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Altered response to mirtazapine on gene expression profile of lymphocytes from Alzheimer's patients

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Abstract

Antidepressants are widely used in the treatment of mood disorders associated with dementia, however little information is available on their effect at the molecular level. We have demonstrated that gene expression profiles of lymphocytes from patients with Alzheimer dementia differ from that seen with controls, with α_2-adrenoceptor being the most highly repressed transcript. To address this issue in light of antidepressant treatment, we used lymphocytes derived from Alzheimer patients and control individuals to assess the impact of mirtazapine, the novel antidepressant with α_2-adrenoceptor antagonistic activities, on gene expression using a cDNA microarray representing 3200 distinct human genes. Sequences that are differentially regulated after treatment with mirtazapine were identified and categorized based on similarities in biological functions. This analysis revealed that selected biological processes, including protein metabolism, cytoskeleton integrity, immune response, cellular plasticity, and neurotransmission, are involved in early phases of administration of this antidepressant. In addition, although it was possible to identify common targets, the expression profiles of Alzheimer lymphocytes differed mainly in their magnitude from those seen with controls. These results confirm the usefulness of the gene array approach for studying Alzheimer-specific changes in the periphery and suggest that the expression of genes of Alzheimer lymphocytes is modulated differently by mirtazapine, which correlates with the pathology.

Keywords: Alzheimer's disease; Antidepressant; Gene expression; Lymphocyte; Microarray; Mirtazapine

1. Introduction

Cerebral β-amyloidosis leads to cellular alterations in the brain of Alzheimer's disease patients. Disruption of ionic homeostasis (Mattson et al., 1992), generation of reactive oxygen species (Hensley et al., 1994), activation of inflammatory response (Arvin et al., 1996) and interfering with various signaling pathways (Williamson et al., 2002) are some of the many cytotoxic actions of amyloids. Based on the biochemical findings in both neural and extraneural tissues, Alzheimer's disease is thought to be a systemic disorder with the most prominent

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pathology in the brain. In light of this, peripheral cells such as lymphocytes were proposed as models for studying the biochemistry and molecular biology of the central nervous system and for investigating possible systemic derangements in various neuropsychiatric disorders (Dolman, 1984).

In line with that, we have previously reported that 20 of the 3200 sequences on a broadly representative cDNA microarray were differentially expressed when lymphocytes from demented patients were compared with those from healthy individuals (Kálmán et al., 2004). We demonstrated that the most highly repressed gene in Alzheimer's disease cells encoded for the α_{2C}-adrenoceptor; selected genes involved in immune response were also down-regulated.

Although cognitive and functional decline is the hallmark of the disease, recent consensus-based data from clinical settings suggest that psychiatric disturbances afflict approximately 90% of Alzheimer's disease patients over the course of their illness (Lyketsos et al., 2000). Roughly half of the Alzheimer's disease population suffers from mood disorders in which the use of antidepressants is required (Olin et al., 2002).

Low serotonergic and noradrenergic activities are associated with mood disorders (Duman et al., 1997). Importantly, these neurotransmitter alterations, along with profound cholinergic depletion, are also implicated in Alzheimer's disease (Berger, 1984; Bowen et al., 1983).

Apart from serotonin (5-hydroxytryptamine, 5HT) and noradrenalin, control of mood and behavior involves many different neurotransmitter systems, including glutamate, γ-amino-butyric-acid (GABA), acetylcholine, dopamine, and neuropeptides. The pharmacological profile of the novel noradrenergic and selective serotonergic antidepressant mirtazapine is characterized by potent α_2-adrenergic, $5HT_2$-, $5HT_3$-, and histamine H_1-receptor antagonistic activities. Therefore, it increases both noradrenergic and serotonergic neurotransmission via the blockade of the central α_2-autoreceptors, and the increased release of 5HT stimulates only the $5HT_1$-receptors ($5HT_1$-R). Because α_{2C}-adrenoceptors are down-regulated in Alzheimer's disease lymphocytes, we investigated the impact of the novel noradrenergic and selective serotonergic antidepressant—with α_2-receptor antagonistic activity on white blood cells.

Based on the reported abnormalities in lymphocyte physiology, function and differing gene expression in Alzheimer's disease, we used the microarray technique to address the question whether the gene expression profile of Alzheimer's disease lymphocytes responds differently to mirtazapine than control cells. DNA microarray technology was used because it is the most informative and most effective functional method among molecular biological techniques regarding the time and work necessity for analyzing gene-expression changes in diverse clinical samples (Nees and Woodworth, 2001).

2. Methods

2.1. Participants

Thirty-two individuals were carefully selected from a 150-resident hospice for the elderly. Being from the very same nursing home, all of them were on the same diet and their daily activities were also very similar. All participants underwent a thorough medical, including neurological examination, computed tomography (CT) or magnetic resonance imaging (MRI scan). Their routine laboratory tests were within the normal values. All of them had normal blood chemistry, white blood cell count, differential blood count and sedimentation rate. None of them had history of any kind of immune or autoimmune disorders. None of them suffered from acute or chronic inflammation during the experiments.

