeed, in contrast to prophylactic vaccines against childhood infections, AD vaccine preparations are composed of antigens to which the immune system of AD patients has undoubtedly been exposed over time. Thus, analysis of the baseline immune status of AD patients constitutes an essential component in the development of vaccines aimed at disease modulation.

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The possibility of altered immune function in AD was suggested by our earlier demonstration of accelerated telomere shortening in peripheral blood mononuclear cells (PBMC) from persons with Down syndrome, who invariably develop AD by the age of 40 [14,36]. To further explore possible immune components of AD, in the present study, we performed telomere length measurement of specific leukocyte populations from community dwelling AD patients and age-matched controls. The mean telomere length of unseparated PBMC from the AD group was significantly shorter than that of the controls. Moreover, within the AD group, there was a significant correlation between telomere length of T cells and Mini Mental Status Exam (MMSE) scores, a measure of disease status. The absence of telomere length/MMSE score correlations for B cells and monocytes further underscores the importance of the T cell observations, and suggests that the observed relationship is not due to potential telomere effects that might be caused by serum inflammatory cytokines or oxidative stress [38]. These telomere results, together with additional T cell alterations observed in the AD patients, provide evidence suggestive of specific immune involvement in AD.

2. Materials and methods

2.1. Study population

Subjects for this study were part of an ongoing, unrelated study on males with AD, and consisted of 15 AD patients from the UCLA Alzheimer's Disease Center (mean age: 73 years, range: 59–84) and 15 healthy controls (11 male, 4 female; mean age: 70 years, range: 64–89). The AD patients were diagnosed with mild or moderate AD, as evidenced by MMSE scores ranging from 14 to 29 out of 30. Subjects with MMSE scores below 14 were excluded from the study due to the possibility of poor self-care that might increase the risk of infections and affect immune status. Each subject was given a thorough physical and neurological examination, laboratory blood tests, an MRI and a SPECT brain scan, and the MMSE, a measure of cognitive functioning. All donors were community dwelling and had no known immune disorders. The groups were very similar with respect to smoking status, family income, current/past type of occupation, employment status, marital status, level of physical activity, and socio-economic status. Both groups had similar educational levels (average of 16 total years of education for both groups, range 12–18). The UCLA Internal Review Board approved this study and all participants gave written, informed consent.

2.2. Peripheral blood mononuclear cells

Venipuncture was performed in the morning between 08:00 and 10:00 h (after breakfast, but not following strenuous exercise). Tubes were kept at room temperature and transported to the laboratory for processing within 1 h of collection. PBMC from each donor were separated from heparinized whole blood by Ficoll Hypaque density gradient centrifugation (IsoPrep, Robbins Scientific, Sunnyvale, CA), washed in Hank's Balanced Salt Solution (Cellgro, Life Technologies, Rockville, MD), and resuspended in RPMI 1640 supplemented with 20% heat-inactivated (56 °C, 30 min) human AB serum (Gemini Bio-Products, Woodland, CA), 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 0.1 M Hepes Buffer (complete medium).

2.3. Telomere length analysis (TRF assays)

Telomere length analysis was performed by the standard terminal restriction fragment (TRF) protocol as previously described [35]. Two million PBMC from each donor were washed with phosphate buffered salt solution (PBS), pelleted by high-speed centrifugation, shock frozen with no supernatant for 5 s in liquid nitrogen, and stored at −80 °C for subsequent DNA isolation. Purified T cells, B cells, and monocytes were isolated from PBMC by magnetic microbead positive selection (Miltenyi Biotech, Auburn, CA). The purified cell subsets were pelleted and frozen in the same manner as the PBMC. To measure telomere lengths, genomic DNA was isolated from the cell pellets and digested with the restriction endonucleases *Hin*fI and *Rsa*I. A 1.5 μg aliquot of digested DNA (and ^{32}P-ATP labeled 1 kb and Lambda DNA *Hin*dIII digest ladders) were run on a 0.5% agarose gel at 40 V for 19 h and placed in a gel dryer for 1 h at 60 °C. The DNA was denatured directly on the gel by treatment with denaturing and neutralization buffers, and probed overnight with a ^{32}P-ATP labeled oligomer specific for the telomeric sequence (TTAGGG). Unbound probe was removed by three 10 min washes with 0.5× SSC. The gel was scanned and analyzed by Packard InstantImager and software. Telomere length is given as the average TRF length for each sample. Mean TRF length was quantified by integrating the signal intensity of the TRF smear on the gel as a function of its mean molecular weight, which is determined based on the standard ladders of known molecular weight.

