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A Relationship Between Alzheimer's Disease and Type 2 Diabetes Mellitus Through the Measurement of Serum Amyloid-β Autoantibodies

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Abstract. Increasing evidence suggests that type 2 diabetes mellitus (T2DM) is strongly correlated with Alzheimer's disease (AD). To examine the relationship between T2DM and AD, autoantibodies against amyloid-Aβ were measured in the serum of T2DM patients and age-matched controls. Levels of Aβ autoantibody were measured by ELISA in serum samples of T2DM patients ($n = 92$) and age-matched control group ($n = 106$). Aβ autoantibody levels were increased in T2DM compared with age-matched controls by $45.4 \pm 8.1\%$ ($p < 0.001$). Females had higher Aβ autoantibody levels than males in both T2DM and control group. Aβ autoantibody levels in the T2DM group were positively correlated with the levels of cholesterol ($p = 0.011$), low density lipoprotein cholesterol ($p = 0.020$), and triglycerides ($p = 0.039$). In conclusion, the level of Aβ autoantibody is dramatically elevated in patient serum of T2DM, and, as such, might be used as a possible biomarker for T2DM.

Keywords: Aβ Autoantibody, Alzheimer's disease, biomarker, cholesterol, type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is one of the most common and increasing metabolic diseases characterized by elevated blood glucose concentration [1]. Even though DM is indicated by hyperglycemic status, DM can lead to micro- or macrovascular complications associated with morbidity and mortality of these individuals. Moreover, DM is often associated with central obesity, hypertension, and dyslipidemia, which collectively constitutes metabolic syndrome. Each component of metabolic syndrome is related, and they each represent risk factors for multiple life threatening diseases, such as myocardial infarction and cerebrovascular diseases. DM is classified as type 1 DM (T1DM) and type 2 DM (T2DM). In T1DM, pancreatic β-cells are depleted due to abnormal autoimmune response. On the other hand, T2DM is normally characterized by insulin resistance, in which even increased insulin levels do not elicit proper signaling responses. In advanced state of T2DM, however, pancreatic β-cells can also be depleted [1].

Alzheimer's disease (AD) is an age-related neurodegenerative disorder, characterized by senile plaques formed from the Aβ peptide and neurofibrillary tangles derived from hyperphosphorylated tau [2]. Although these two diseases appear to bear no pathological relationship at first sight, increasing evidence suggests

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that DM and AD are strongly correlated [3,4]. For example, the incidence of AD is 2 to 5 times higher in T2DM patients than normal population [5]. Moreover, these two diseases share uncanny similarities in their pathogenic mechanisms (i.e., age-related processes, metabolic changes, degenerative processes, and aggregation of Aβ) [5]. Like AD whose pathological hallmark is Aβ plaques, amyloid in islet (amylin) is partly responsible for the destruction of pancreatic β-cell in T2DM [6]. Moreover, islet amyloid is derived from the islet Aβ protein precursor (IAβPP), which has about high homology to the Aβ protein precursor (AβPP). In the same way that Aβ is derived from AβPP, islet amyloid is derived from IAβPP [7]. Islet amyloid toxicity is also linked to the induction of inflammatory processes and neurotoxicity [8]. As such, intra-cerebroventricular injections of streptozotocin, a diabetogenic compound, has recently been used as a novel animal model of AD [9]. Conversely, it has also been suggested that AD represents a 'brain-specific form of diabetes' (i.e., 'Type 3 diabetes') by several reports [9–11].

Based on the background described above, we designed this study to determine the relationship between Aβ autoantibodies and T2DM. Although the deposition of Aβ in the brain is a cardinal marker for AD, its levels in AD patient serum are controversial – increased or decreased. On the other hand, autoantibodies against Aβ are consistently reduced in AD patient serum [12–15]. In this study, we evaluated the levels of Aβ autoantibodies in serum of T2DM and healthy age-matched control subjects.

MATERIALS AND METHODS

Subjects

Serum samples of T2DM patients ($n = 92$) were obtained from Seoul National University Hospital (SNUH), and age-matched control groups ($n = 106$) were obtained from Seoul National University Healthcare System Gangnam Center. Each sample was collected after informed consent under the approval of the Institutional Review Board. Height, weight, body mass index (BMI), waist, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose, HbA1c, total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), and creatinine of samples were determined (Table 1).