In case of every individual, the use of psychotropic medications—e.g., cholinesterase inhibitors, nootropic agents, anxiolytics, antidepressants—or other drugs known to interfere with the immune system were terminated 6 weeks prior to and during the experiments. All participants were free of any medication during the study, including vitamins or antioxidants. All of them were abstinent and non-smokers.

Sixteen probable Alzheimer's disease patients participated in the study. All of them met the criteria of International Classification of Diseases (ICD-10) and NINCDS-ADRDA for probable Alzheimer's disease. The clinical diagnosis of Alzheimer's disease was not confirmed neuropathologically. They were all considered sporadic, late-onset Alzheimer's disease cases (onset of symptoms >65 years; family history negative for neuro-psychiatric disorders). Probable Alzheimer's disease patients had moderate dementia syndrome (MMSE score: 16 ± 5.1 points).

Sixteen participants were considered non-demented, non-depressed healthy elderly control persons based on their former medical history, somatic and neuropsychiatric examination, CT or MRI scans. Their Mini-Mental State Exam (MMSE) score was higher than 26; therefore, none of them was considered demented. None of them was diagnosed with depression, based on their Beck Depression Inventory scores and neuro-psychiatric history.

All subjects gave informed consent to participate according to the Declaration of Helsinki. The study protocol was approved by the local Human Biomedical Research Ethics Committee (IRB).

2.2. Treatment

To monitor the effect of the noradrenergic and selective serotonergic antidepressant, eight participants from the control group (50%) and eight patients from the Alzheimer's disease group (50%) were orally treated with 30 mg of mirtazapine (Remeron®; Organon).

2.3. Samples and RNA preparation

Total RNAs were purified from lymphocytes obtained from 5 ml of blood from each individual with NucleoSpin RNA purification kit (Macherey-Nagel, Dürren, Germany) according to the manufacturer's instructions. Total RNA of each individual was pooled according to control and Alzheimer's disease groups. Four control and four Alzheimer's disease groups were made, each containing four individuals. The quantity and the quality of all RNA preparations were assessed by gel electrophoresis and OD_{260}/OD_{280} ratios. Total RNA was used for microarray analysis as well as for reverse transcription quantitative polymerase chain reaction (PCR).

2.4. Microarray protocols

2.4.1. Construction of microarrays

Construction and use of microarrays were performed as described previously (Kitajka et al., 2002). Briefly, 3200 cDNA inserts from human cDNA libraries (peripherial ganglion, brain, heart, and mixed tissue libraries) were amplified, and purified with MultiScreen-PCR plate (Millipore), resuspended in 50% dimethyl-sulfoxide/water, and arrayed on amino-silanized slides (Sigma-Aldrich) by using a MicroGrid Total Array System (BioRobotics, Cambridge, UK) spotter with 16 pins in a 4×4 format. DNA elements were deposited in duplicate. The diameter of each spot was approximately 200 μm. After printing, DNA was ultraviolet (UV) cross-linked to the slides (Stratagene, Stratalinker, 700 mJ). Prior to hybridization, the slides were blocked in 1× sodium-chloride–sodium-citrate buffer (SSC), 0.2% sodium-dodecyl-sulphate (SDS), 1% bovine serum albumin (BSA) for 30 min at 42 °C, rinsed with water and dried.

2.4.2. Microarray probe preparation and hybridization

Three micrograms of total RNA from each sample was amplified by a linear antisense RNA amplification method, and labeled with Cy3 or Cy5 fluorescent dye during reverse transcription as described previously (Puskás et al., 2002a). Briefly, 2.5 μg of amplified RNA was labeled in a reaction, which contained 0.4 μM random nonamers, 0.1 mM d(G/T/A)TPs, 0.05 mM desoxy-cytosine 5′ -triphosphate (dCTP) (Amersham Pharmacia Biotech, UK), 20 units of RNAsin (Fermentas, Vilnius, Lithuania), 1× first-strand buffer, 200 units of RNAse H(−) point mutant murine myeloid leukemia virus (M-MLV) reverse transcriptase (Fermentas), and 0.05 mM Cy3-dCTP or Cy5-dCTP (NEN Life Science Products, Boston, MA) in a 20-μl total volume. The RNA, primer and RNAsin were denatured at 80 °C for 5 min and cooled on ice before adding the remaining reaction components. After 2 h of incubation at 37 °C, the hetero-duplexes were purified for recovery as described (Puskás et al., 2002b), denatured and the mRNA was hydrolyzed with NaOH for 15 min at 37 °C and neutralized with 3-(*N*-morpholino)-propanesulphonate (MOPS, pH: 6.0). The labeled cDNA was purified with a PCR purification kit (Macherey-Nagel) according to the manufacturer's instructions. Probes, generated from control and Alzheimer's disease samples, were mixed and dissolved in 15 μl of hybridization buffer (50% formamide, 5× SSC, 0.1% SDS, 100 mg/ml salmon sperm DNA) and applied onto the array after denaturation by heating for 1 min at 90 °C. The slide was covered by a 24×32-mm coverslip and sealed with DPX mountant (Fluka, Buchs, Switzerland) in order to prevent evaporation. Slides were incubated at 42 °C for 20 h in a humid hybridization chamber. After hybridization, the mountant was removed and the arrays were washed as published (Puskás et al., 2002a).