2.4. Telomerase activity

The telomerase PCR ELISA kit was used according to the manufacturer's instructions (Roche Molecular Biochemicals, Germany). Pellets of 0.5 million PBMC were collected both before and after 72 h stimulation in vitro with the mitogen PHA, and shock frozen as described previously (for the TRF Assay). RNA was extracted by resuspending cell pellets in 200 μl lysis reagent and maintaining on ice for 30 min. The cell extracts were centrifuged at 16,000 × *g* for 20 min at 4 °C to remove cellular debris. Protein concentration of the cell extracts was determined by Bradford Assay using Bio-Rad protein assay reagent

(Hercules, CA). A 1 μg of protein from each extract (determined in this lab to be the aliquot optimum concentration) was used for the telomerase PCR ELISA assay. Negative controls were prepared by heat inactivation of each test sample at 85 °C for 20 min. The positive control was provided in the kit as a cell extract prepared from immortalized telomerase-expressing human kidney cells (293 cell line). To measure telomerase activity of the extracts, a reaction mixture containing telomerase substrates, primers, nucleotides, and Taq polymerase was added to each extract. Telomerase, a reverse transcriptase, synthesizes telomeric repeats onto the primers. After 30 min at 25 °C, telomerase-mediated extension of the primer was stopped by heating the mixture to 94 °C for 5 min. The telomeric repeats were subsequently amplified by PCR in the same tubes. Aliquots of the PCR product were denatured and hybridized to a digoxygenin-labeled, telomeric repeat specific detection probe. The product was immobilized onto the wells of a streptavidin-coated microtiter plate (supplied with the kit), and was bound to a peroxidase-conjugated antibody against digoxigenin. A solution containing the peroxidase substrate, 3,3′,5,5′-tetramethyl benzidine (TMB), was added to the wells to produce a color change directly proportional to the amount of PCR product. Absorbances were read with a Bio-Rad Benchmark Microplate Reader (Hercules, CA) at 450 nm (reference 690 nm). Telomerase activity was represented by the mean absorbance reading, in units of optical density (OD), of each sample minus the mean OD of the negative control.

2.5. Flow cytometry

Cell staining and cytofluorometric analyses were performed on whole peripheral blood collected in sodium heparin anticoagulant. The following antihuman monoclonal antibodies were used in three-color combinations to detect the cell surface markers on gated populations of lymphocytes and monocytes: CD3, CD8, CD4, CD19, CD16/56, CD25, HLA-DR, CD45RO, CD14, CD16, IgG1, IgG2A (Becton Dickinson Immunocytometry Systems, BDIS), CD28 (BioSource, Camarillo, CA). Five microliters of each monoclonal antibody was added to 50 μl of whole blood in 12 mm × 75 mm test tubes, vortexed gently, and incubated at room temperature for 20 min in the dark. Red blood cells were lysed during two separate incubations with 1× FACS lysing buffer, 500 and 200 μl, respectively for 2 min each at room temperature in the dark. The cells were washed with Dulbecco's PBS containing 0.1% sodium azide, resuspended in PBS, and analyzed using a FACScan flow cytometer (BDIS) with a 15 mW 488 nm air-cooled argon-ion laser. Performance of the instrument was monitored daily by running Calibrate beads™ (BDIS), using Autocomp software (BDIS). At least 5000 events were acquired using CELLQuest software. Data analysis was performed on a Macintosh Power PC using CELLQuest software.