Measurement of Aβ autoantibody

Levels of Aβ autoantibody in serum of the T2DM patients and control group were measured by direct enzyme-linked immunosorbent assay (ELISA) established as previously reported [11]. Briefly, microtiter wells (Maxisorp, Nunc, Roskilde, Denmark) were coated overnight with 1 μg/well monomeric human Aβ_{1-42} (Bachem, Bubendorf, Switzerland) in 0.1 M sodium bicarbonate buffer (pH 9.6) at 4 °C. Following washing with phosphate-buffered saline (PBS)/0.05% tween20, the plates were blocked with fetal bovine serum (FBS)/PBS buffer (10% FBS in PBS) at room temperature (RT) for 1 h. The plates were washed and then incubated with serum diluted at 1:100 in FBS/PBS buffer for 1 h at 37 °C. After washing, the wells were incubated with a 1:2000 dilution of sheep anti-human IgG antibody conjugated to HRP (Amershan Pharmacia Biotech, Buckinghamshire, UK) for 30 min at 37 °C. The well were washed, and a color substrate, 3,3',5,5'-tetramethylbenzidine (TMB) (Pierce, Rockford, IL) was added to the wells. The plates were read on a plate reader at 450 nm.

Measurement of total IgG

Amounts of total IgG in serum of T2DM patients and control group were measured by direct ELISA as described in the manufacturer's protocols of "human IgG kit" (Standard Diagnostics, INC., Korea)

Statistical analysis

Differences in Aβ-autoantibody levels between T2DM patients and control groups were examined for statistical significance using the unpaired t-test. The relationship between Aβ autoantibody and other variables in DM group were examined using the correlation analysis and linear regression analysis. Statistical analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL).

RESULTS

Subject characteristics

There was no statistical difference between T2DM patients and control groups in age, BMI, waist, blood pressure, blood cholesterol level, blood LDL level, and creatinine (Table 1). However, the DM group had greater mean fasting blood glucose ($p < 0.001$), HbA1c ($p < 0.001$), and TG ($p < 0.005$) than control group and reduced mean HDL ($p < 0.005$) than control group (Table 1).

Table 1
Clinical data of control and T2DM group. Data are means ± SD

	Control ($n = 106$)	DM ($n = 91$)
Gender (M:F)	(62:44)	(53:38)
Age (year)	55.1 ± 7.1	55.8 ± 8.0
Height (cm)	163.2 ± 7.0	163.2 ± 8.8
Weight (kg)	62.6 ± 9.0	64.3 ± 11.3
BMI (kg/m^2)	23.4 ± 2.4	24.0 ± 3.0
TG (mg/dL)	103.8 ± 42.9	130.9 ± 71.7**
TC (mg/dL)	197.5 ± 28.8	192.3 ± 33.8
HDL (mg/dL)	55.0 ± 12.5	49.4 ± 12.3**
LDL (mg/dL)	121.7 ± 27.7	116.8 ± 30.1
SBP (mmHg)	118.7 ± 16.6	123.0 ± 16.2
DBP (mmHg)	75.4 ± 11.2	76.3 ± 9.3
Fasting blood glucose (mg/dL)	90.0 ± 6.3	136.30 ± 37.5***
HbA1c (ng/mL)	5.5 ± 0.2	7.0 ± 1.2***
Creatinine (mg/mL)	1.0 ± 0.2	1.0 ± 0.2

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, by unpaired t-test.

Levels of Aβ autoantibody

Levels of Aβ autoantibody measured by ELISA were calculated by the mean of control group to be 100%. Aβ autoantibody levels were significantly elevated in the T2DM group by 45.4 ± 7.7% compared to the control group ($p < 0.001$) (Fig. 1A). Surprisingly, females had 63.0 ± 13.7% higher Aβ autoantibody levels than males in the T2DM group ($p < 0.001$). The reason for this difference is currently unclear. A similar pattern of increased Aβ autoantibodies in females (12.7 ± 6.4%) was also seen in the control group ($p = 0.048$) (Fig. 1B). Nonetheless, there remained a significant increase in Aβ autoantibody levels in the T2DM group compared to the control group among both females (74.9 ± 12.0%, $p < 0.001$) and males (24.6 ± 8.7%, $p = 0.006$), albeit with much greater magnitude among females.