2.4.3. Scanning and data analysis

Each array was scanned under a green laser (532 nm for Cy3 labeling) and under a red laser (660 nm for Cy5 labeling) by using a ScanArray Lite (GSI Lumonics, Billerica, MA) scanning confocal fluorescent microscope with 10-μm resolution. Image analysis was performed by the ScanAlyze2 software (http://www.microarrays.org/software.html). Each spot was defined by manual positioning of a grid of circles over the image. The average signal intensities of the replica spots were determined after subtraction of the local background intensities. From each sample, a replica experiment was performed and the average ratio was used for data analysis.

2.5. Expression profile verification

Real-time quantitative reverse transcription-PCR (QRT-PCR) was performed on a RotorGene 2000 instrument (Corbett Research, Sydney, Australia) with gene-specific primers and SybrGreen protocol to confirm the gene expression changes observed by microarrays. Two micrograms of total RNA from each sample was reverse transcribed in the presence of poly(dT) sequences in a total volume of 20 μl. After dilution with 20 μl of water, 1 μl of the diluted reaction mix was used as template in QRT-PCR. The 20-μl reaction volume contained 0.2 mM of desoxynucleotide 5′ -triphosphate (dNTP), 1× PCR reaction buffer (ABGene, Epsom, UK), 6 mM of each primer, 4 mM of $MgCl_2$, 1× SYBR Green I (Molecular Probes, Eugene, OR) at final concentration, and 0.5 units of thermostart Taq DNA polymerase (ABGene). The amplification was carried out with the following cycling parameters: 360-s heat start, 45 cycles of denaturation at 95 °C for 25 s, annealing at 60 °C for 25 s and fluorescence detection at 77 °C for 15 s. Relative expression ratios were normalized to β-actin.

2.6. Statistical analysis

GenePix Pro3.0 software and Excel for Windows programs were utilized for data analysis of gene expression,

Table 1
Characteristics of control and Alzheimer's disease cases treated with mirtazapine and selected for microarray analysis (average±S.D.)

	Control, n=8	Alzheimer's disease, n=8
Age (years)	76±5.7	78±3.5
Gender (male/female)	2/6	3/5
Education (years)	6–12	6–12
MMSE score	28±0.8	16±6.3[a]
Clock Drawing Test score	6±0.7	3.4±1.6[a]

[a] P<0.0001.

and the program SPSS 9.0 for Windows was used for statistical analysis of QRT-PCR results. The age, MMSE and Clock Drawing Test (CDT) results are expressed as mean±S.D. The differences between these values were assessed by t-test for independent samples. The significance was set at P<0.05. Each and every data presented in this study is considered significant.

Table 2
List of differentially expressed genes in human control lymphocytes after treatment with mirtazapine

Clone name (mRNA)	Δ Fold (mean)	Accession number
Human G0S3 mRNA, complete cds	0.263228844	L49169
Human scaffold protein Pbp1 mRNA, complete cds	0.301676081	AF000652
Homo sapiens bet3 (BET3) mRNA, complete cds	0.337401457	AF041432
Epidermal growth factor	0.348358414	X04571
Formyl peptide receptor-like 2	0.418960512	D10922
Ubiquitin-conjugating enzyme E2B (RAD6 homolog)	0.428366172	M74525
H. sapiens Pyrin (MEFV) mRNA, complete cds	0.433999349	AF018080
Tumor necrosis factor-inducible protein tsg-6 precursor	0.434799342	M31165
Zinc finger protein 2 (A1-5)	0.442731034	X78925
Image EST	0.447361855	R78712
Peptidyl-prolyl *cis*-trans isomerase, mitochondrial precursor	0.462015869	M80254
Human mRNA for KIAA0227 gene, partial cds	2.185085633	D86980
CD3G antigen, gamma polypeptide (TiT3 complex)	2.19257437	X04145
Proliferation-associated gene A (natural killer-enhancing factor A)	2.202196919	X67951
T-cell receptor, beta cluster	2.282518168	K01571
Mannose phosphate isomerase	2.335706486	X76057
60S ribosomal protein L41	2.414130367	Z12962
Human ring zinc-finger protein (ZNF127-Xp) gene and 5′ flanking sequence	2.625917018	U41315
Finkel–Biskis–Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived)	2.628341865	X65923
Human mRNA for NADPH-flavin reductase, complete cds	2.868339481	D32143

3. Results

The demographic and clinical characteristics of participants in are summarized in Table 1. There were no significant differences between the control and Alzheimer's disease groups with respect their demographic variables. The Alzheimer's disease group's performance was significantly worse in both neuro-cognitive screening tests: MMSE and Clock Drawing Test.