2.6. Cytokine assays

Ten cc of whole blood without anticoagulant was centrifuged at 1800 rpm for 10 min. The serum was removed, aliquoted, and stored at −80 °C until the day of assay. TNFα and IL-6 levels were determined in serum samples by high sensitivity ELISA, and IL-1α levels determined using standard ELISA according to the manufacturer's kit instructions (R&D Systems, Minneapolis, MN). Absorbances were read with a Bio-Rad Benchmark Microplate Reader at 490 nm (reference 655 nm) for TNFα and IL-6, and at 450 nm (reference filter 490 nm) for IL-1α.

2.7. T cell proliferation assay

PBMCs (5×10^5) in complete medium were placed in a total volume of 200 μl in 96 well flat-bottom microtiter culture plates (Costar, Cambridge, MA). Cells were stimulated with 0 or 10 μg/ml PHA (L-9132, Sigma) for 48 h in humidified 5% CO_2 incubator at 37 °C. Mitogen concentration, PBMC number, and incubation times were determined as the optimum conditions for mitogen stimulation of PBMC in our laboratory. Each well was pulsed for an additional 18 h with 0.55 μCi of methyl-^{3}H-thymidine TdR (Nen™, Boston, MA), and harvested in a Multisample Automated Sample Harvester (MASH, PHD, Cambridge, MA). Incorporated radioactivity was measured as counts per minute (cpm) in a Beckman Liquid Scintillation Counter. The results are expressed as Stimulation Index, calculated from mean cpm of quadruplicate wells with mitogen/mean cpm of quadruplicate wells without mitogen.

2.8. Apoptosis assay

Heat shock-induced apoptosis was evaluated by exposing the cells to a mild heat stress (a 1 h 42 °C incubation in a water bath) followed by a 16–20 h incubation in a 5% CO_2 incubator at 37 °C. The cells were then washed in PBS and resuspended in 50 μl of 0.1% sodium azide containing 2% newborn calf serum (PBSAz). Five μl of anti-CD3 PE monoclonal antibody (Becton Dickinson) was incubated with the cells for 20 min at room temperature in the dark. The cells were washed in 1 ml PBSAz, then resuspended in 200 μl of 1× Binding Buffer supplied in the ApoAlert Annexin V-FITC Apoptosis Kit (Clontech Laboratories, Palo Alto, CA), 1 μg/ml Annexin V-FITC, and 20 μg/ml of 7-amino-actinomycin D (7AAD) (Calbiochem–Novabiochem Co., La Jolla, CA). Three tubes were used as controls for flow cytometry calibration, each containing 1×10^6 PBMC and either 7AAD only, 7AAD and anti-CD3 PE, or 7AAD and Annexin V-FITC. After 20 min of incubation in the dark at 4 °C, apoptosis was evaluated by flow cytometry. At least 5000 events were acquired using CELLQuest software. Data analysis was performed on a Macintosh Power PC using CELLQuest software. Only

the gated population of lymphocytes was analyzed. Early apoptosis was defined as the lymphocyte population that was Annexin V-positive and 7AAD-negative.

3. Results

Initial analysis was performed to compare the overall PBMC telomere lengths of the AD and control groups. The mean TRF length of the PBMC from the AD patients was significantly shorter than that of the controls (Fig. 1; AD: 6.22 kb ± 0.23; controls: 7.15 kb ± 0.37; $P = 0.04$). For a subset of donors from whom we obtained sufficient cells to isolate specific cell subpopulations, a trend toward shorter telomeres from AD patients in T cells (AD: 6.09 kb ± 0.47, controls: 6.72 kb ± 0.33), monocytes (AD: 6.66 kb ± 0.20, controls: 7.57 kb ± 0.44), and B cells (AD: 7.32 kb ± 0.39, controls: 8.36 kb ± 0.46) was observed, although this did not reach statistical significance ($P > 0.05$).