Amounts of total IgG

Total IgG levels were 14.6 ± 0.7 mg/mL in the T2DM group and 13.8 ± 0.6 mg/mL in the control group, which did not significantly differ from each other ($p = 0.237$) (Fig. 1C).

Correlation for variables and level of Aβ autoantibody in T2DM group

Correlation analyses were performed to determine whether Aβ autoantibody levels correlate with various other serum measures among the T2DM group. Specifically, linear regression analysis showed that the level of Aβ autoantibody was positively correlated with TC ($R = 0.264$, $p = 0.011$), LDL ($R = 0.244$, $p = 0.020$), and TG ($R = 0.217$, $p = 0.039$) in the T2DM group (Fig. 2). In control subjects, the level of Aβ autoantibody was not significantly correlated with any of these variables (TC, $R = -0.064$, $p = 0.513$; LDL, $R = -0.040$, $p = 0.684$; TG, $R = -0.040$, $p = 0.686$). R stands for Pearson's correlation coefficient (R).

DISCUSSION

T2DM is characterized by insulin resistance and dyslipidemic state [1]. Under these conditions, Aβ autoantibody levels in serum were elevated. This result suggests an interesting pathobiological relationship between AD and T2DM. We previously showed that Aβ autoantibody levels were decreased in Tg2576 mice (AD animal model), and serum level of Aβ autoantibody was lower in older mice that had higher Aβ level in the brain [12]. Similarly, other studies also reported that AD patients had reduced serum level of Aβ autoantibodies [13–15]. Given that immunization with Aβ peptides could prevent amyloid deposition and clear preformed plaques from brain [16–19], reduced level of Aβ autoantibodies would be expected to attenuate the capacity to clear Aβ from AD brains, and thereby further promote Aβ deposition [20]. In contrast, our results showed increased level of Aβ autoantibodies in T2DM. This might be interpreted to indicate that DM may be protective against the development of AD. However, this simple interpretation is contrary to findings from many epidemiological studies in which DM patients are far more likely to develop AD [3,4]. To find a more suitable interpretation of this phenomenon, it is important to also consider the effects of DM on