Among the 3200 genes examined in the present study, the expression of only 46 genes (1.437%) was found to be significantly altered after treatment with mirtazapine. In case

Table 3
List of differentially expressed genes in human lymphocytes derived from Alzheimer patients after treatment with mirtazapine

Clone name (mRNA)	Δ Fold (mean)	Accession number
H. sapiens defensin 5 gene, complete cds	1.108728367	M97925
Human cell death protein (*rip*) mRNA, partial cds	0.763355907	U50062
Tumor necrosis factor-inducible protein TSG-6 precursor	0.779006772	M31165
Carbonic anhydrase II	0.855154251	J03037
Putative DNA-binding protein A20	1.270504138	M59465
H. sapiens stimulator of Fe-transport mRNA, complete cds	0.726222724	AF020761
$\alpha_{2C\text{-}1}$ adrenoceptor	1.062829195	J03853
Human retinoic acid-responsive protein (NN8-4AG) mRNA, complete cds	0.939051919	U50383
Human mRNA for KIAA0059 gene, complete cds	2.526335591	D31883
H. sapiens HP protein (HP) mRNA, complete cds	1.060195636	AF035119
Human BAC clone GS025M02 from 7q21-q22	1.017682468	AC002540
H. sapiens mRNA for villin-like protein, complete cds	1.031602709	D88154
EST	1.090293454	AA610122
Cysteine-rich protein	1.090293454	M33146
Bone morphogenetic protein 4	1.086907449	M22490
H. sapiens atrophin-1 interacting protein 4 (AIP4) mRNA, partial cds	0.94055681	AF038564
EST	1.069601204	AA938448
H. sapiens torsinB (DQ1) mRNA, partial cds	1.009029345	AF007872
EST	0.920617005	AA918616
Homolog of *Drosophila* slowpoke (potassium channel, calcium-activated)	1.019563582	U13913
H. sapiens mitotic feedback control protein Madp2 homolog mRNA, complete cds	1.097817908	U65410
EST	1.100075245	AA845426
Glucosaminyl (*N*-acetyl) transferase 1, core 2 (β-1,6-*N*-acetylglucosaminyltransferase)	1.0331076	M97347
EST	1.220466516	AA648525
H. sapiens putative tumor suppressor protein unspliced form (Fus-2) mRNA, complete cds	0.92400301	AF040705
EST	1.124153499	AA886792