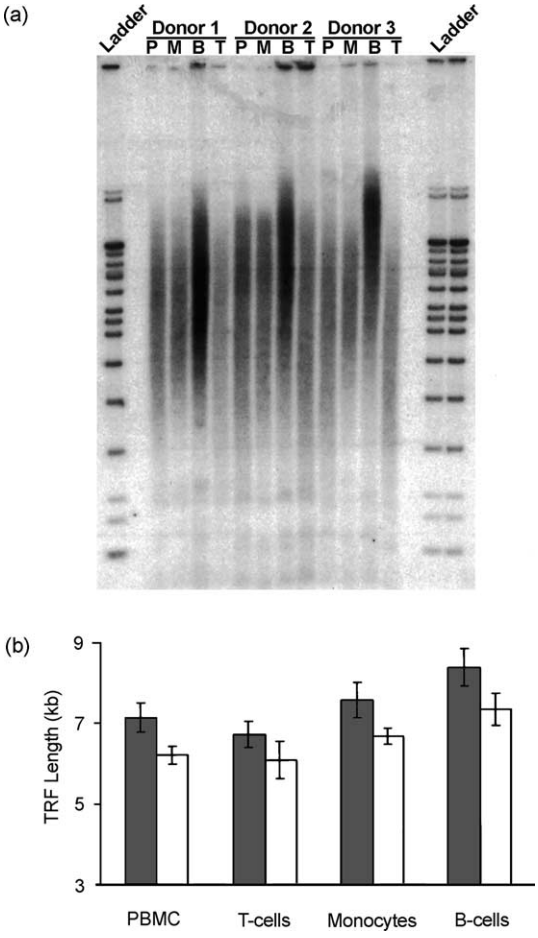

Fig. 1. (a) Example of telomere length Southern blot of three representative donors from whom sufficient cells were obtained to allow magnetic bead separation and telomere analysis of PBMC (P), monocytes (M), T cells and B cells. (b) Shortened telomeres in PBMC of AD patients. Telomeres lengths (expressed as TRF) were measured in total PBMC and bead-separated subsets (where cell numbers were sufficient) from AD patients (open bars) and controls (shaded bars). For PBMC ($n = 13$), $P = 0.04$; for T cells, monocytes, and B cells ($n = 6$–9), $P > 0.05$ (Student's t-test for equal variances).

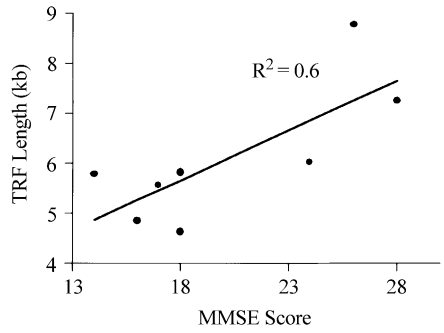

Fig. 2. Relationship of telomere length to cognitive function. T cells were isolated from blood samples that had sufficient number of cells to allow for bead separation; purity of separated population was verified to be >95% by flow cytometry. The telomere length (expressed as TRF) of T cells from AD patients ($n = 7$) is plotted against the MMSE score, a measure of cognitive functioning ($P = 0.025$, ANOVA regression analysis).

Telomere shortening is accelerated in cells cultured under increased oxygen concentration [37], raising the possibility that the observed telomere changes, rather than signifying specific immune involvement, might be the outcome of overall oxidative stress associated with AD. If this were the case, telomere length of PBMC, T cells, monocytes and B cells would all be predicted to correlate with disease severity. However, using the MMSE as a marker of AD disease status, a significant positive linear correlation was observed only between T cell telomere length (and not other cell types) and MMSE score (Fig. 2; $R^2 = 0.6$, $P = 0.025$). Thus, AD patients with lower MMSE scores, signifying more severe disease, have shorter T cell telomeres than patients with less severe disease. The absence of correlation between MMSE score and telomere length for PBMC ($R^2 = 0.0528$; $P = 0.45$), B cells ($R^2 = 0.0309$; $P = 0.13$) or monocytes ($R^2 = 0.0197$; $P = 0.8$) suggests that the T cell association is not simply due to oxidative stress or proinflammatory cytokine milieu, which would have produced equivalent telomere/MMSE correlations for all cell types.