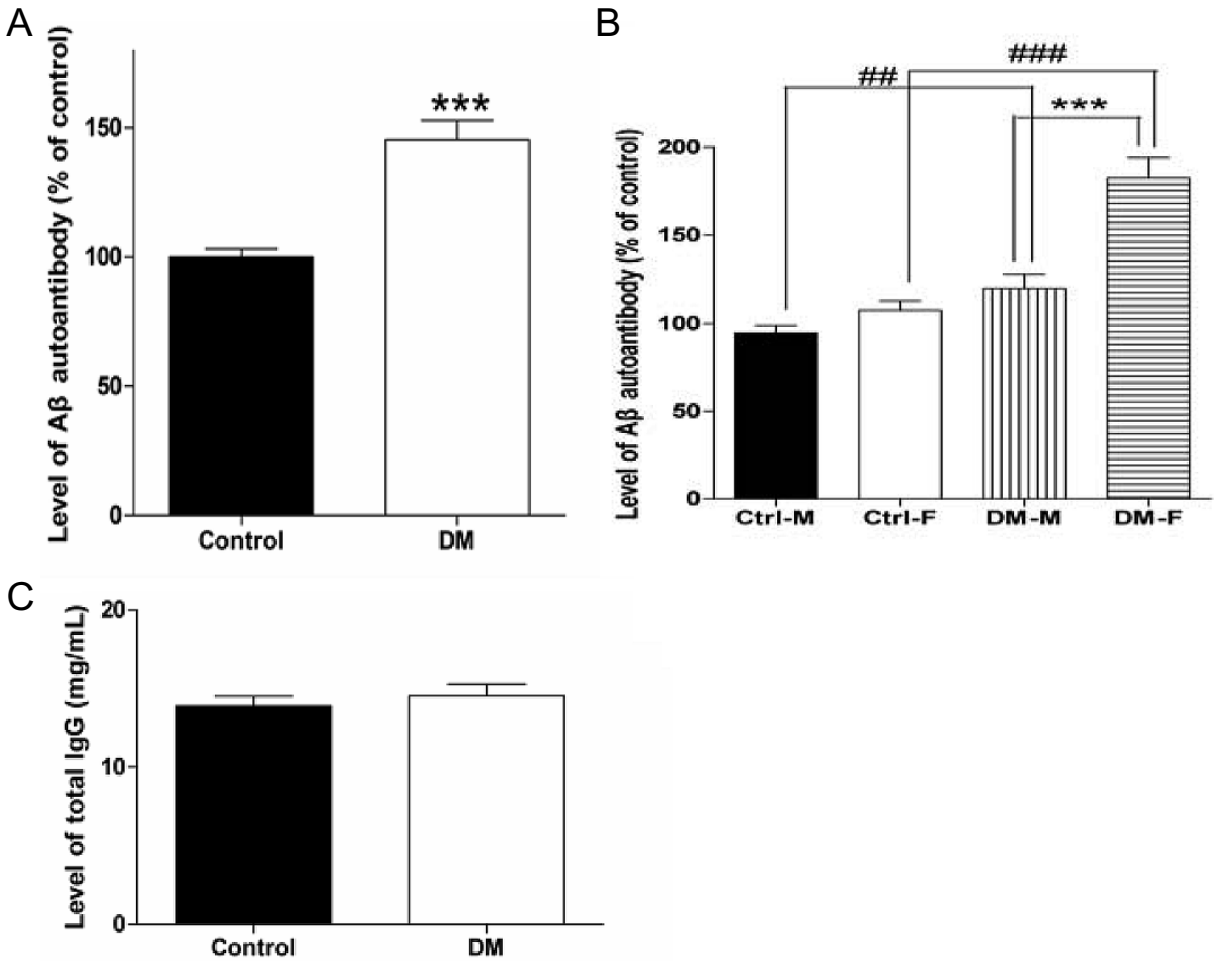

Fig. 1. Serum level of Aβ autoantibody and amounts of IgG. A) Difference of serum level of Aβ autoantibody between control and T2DM group. Data are mean $\pm$ SEM. $^{***}p < 0.001$, by unpaired t-test. B) Gender difference of serum level of Aβ autoantibody. Data are mean $\pm$ SEM. $^{***}p < 0.001$, $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, by one-way ANOVA followed by post-hoc test. C) Difference of amounts of IgG between control and DM group. Data are mean $\pm$ SEM. No significant difference, by unpaired t-test.

dyslipidemia and immune function. T2DM is frequently associated with dyslipidemia which is characterized by elevated levels of TG, LDL, and cholesterol. Dyslipidemia in DM results from insulin resistance and reduced activity of lipoprotein lipase, leading to slower degradation of VLDL (very low density lipoprotein) as well as increased TG and decreased HDL [21]. Indeed, the relationship between cholesterol/lipids and AD is well documented. In AD animal models, diet-induced hypercholesterolemia caused accumulation of Aβ in the central nervous system [22]. Hypercholesterolemia is thought to promote β-secretase cleavage of AβPP in membrane rafts [2] and thereby promote Aβ generation. The non-amyloidogenic α-secretase resides outside of membrane rafts, whereas β- and γ-secretases are concentrated in membrane rafts (cholesterol rich area) [2]. Membrane cholesterol level is a key factor determining raft stability [23] and membrane cholesterol content is regulated by serum cholesterol. Consequently, the level of Aβ could be increased in hypercholesterolemic state. In this study, since only TG, not TC or LDL, was significantly elevated in T2DM group, the effect of elevated TG on Aβ autoantibody remains to be clarified in future studies.

Although Aβ levels in peripheral blood may fluctuate, it is conceivable that Aβ autoantibody levels might reflect Aβ content within a defined period. Given that increased TC, LDL, or TG is positively correlated with level of Aβ autoantibody, we hypothesize that chronic dyslipidemia causes enhances Aβ levels, which in turn stimulates B-cells to produce more autoantibodies against Aβ. Our observation that total IgGs were