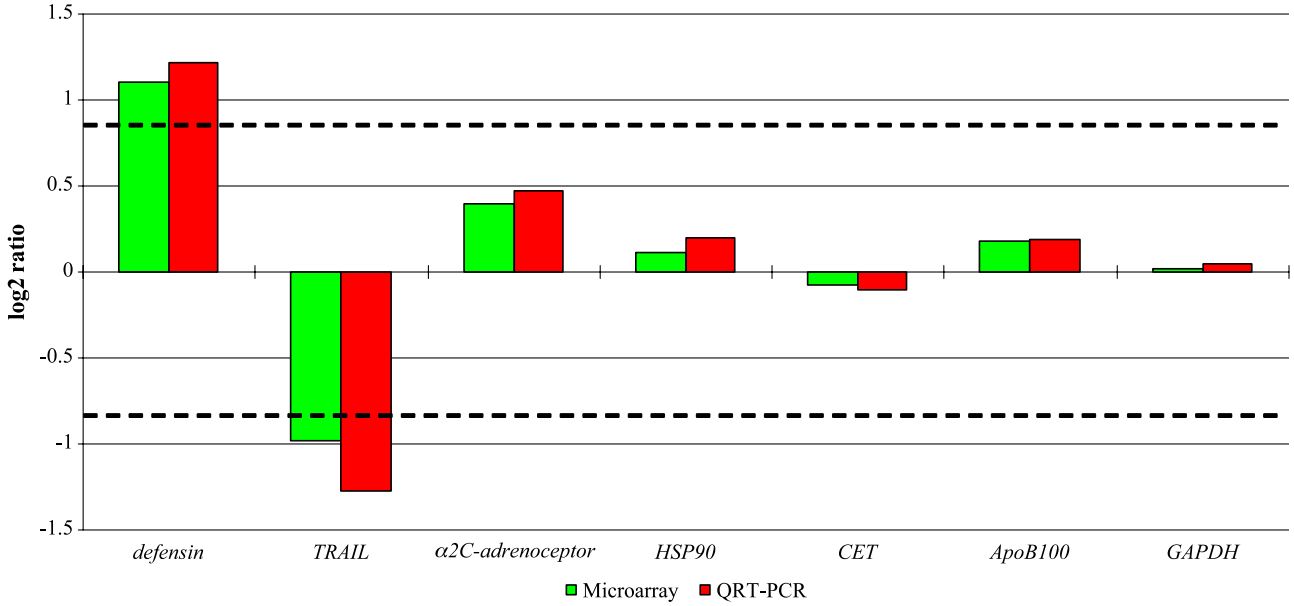

Fig. 1. Quantitative determination of transcript levels by real-time PCR. Changes in transcript levels in lymphocytes after treatment of Alzheimer's patients with mirtazapine (green box) were confirmed by real-time PCR (red box). The expression of defensin, TNF-related apoptosis inducing ligand (TRAIL), α_{2C}-adrenoceptor, heat-shock protein 90 (HSP90), cholesteryl-ester transfer-protein (CET), apolipoprotein B100 ($ApoB_{100}$), and glyceraldehyde-phosphate-dehydrogenase (GAPDH) was determined. Dashed lines indicate the interval −1.8- to 1.8-fold regulation (corresponding to $\log_2$=0.85) in which changes in expression were considered not significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of control cells, 9 genes showed down-regulation in their expression while 11 were over-expressed. Alzheimer lymphocytes, however, responded to mirtazapine by repressing 7 and inducing 19 genes. The list of genes with significantly altered expression is delineated in Tables 2 and 3. Values are presented as the expression ratio of treated/untreated cases for a certain gene.

To validate these semiquantitative results, the expression of seven genes was measured by real-time quantitative PCR (QRT-PCR) in cDNA samples derived from lymphocytes. Defensin, TNF-related apoptosis inducing ligand (TRAIL), and α_{2C}-adrenoceptor—all down-regulated in Alzheimer's disease lymphocytes—and three other genes coding for proteins that are differentially expressed in Alzheimer's disease brain or have been correlated to Alzheimer's disease—heat-shock protein 90 (hsp90), cholesteryl-ester transfer-protein (CET), and apolipoprotein B100 ($ApoB_{100}$)—were selected. An additional control gene, glyceraldehyde-phosphate-dehydrogenase (GAPDH), was also chosen for QRT-PCR. Relative expression ratios did not significantly differ from and were in accordance with the expression of the respective gene (Fig. 1). Therefore, the differential expression of genes revealed by microarray analysis of human lymphocytes was truly confirmed by QRT-PCR technique.

4. Discussion

Treatment with mirtazapine changed the gene expression profile of both control and Alzheimer's disease lymphocytes. Because the drug apparently does not have an impact on the expression of selected genes that would have been altered as a result of general toxicity, we conclude that the administration of mirtazapine at the dose used does not exert toxic effect. Therefore, the results observed in this study reflect a real effect of this noradrenergic and selective serotonergic antidepressant, and not a response to toxicity or drug-induced stress.

The most highly induced genes in control lymphocytes had been identified as being involved in immune functions. Cells exhibited up-regulation of natural killer-enhancing factor A, along with induction of subunits of cluster of differentiation antigen 3G (CD3G antigen) and T-cell receptors. A number of genes, whose expression is associated with apoptosis and metabolism in general, were down-regulated. This might be attributable to mirtazapine's impact on the serotonergic system.

5HT plays roles as a neurotransmitter and a neuromodulator with functions in both the nervous system and in the periphery (Mossner and Lesch, 1998). It has been found to influence several functions of the immune system (Plaut, 1987), notably activation of natural killer (NK)-cells, macrophages, T-cells and pre-B lymphocytes (Aune et al., 1990; Bonnet et al., 1984; Hellstrand and Hermodsson, 1987). Modulation of the immune system by 5HT occurs via membrane receptors, i.e. $5HT_{1A}$ and $5HT_3$, present on lymphocytes (Ferriere et al., 1996; Meyniel et al., 1997). Moreover, the mammalian lymphoid organs such as spleen and thymus are innervated with serotonergic and catecholaminergic neurons, suggesting that leukocytes are directly

exposed to 5HT flow even at early stage of differentiation (Bellinger et al., 1988). Another way to influence the functions of immune cells is the uptake of this biogenic amine from the extracellular environment. A high-affinity serotonin transporter, sensitive to inhibition by a selective group of antidepressants, has been reported in different cell types such as platelets (Olson et al., 1974), lymphocytes (Faraj et al., 1993), endothelial cells (Myers et al., 1989), intestinal brush-border vesicles (Ramamoorthy et al., 1993) and human placental choriocarcinoma cells (Cool et al., 1991). However, mirtazapine might have an immune-modulatory effect by not only acting on the serotonergic system, but also by increasing noradrenergic transmission via the adapting α_2-antagonism.