Telomerase, a reverse transcriptase enzyme complex that functions to restore telomere sequences in dividing cells, has been shown to protect neurons from amyloid Aβ-induced apoptosis [44]. In T cells, telomerase activity, which is upregulated during early activation, functions to retard telomere shortening that would otherwise result from the normal clonal expansion required for an immune response [40]. To determine if the observed association between T cell telomere shortening and AD disease status might be due to global dysregulation of telomerase in persons with AD, which could potentially affect both the brain and the immune system, we compared the telomerase activity in T cells from AD and controls. The data in Fig. 3 show no difference in either the baseline or mitogen-induced telomerase activity levels between the AD and controls ($P > 0.05$). Thus,

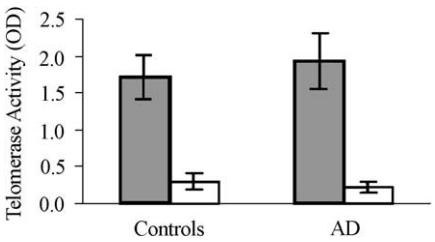

Fig. 3. Mean telomerase activity in AD patients ($n = 8$) and controls ($n = 12$). Telomerase activity was measured in PBMC stimulated with 10 μg/ml PHA for 72 h (shaded bars), and in unstimulated PBMC (open bars). The Telomerase PCR ELISA assay was used to measure telomerase activity. There was no difference in either the baseline or mitogen-induced telomerase activity levels between the AD and controls ($P > 0.05$, two-tailed Student's *t*-test for equal variances).

defective upregulation of telomerase cannot be implicated in the observed correlation of shortened telomeres in T cells with disease status. Interestingly, the T cells from AD patients respond more robustly than T cells from age-matched controls to a mitogenic stimulus (Fig. 4). These data indicate that T cells from AD patients may be "hyperproliferative", at least in response to the PHA mitogen used in this study. Nevertheless, based on this in vitro observation, one can speculate that the association of short T cell telomeres and more severe disease may reflect a history of ongoing enhanced T cell proliferative responses in vivo.

Based on the putative association between T cell replicative history and AD disease status (Fig. 2), we sought to identify additional features of T cells that might be altered in the patient group. Using flow cytometry, we analyzed a variety of markers (see Section 2.5) that reflect activation status, memory, the proportion of CD4 (helper) and CD8 (cytotoxic) T cells, and the expression of the T cell-specific costimulatory molecule, CD28. It had been previously shown that loss of CD28 expression on T cells is associated with telomere shortening both in vivo and in vitro [17,18,27]. Interestingly, in a subgroup of AD patients from whom we obtained sufficient PBMC to allow purification of CD4 and CD8 T cell subsets, we observed a highly statistically significant correlation ($R^2 = 0.8$, $P = 0.004$) between telomere length and CD28 expression within the CD8 T cell

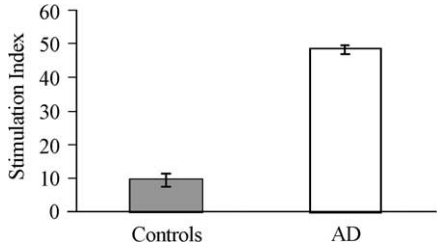

Fig. 4. Proliferative responses for AD patients ($n = 14$) and controls ($n = 10$). PBMC were stimulated with 10 μg/ml PHA (a T cell mitogen) for 72 h, and ^{3}H thymidine was added during the final 18 h. Stimulation Index = ^{3}H incorporation in the presence of PHA/^{3}H incorporation in medium alone. Data is presented as the geometric mean ± S.E.M.