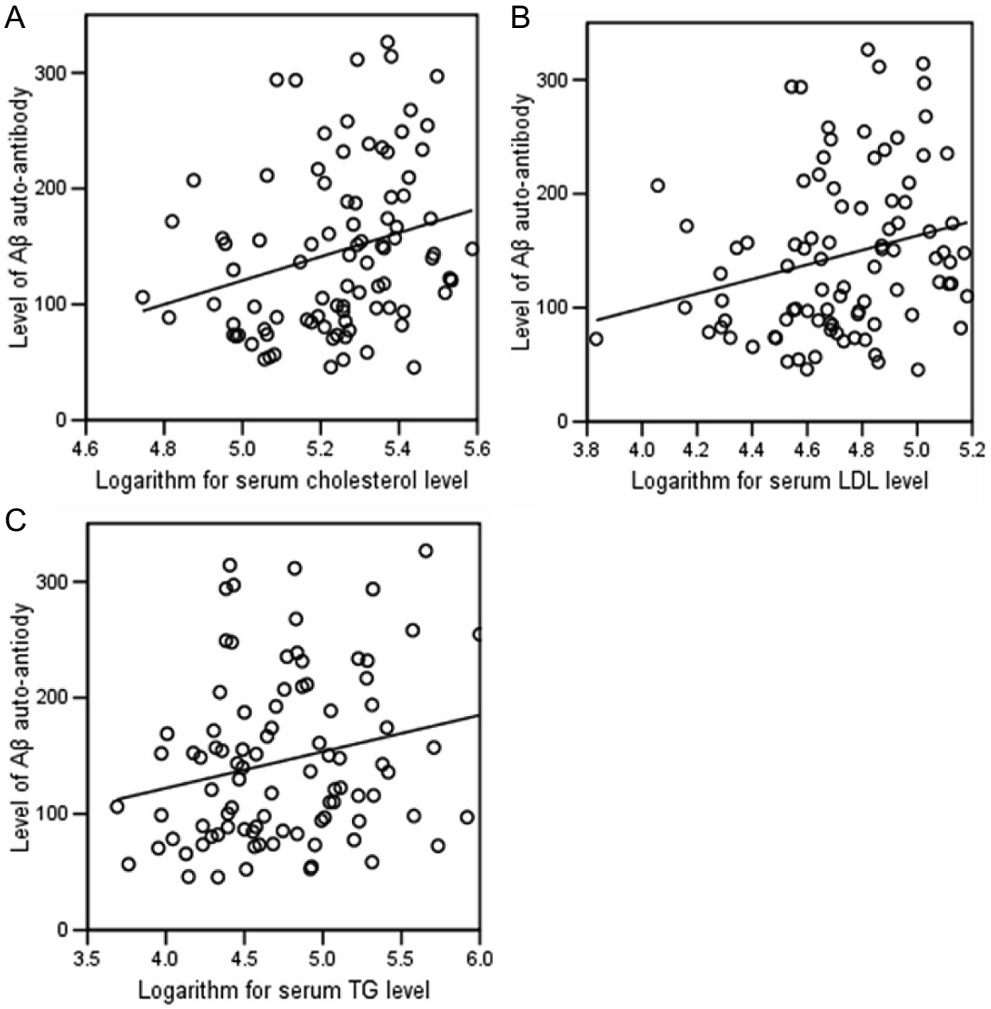

Fig. 2. Correlation for variables and level of Aβ autoantibody in T2DM group. A) Plot of logarithm for serum cholesterol levels against serum level of Aβ autoantibody in T2DM group. $R = 0.264$, $p = 0.011$, by linear regression. B) Plot of logarithm for serum LDL levels against serum level of Aβ autoantibody in DM group. $R = 0.244$, $p = 0.020$, by linear regression. C) Plot of logarithm for serum TG levels against serum level of Aβ autoantibody in DM group. $R = 0.217$, $p = 0.039$.

not different between T2DM and normal controls indicates that global B-cell immune responses are not altered in T2DM. Therefore, the significant increase in Aβ autoantibodies in T2DM suggests a B-cell response dependent on the level of the antigen, Aβ. The mean age of our subjects was about 55 years old, an age that is $\sim$20 years younger than that of typical sporadic AD. Therefore, the increased Aβ autoantibody level in T2DM not only reflects the level of the antigen but also might be an early biomarker related to AD risk. Although the precise mechanism underlying this phenotype is not known, our study showed a possibility that the Aβ autoantibody level might serve as an additive biomarker for T2DM. Additional longitudinal studies will be needed to clarify the correlation of the Aβ autoantibody level with T2DM and AD.

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REFERENCES

[1] Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of WHO consultation. *Diabet Med* **15**, 539-553.

[2] Yankner BA (1996) Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron* **16**, 921-932.