The function of a given cell is influenced by different types of receptors, which commonly do not operate independently from each other; thus, activation of a first type of receptor may influence the effect mediated by a second type of receptor. Such interactions have been extensively studied for noradrenergic axon terminals. They are endowed with receptors by which the released noradrenalin inhibits (via an α_2-adrenoceptor) its own release. In addition to the α_2-adrenoceptor (the autoreceptor) (Starke et al., 1989), the noradrenergic nerve terminals possess heteroreceptors by which mediators and transmitters (other than noradrenalin) released from adjacent neurones or borne with the bloodstream influence noradrenalin release (Vizi et al., 1991; Westfall and Martin, 1991). Activation of α_2-receptors leads to adrenergic suppression of peripheral blood T-cell reactivity (Felsner et al., 1995). α-Adrenergic treatment reduces the number of all lymphocyte classes except NK cells (Stevenson et al., 2001), which respond very differently from T cells to catecholamine. NK cells respond by decreasing their β_2- and α_1-adrenoceptor numbers, while T cells respond by increasing their α_2-receptors (Jetschmann et al., 1996). This is in line with our finding indicating that mirtazapine differently induced genes associated with NK- or T-cell functions, e.g. natural-killer-enhancing factor A, CD3G antigen, etc.

Oxidative damage and enhanced vulnerability to apoptosis have been reported in Alzheimer's disease lymphocytes (Eckert et al., 1998; Mórocz et al., 2002). α_2-Agonists have been shown to potentiate apoptosis in splenocytes (Haberfeld et al., 1999). Because the effect mediated by many types of heteroreceptors is increased by simultaneous blockade of the α_2-autoreceptors, down-regulation of α_2-adrenoceptors might be a functional compensatory mechanism in Alzheimer's disease to promote survival of immune cells. This effect is supported by the administration of mirtazapine to block α_2-autoreceptors which, together with inducing defensin, and repressing human cell death protein *rip* and tumor necrosis factor (TNF)-associated apoptosis inducer TSG-6, increase survival of cells. Moreover, mirtazapine apparently normalizes the expression of α_2-receptor, which is markedly repressed in Alzheimer's disease.

Stress clearly perturbs immune function, as does mood and emotion. The existence of bi-directional communication among the immune, neuroendocrine and central nervous systems is beyond dispute, even if current insight probably represents just the tip of an iceberg (Serafeim and Gordon, 2001). This interaction has been documented at many levels. Main players mooted responsible for such crosstalk between mind and body are direct nervous connection in lymphoid organs (Felten et al., 1987), modulation of the hypothalamic–pituitary–adrenal axis by neurotransmitters and/or cytokines (Merrill and Jonakait, 1995), by the production of cytokines in the central nervous system and, in turn, by the expression of neural markers in a variety of cells of the immune system (Koenig, 1991). Therefore, the use of antidepressants such as mirtazapine might beneficially affect both mood and immune functions.

5. Conclusion

The gene expression profile analysis of Alzheimer's disease brains revealed that genes implicated in signal transduction, energy metabolism, stress response, synaptic vesicle transport, calcium homeostasis, and cytoskeletal activities are down-regulated during the neurodegenerative process (Loring et al., 2001; Pasinetti, 2001). Alzheimer's disease lymphocytes, as we have previously reported (Kálmán et al., 2004), also exhibit similar changes. Some of the disease-specific alterations in the periphery, most conspicuously the decreased activities of immune-responses associated with Alzheimer's disease, are efficiently counterbalanced by mirtazapine at the gene expression level.

Because gene expression profile and the response to certain drugs of lymphocytes at the nucleic acid level might differ from those seen in the brain, it is unlikely that the very same changes could be observed in neurons. However, differences in the impact of drug administration on Alzheimer's disease and control lymphocytic gene expression might help us understand the systemic nature of the disorder and might be a useful lead to identify Alzheimer-specific markers both in the central nervous system and in the periphery.

DNA-chips, however, like any other methods, do have a number of limitations that exist in interpretation of the data from cDNA array studies. These include possibility of false negative results due to low specificity; downregulation of genes in one subset of cell-type (e.g. T-killer, T-cytotoxic, B-cell, etc.) may well be masked by upregulation of the same gene in another subgroup. In addition, a possible dissociation of gene and protein expression might mislead in understanding certain molecular processes. Despite these limitations, cDNA micro-chips remain useful tools for identifying novel Alzheimer's disease biomarkers from lymphocytes.

References

Arvin, B., Neville, L.F., Barone, F.C., Feuerstein, G.Z., 1996. The role of inflammation and cytokines in brain injury. Neurosci. Biobehav. Rev. 20, 445–452.

Aune, T.M., Kelley, K.A., Ranges, G.E., Bombara, M.P., 1990. Serotonin-activated signal transduction via serotonin receptors on Jurkat cells. J. Immunol. 145, 1826–1831.

Bellinger, D.L., Felten, S.Y., Felten, D.L., 1988. Maintenance of non-adrenergic sympathetic innervation in the involuted thymus of the aged Fisher 344 rat. Brain Behav. Immun. 2, 133–150.