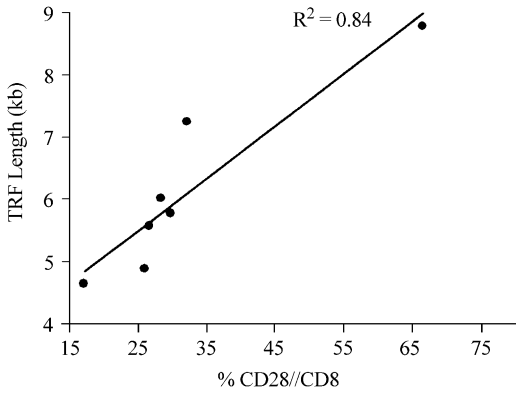

Fig. 5. Relationship of T cell telomere length to CD28 expression in CD8 T cells in AD patients. The percent expression of CD28 in CD8 T cells from AD patients, as measured by antibody staining and flow cytometry, is plotted against CD3 T cell telomere length (expressed as TRF) ($P = 0.004$; ANOVA regression analysis).

(the so-called cytotoxic) subset (Fig. 5). There was no significant correlation between telomere length and any other immune cell markers tested. Also, consistent with the enhanced production of TNFα by T cells that have undergone telomere shortening in cell culture in response to chronic antigenic stimulation [18], there was a significant inverse correlation between T cell telomere length and serum TNFα levels (Fig. 6), although the cellular source of this cytokine was not determined. No correlation was seen between T cell telomere length and serum levels of IL-6 or IL-1α.

Appropriately timed and efficient apoptosis is a critical aspect of normal immune function [14]. The size and quality of the T cell pool is dependent on the balance between extensive antigen-induced clonal expansion and subsequent efficient apoptosis of most of the responding cells, leaving only a small number of memory cells to respond to repeated encounter with the same antigen. Thus, to further investigate the potential relationship between telomere length and altered T cell function in the context of AD, we evaluated

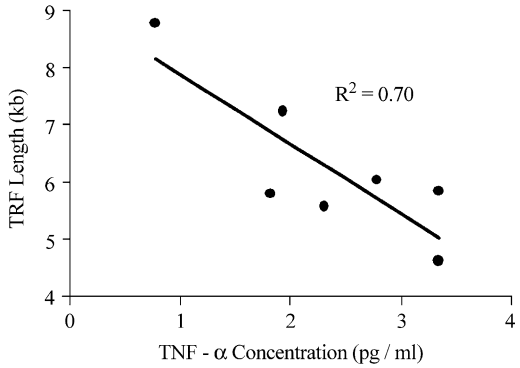

Fig. 6. Relationship of telomere length to TNFα concentration in AD patients ($n = 7$). The serum TNFα concentrations of AD patients, as measured by ELISA, are plotted against T cell telomere lengths (expressed as TRF) ($P = 0.018$; ANOVA regression analysis).

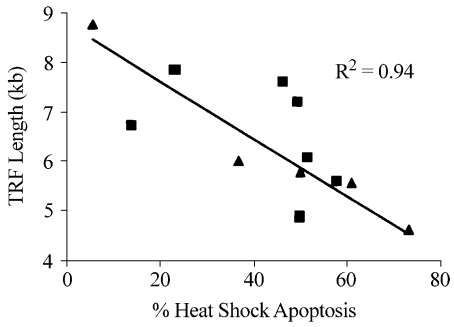

Fig. 7. Relationship of telomere length to apoptosis. T cell telomere lengths (expressed as TRF) from AD patients (square symbols) and controls (triangle symbols) are plotted against percent heat shock-induced apoptosis in lymphocytes ($P = 0.003$ for all subjects). The linear trend line describes the relationship between telomere length and percent apoptosis in AD patients alone ($R^2 = 0.94$, $P = 0.007$; ANOVA regression analysis).

apoptosis in response to a mild heat shock. There was a statistically significant negative linear correlation between the percentage of lymphocytes undergoing heat shock-induced apoptosis and T cell telomere length (expressed as TRF) (Fig. 7). This correlation was apparent for all subjects ($R^2 = 0.6$, $P = 0.003$), but became even stronger for AD patients alone (square symbols, $R^2 = 0.94$, $P = 0.007$). Interestingly, apoptosis dysregulation has been previously reported to be associated with telomere shortening in a variety of cell types [18,39].