[3] Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A (1999) Breteler MM. Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology* **53**, 1937-1942.

[4] Pelia R, Rodriguez BL, Launer LJ (2002) Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes* **51**, 1256-1262.

[5] Li L, Hölscher C (2007) Common pathological processes in Alzheimer disease and type 2 diabetes: a review. *Brain Res Rev* **56**, 384-402.

[6] Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, Copper GJ, Holman RR, Turner RC (1988) Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. *Diabetes Res* **9**, 151-159.

[7] Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB (1987) Purification and characterization of a peptide from amyloid-rich pancreas of type 2 diabetic patients. *Proc Natl Acad Sci U S A* **84**, 8628-8632.

[8] Gotz J, Ittner LM, Lim YA (2009) Common features between diabetes mellitus and Alzheimer's disease. *Cell Mol Life Sci* **66**, 1321-1325.

[9] Lester-Coll N, Rivera EJ, Soscia SJ, Doiron K, Wands JR, de la Monte SM (2006) Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. *J Alzheimers Dis* **9**, 13-33.

[10] Steen E, Terry BM, Rivera EJ, Cannon JL, Neely, TR, Tavares R, Xu XJ, Wands JR, de la Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease – is this type 3 diabetes? *J Alzheimers Dis* **7**, 63-80.

[11] de la Monte SM, Tong M, Lester-Coll N, Plater M Jr, Wands JR (2006) Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: Relevance to Alzheimer's disease. *J Alzheimers Dis* **10**, 89-109.

[12] Sohn JH, So JO., Kim H, Nam EJ, Ha HJ, Kim YH, Mook-Jung I (2007) Reduced serum level of antibodies against amyloid beta peptide is associated with aging in Tg2576 mice. *Biochem Biophys Res Commun* **361**, 800-804.

[13] Sohn JH, So JO, Hong HJ, Kim JW, Na DR, Kim M, Nam E, Ha HJ, Kim YH, Mook-Jung I (2009) Identification of autoantibody against beta-amyloid peptide in the serum of elderly. *Front Biosci* **14**, 3879-3883.

[14] Weksler ME, Relkin N, Turkenich R, LaRusse S, Zhou L, Szabo P (2002) Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp Gerontol* **37**, 943-948.

[15] Moir RD, Tesitlin KA, Soscia S, Hyman BT, Irizarry MC, Tanzi RE (2005) Autoantibodies to redox-modified oligomeric Abeta are attenuated in the plasma of Alzheimer's disease patients. *J Biol Chem* **280**, 17458-17463.

[16] Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* **6**, 916-919.

[17] DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM (2002) Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* **295**, 2264-2267.

[18] Dickstein DL, Biron KE, Ujiie M, Pfeifer CG, Jeffries AR, Jefferies WA (2006) Abeta peptide immunization restores blood-brain barrier integrity in Alzheimer disease. *FASEB J* **20**, 426-433.

[19] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, Walker S, Wogulis M, Yednock T, Games D, Seubert P (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400**, 173-177.

[20] Pan W, Solomon B, Maness LM, Kastin AJ (2002) Antibodies to beta-amyloid decrease the blood-to-brain transfer of beta-amyloid peptide. *Exp Biol Med* (*Maywood*) **227**, 609-615.

[21] Krentz AJ (2003) Lipoprotein abnormalities and their consequences for patients with type 2 diabetes. *Diabetes Obes Metab* **5** (**Suppl 1**), S19-27.

[22] Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K, Pappolla MA (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* **7**, 321-331.

[23] Silvius JR (2003) Role of cholesterol in lipid raft formation: lessons from lipid model system. *Biochim Biophys Acta* **1610**, 174-183.

[24] Rubinstein R, Genaro AM, Motta A, Cremaschi G, Wald MR (2008) Impaired immune response in streptozotocin-induced type I diabetes in mice. Involvement of high glucose. *Clin Exp Immunol* **154**, 235-246.

[25] Sakowicz M, Szutowicz A, Pawelczyk T (2005) Differential effect of insulin and elevated glucose level on adenosine transport in rat B lymphocytes. *Int Immunol* **17**, 145-154.

[26] Alexiewicz JM, Kumar D, Smogorzewski M, Massry SG (1997) Elevated cytosolic calcium and impaired proliferation of B lymphocytes in type II diabetes mellitus. *Am J Kidney Dis* **30**, 98-104.