Berger, B., 1984. Neurotransmitter anomalies in Alzheimer's disease. Rev. Neurol. 10, 539–552.

Bonnet, M., Lespinats, G., Burtin, C., 1984. Histamine and serotonin suppression of lymphocyte response to phytohemagglutinin and allogenic cells. Cell. Immunol. 83, 280–291.

Bowen, D.M., Allen, S.J., Benton, J.S., Goodhardt, M.J., Haan, E.A., Palmer, A.M., Sims, N.R., Smith, C.C., Spillane, J.A., Esiri, M.M., Neary, D., Snowdo, J.S., Wilcock, G.K., Davison, A.N., 1983. Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. J. Neurochem. 41, 266–272.

Cool, D.R., Leibach, F.H., Bhalla, V.B., Mahesh, V.B., Ganapathy, V., 1991. Expression and cyclic AMP-dependant regulation of a high affinity serotonin transporter in the human placental choriocarcinoma cell line (JAR). J. Biol. Chem. 266, 15750–15757.

Dolman, C.L., 1984. Diagnosis of neurometabolic disorders by examination of skin biopsies and lymphocytes. Semin. Diagn. Pathol. 1, 82–97.

Duman, R.S., Heninger, G.R., Nestler, E.J., 1997. A molecular and cellular theory of depression. Arch. Gen. Psychiatry 54, 597–606.

Eckert, A., Cotman, C.W., Zerfass, R., Hennerici, M., Muller, W.E., 1998. Enhanced vulnerability to apoptotic cell death in sporadic Alzheimer's disease. NeuroReport 9, 2443–2446.

Faraj, B.A., Olkowski, Z.L., Jackson, R.T., 1993. A high affinity serotonin transporter in human lymphocytes. FASEB J. 7, A264–A272.

Felsner, P., Hofer, D., Rinner, I., Porta, S., Korsatko, W., Schauenstein, K., 1995. Adrenergic suppression of peripheral blood T cell reactivity in the rat is due to activation of peripheral α2-receptors. J. Neuroimmunol. 57, 27–34.

Felten, D.L., Felten, S.Y., Bellinger, D.L., Carlsson, S.L., Ackerman, K.D., Madden, K.S., Olschowka, J.A., Livnat, S., 1987. Noradrenergic sympathetic neuronal interactions with the immune system: structure and function. Immunol. Rev. 100, 225–260.

Ferriere, F., Khan, N.A., Troutaud, D., Deschaux, P., 1996. Serotonin modulation of lymphocyte proliferation via 5-HT_{1A} receptors in rainbow trout (*Oncorhynchus mykiss*). Dev. Comp. Immunol. 20, 273–283.

Haberfeld, M., Johnson, R.O., Ruben, L.N., Clothier, R.H., Shiigi, S., 1999. Adrenergic modulation of apoptosis in splenocytes of *Xenopus laevis* in vitro. Neuroimmunomodulation 6, 175–181.

Hellstrand, K., Hermodsson, S., 1987. Role of serotonin in the regulation of human natural killer cell cytotoxicity. J. Immunol. 139, 869–875.

Hensley, K., Carney, J.M., Mattson, M.P., Aksenova, M., Harris, M., Wu, J.F., Floyd, R.A., Butterfield, D.A., 1994. A model for β-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 91, 3270–3274.

Jetschmann, J.U., Benschop, R.J., Jacobs, R., Kemper, A., Oberback, R., Schmidt, R.E., Schedlowski, M., 1996. Expression and in vivo modulation of α- and β-adrenoceptors on human natural killer (CD16+) cells. J. Neuroimmunol. 74, 159–164.

Kálmán, J., Kitajka, K., Pákáski, M., Zvara, Á., Juhász, A., Vincze, G., Janka, Z., Puskás, L.G., 2004. Gene expression profile analysis of lymphocytes from Alzheimer's patients. Psych. Genet. (in press).

Kitajka, K., Puskás, L.G., Zvara, Á., Hackler Jr., L., Barcelo-Coblijn, G., Yeo, Y.K., Farkas, T., 2002. The role of *n*−3 polyunsaturated fatty acids in brain: modulation of rat brain gene expression by dietary *n*−3 fatty acids. Proc. Natl. Acad. Sci. U. S. A. 99, 2619–2624.

Koenig, J.I., 1991. Presence of cytokines in the hypothalamic pituitary axis. Prog. Neuroendocrinol. Immunol. 4, 143–153.

Loring, J.F., Wen, X., Lee, J.M., Seilhamer, J., Somogyi, R., 2001. A gene expression profile of Alzheimer's disease. DNA Cell Biol. 20, 683–695.

Lyketsos, C.G., Steinberg, M., Tschantz, J., Norton, M., Steffens, D., Breitner, J.C.S., 2000. Mental and behavioral disturbances in dementia: findings from the Cache County Study on Memory in Aging. Am. J. Psychiatry 157, 708–714.