4. Discussion

To our knowledge, this is the first study that relates telomere length changes to mental status in the context of AD. We observed an overall telomere shortening in PBMC from AD patients versus age-matched controls, in accord with our earlier findings in persons with DS [36]. Importantly, within the AD group, there was a significant correlation between telomere length of T cells and MMSE scores, a measure of disease status. The fact that this correlation is observed for T cells, and not for other immune cell types, underscores the importance of the T cell findings, and suggests that serum cytokines or systemic increase in oxidative stress, which could affect telomere length [38], are probably not involved. The telomere results, along with additional T cell alterations observed in the AD patients, are indicative of perturbations in the immune system of persons with AD. Whether these changes signify specific involvement in the initiation of disease or are secondary changes in response to inflammation and brain pathology, they will undoubtedly affect vaccine-based therapeutic approaches to the disease, and therefore merit continued investigation.

A potential role for T cells in AD pathology has been suggested in previous reports. The studies of Itagaki et al. [21] documenting significant numbers of both CD4 and CD8 T cells in the hippocampus and temporal cortex of Alzheimer brain but not normal brain tissue indicate that, at the very least, T cell trafficking is altered in the course of AD. The activated status of the brain-localized T cells, as indicated by their increased expression of MHC Class II antigens, is consistent with the ability of activated, but not resting, T cells to cross the blood brain barrier. The activated microglia present in the brain of AD patients may further enhance a putative T cell immune response both by presenting antigen to the infiltrating cells and also by augmenting their effector function [33]. The fact that other studies have failed to identify T cells in AD brains may relate to the stage of disease at which the autopsies were performed or differences between the cohorts studied [2]. The absence of T cells in the brain at the time of autopsy does not preclude their possible role at an earlier stage of the disease. Indeed, it is entirely possible that the T cells analyzed in the present study had undergone their activation and proliferation within the brain and subsequently re-entered the peripheral blood circulation.

A variety of studies have shown that T lymphocytes isolated from peripheral blood are altered in AD patients, consistent with the notion that T cells might play a role in AD without necessarily entering and/or taking up residence in the brain. Moreover, these changes in function and phenotype could potentially affect vaccine-based efforts to modulate AD. For example, altered intracellular calcium responses of T cells from both AD patients and older DS patients [20], as well as increased expression of activation markers [32] and increased proportions of T cells from AD patients coated with IgM [23] might negatively influence the T cell help required to generate an effective antibody response to immunization with Aβ. Similarly, the heat shock-induced altered apoptosis shown in Fig. 7 and the reported dexamethasone-induced apoptosis deregulation observed in AD patients [28] would also be predicted to impact the response to vaccines. Coincidentally, hypersensitivity to cell death has also been observed in T cells from presenilin-1 transgenic mice, a model for early-onset inherited AD [13].

An intriguing link between presenilin and T cells is the observation that presenilin-dependent γ-secretase activity modulates T cell development in the thymus via the Notch pathway [12]. The γ-secretase enzyme is the same enzyme that catalyzes the generation of amyloid beta peptides from the amyloid precursor protein (APP). Furthermore, members of the Notch protein family affect a variety of stages in T cell development and are also linked to AD, as suggested by similarities in Aβ production between presenilin and Notch knockout mice and loss-of-function mutations of the presenilin homologs in *C. elegans* and *Drosophila* that mimic Notch deficiencies [12]. Thus, there are genetically-based potential interactions between AD-related functions and key aspects of T cell development, consistent with the immune involvement in disease pathogenesis that is suggested by our own data.