Mattson, M.P., Cheng, D., Davis, D., Bryant, K., Lieberburg, I., Rydel, R.E., 1992. β-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J. Neurosci. 12, 376–389.

Merrill, J.E., Jonakait, G.M., 1995. Interactions of the nervous and immune systems in development, normal brain homeostasis, and disease. FASEB J. 9, 611–618.

Meyniel, J.P., Khan, N.A., Ferriere, F., Deschaux, P., 1997. Identification of lymphocyte $5HT_3$ receptor subtype and its implications in fish T-cell proliferation. Immunol. Lett. 55, 151–160.

Mórocz, M., Kálmán, J., Juhász, A., Sinkó, I., McGlynn, A.P., Downes, C.S., Janka, Z., Raskó, I., 2002. Elevated levels of oxidative DNA damage in lymphocytes from patients with Alzheimer's disease. Neurobiol. Aging 23, 47–53.

Mossner, R., Lesch, K.P., 1998. Role of serotonin in the immune system and in neuroimmune interactions. Brain Behav. Immun. 12, 249–271.

Myers, C.L., Lazo, J.S., Pitt, B.R., 1989. Translocation of protein kinase C is associated with inhibition of 5HT uptake by cultured endothelial cells. Am. J. Physiol. 257, L253–L258.

Nees, M., Woodworth, C.D., 2001. Microarrays: spotlight on gene function and pharmacogenomics. Curr. Cancer Drug Targets 1, 155–175.

Olin, J.T., Schneider, L.S., Katz, I.R., Meyers, B.S., Alexopoulos, G.S., Breitner, J.C., Bruce, M.L., Caine, E.D., Cummings, J.L., Devanand, D.P., Krishnan, K.R., Lyketsos, C.G., Lyness, J.M., Rabins, P.V., Reynolds III, C.F., Rovner, B.W., Steffens, D.C., Tariot, P.N., Lebowitz, B.D., 2002. Provisional diagnostic criteria for depression of Alzheimer disease. Am. J. Geriatr. Psychiatry 10, 125–128.

Olson, P.S., Ljungvist, U., Bergentz, S.E., 1974. Analysis of platelet, red cell and fibrin content in experimental arterial and venous thrombi. Thromb. Res. 5, 1–4.

Pasinetti, G.M., 2001. Use of cDNA microarray in the search for molecular markers involved in the onset of Alzheimer's disease dementia. J. Neurosci. Res. 65, 471–475.

Plaut, M., 1987. Lymphocyte hormone receptors. Annu. Rev. Immunol. 5, 621–629.

Puskás, L.G., Hackler Jr., L., Kovács, G., Kupihár, Z., Zvara, Á., Micsik, T., van Hummelen, P., 2002. Recovery of cyanine-dye nucleotide triphosphates. Anal. Biochem. 305, 279–281.

Puskás, L.G., Zvara, Á., Hackler Jr., L., van Hummelen, P., 2002. Comparison of different sample amplification methods for micro-array gene expression analysis. RNA amplification results in reproducible microarray data with slight ratio bias. BioTechniques 32, 1330–1340.

Ramamoorthy, S., Cool, D.R., Mahesh, V.B., Leibach, F.H., Ganapathy, V., 1993. Regulation of the human serotonin transporter. J. Biol. Chem. 268, 21626–21631.

Serafeim, A., Gordon, J., 2001. The immune system gets nervous. Curr. Opin. Pharmacol. 1, 398–403.

Starke, K., Göthert, M., Kilbinger, H., 1989. Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol. Rev. 69, 864–989.

Stevenson, R.J., Westermann, J., Liebmann, P.M., Hörtner, M., Rinner, I., Felsner, P., Wölfler, A., Schauenstein, K., 2001. Prolonged α-adrenergic stimulation causes changes in leukocyte distribution and lymphocyte apoptosis in the rat. J. Neuroimmunol. 120, 50–57.

Vizi, E.S., Kiss, J., Elenkov, I.J., 1991. Presynaptic modulation of cholinergic and noradrenergic neurotransmission: interaction between them. News Physiol. Sci. 6, 119–123.

Westfall, T.C., Martin, J.R., 1991. Presynaptic receptors in the peripheral and central nervous systems. In: Feigenbaum, J., Hanani, M. (Eds.), Presynaptic Regulation of Neurotransmitter Release: A Handbook. Freund Publishing House, Tel Aviv, pp. 311–370.

Williamson, R., Scales, T., Clark, B.R., Gibb, G., Reynolds, C.H., Kellie, S., Bird, I.N., Varndell, I.M., Sheppard, P.W., Everall, I., Anderton, B.H., 2002. Rapid tyrosine phosphorylation of neuronal proteins dincluding tau and focal adhesion kinase in response to amyloid-β peptide exposure: involvement of Src family protein kinases. J. Neurosci. 22, 10–20.