The possibility that antigen-specific T cells play a role in AD pathogenesis has been previously suggested by studies that analyzed T cell reactivity to a panel of metabolic

products of the APP molecule. In one report, the toxic fragment Aβ 25–35 was shown to induce a much smaller increase in intracellular calcium in T cells from AD patients versus healthy controls and depressive patients [8]. Grubeck-Loebenstein and co-workers [34] have documented that T cells from healthy young and aged individuals, but not persons with AD, proliferate in response to these disease-related peptides. Interestingly, the T cells from AD patients, while unable to proliferate, nevertheless do respond to APP-derived peptides by upregulating expression of the alpha chain of the IL2 receptor (CD25), indicative of antigen recognition. The proliferative unresponsiveness of the AD T cells to disease-related antigens, but not to other antigens such as influenza, is highly suggestive of an antigen-specific T cell involvement in some aspect of the disease. One hypothetical scenario that might explain these observations is that T cells reactive with APP and/or its metabolic products may play a role in the elimination of these substances. Based on this scenario, one can speculate that in healthy individuals, autoreactive T cells continually function to eliminate potentially amyloidogenic substances. In person with AD, it is possible that this mechanism is either impaired or, as suggested by the hyper-proliferative response data (Fig. 4) , may have even become "exhausted". Regardless of the validity of these speculated mechanisms, the antigen-specific "hole" in the T cell repertoire of AD patients merits more careful analysis, particularly with respect to vaccine strategies.

The above T cell observations on patients with AD are reminiscent of variety of characteristics documented for T cells that reach a state of replicative senescence after multiple rounds of antigen-induced cell division [16]. It should be emphasized that telomere shortening is accompanied by a host of phenotypic and gene expression changes that result in altered function. Indeed, it is this alteration in function and gene expression, rather that the telomere loss per se, that is believed to exert the most profound effects in vivo [9,29]. For example, telomere shortening in fibroblasts is associated with reduced expression of genes involved in maintaining intracellular matrix, and upregulation of genes that favor collagen degradation—changes that affect the integrity of numerous organ systems [10]. Similarly, T cell telomere shortening is accompanied by a broad spectrum of changes that can profoundly affect immune response to both environmental pathogens as well as self-antigens [18]. These changes include reduced gene transcription of *hsp70* in response to stress [19], loss of CD28 expression [15], acquisition of NK-like activities [1], and increased spontaneous production of TNFα and IL-6. In that regard, it is relevant that TNFα in combination with IFNγ has been shown to alter the metabolism of APP, triggering production of Aβ peptides and inhibiting the secretion of soluble APP [6].

The ubiquitous presence of APP and its metabolic products suggests that T cells have had extensive exposure to these AD-related proteins. Indeed, high levels of APP are expressed in activated lymphocytes [24], as well as in platelets and tissues of the central nervous system. Interestingly, although the APP mRNA levels are similar between AD, young controls and age-matched controls [24], there is a trend toward increased APP in PBMC from AD versus age-matched controls [30], suggestive of possible alterations in the processing of this molecule. Reports showing the increased levels of Aβ 1–42 in the first-degree relatives of patients with AD as well as in several cognitively normal individuals in whom AD later developed are also consistent with possible alterations in APP processing [25]. Increased and prolonged exposure to Aβ might be setting the stage for possible aberrant immune reactivity. Indeed, the increased incidence of antibodies to Aβ produced by B cell lines established from AD patients is consistent with some sort of altered immune reactivity to this AD-related peptide [42]. In addition, the deposition of Aβ in extraneuronal organs of AD patients suggests that this peptide may be derived from cells outside the brain [22]. The absence in correlation between the levels of Aβ 1–40 and 1–42 present in the plasma versus the cerebrospinal fluid in AD has, in fact, led to the notion that the source of the Aβ synthesis in these two compartments may be different [26].

In conclusion, whereas the accumulation of some type of aggregated Aβ is generally accepted as the central feature of AD pathogenesis, the cascade of antecedent events still remains to be elucidated. The innate immune system has been implicated in the inflammatory and pro-oxidant features of AD [4,7], but the potential role of adaptive immunity has not been extensively analyzed. The observed telomere length changes, the association of T cell telomere length with MMSE scores, and other T cell changes in AD patients suggest a possible antigen-specific involvement at some point of the disease process. More detailed analysis of the possible role of T cells in AD etiology and/or progression is critical both to identify possible disease mechanisms, but also to ensure the rational design of effective vaccine-based therapeutic approaches to this devastating disease.

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