Results

Microarray analysis identified differentially expressed genes in peripheral blood lymphocytes between MS and the controls

Among 1,258 genes examined, 173 genes in T cell fraction and 50 genes in non-T cell fraction were expressed differentially between 72 MS patients and 22 CN subjects (see the supplemental table for all of these genes). In T cell fraction, 25 genes were upregulated, while 148 genes were downregulated in MS. In non-T cell fraction, 11 genes were upregulated, while 39 genes were downregulated in MS. Thus, downregulated genes greatly outnumbered upregulated genes in MS. No genes showed an opposed pattern of regulation between T cell and non-T cell fractions. The top 30 most significant genes are listed in Tables 2 and 3, and the corresponding box and whisker plots are shown in Figs. 1 and 2. Among them, six genes, such as regulator of G protein signaling 14 (RGS14), SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 3 (SMARCA3), transcription factor 17 (TCF17), carbohydrate sulfotransferase 4 (CHST4), cytochrome c oxidase assembly protein (COX15), and death-associated protein 6 (DAXX) were downregulated coordinately in both cell fractions.

The majority of top 30 differentially expressed genes between MS and the controls were categorized into apoptosis signaling-related genes

In T cell fraction, the top 30 contained 25 genes closely related to apoptosis signaling (Table 2). They included upregulation in MS of nuclear receptor subfamily 4, group A, member 2 (NR4A2; No. 1, Table 2), transcription factor 8 (TCF8; No. 2), and cytochrome P450 family 1, subfamily A, polypeptide 2 (CYP1A2; No. 3). They also included downregulation in MS of RGS14 (No. 4), mitogen-activated protein kinase 1 (MAPK1; No. 6), SMARCA3 (No. 7), TCF17 (No. 9), heat shock 70-kD protein 1A

(HSPA1A; No. 10), TRAIL (No. 12), topoisomerase 1 (TOP1; No. 13), protein tyrosine phosphatase, non-receptor type 6 (PTPN6; No. 14), chemokine, CC motif, receptor 5 (CCR5; No. 15), v-erb-a erythroblastic leukemia viral oncogene homolog 4 (ERBB4; No. 17), transcription factor 21 (TCF21; No. 18), ATPase, hydrogen-transporting, lysosomal, 56/58kD, V1 subunit B, isoform 2 (ATP6V1B2; No. 19), cAMP responsive element-binding protein 1 (CREB1; No. 20), integrin, beta 1 (ITGB1; No. 21), COX15 (No. 22), Myc protooncogene (MYC; No. 23), BCL2-associated athanogene 1 (BAG1; No. 24), cell division cycle 16 (CDC16, No. 25), DAXX (No. 27), TGFβ-stimulated gene 22 (TSC22; No. 28), GA-binding protein transcription factor, beta subunit 1 (GABPB1; No. 29), and poly(ADP-ribose) polymerase (PARP; No. 30). Surprisingly, the top 30 included none of Th1-specific marker genes except for CCR5. The concurrent downregulation of proapoptotic and antiapoptotic genes, such as TRAIL, DAXX, and BAG1, suggests that T cells of MS show the gene expression pattern which represents a counterbalance between promoting and preventing apoptosis.

In non-T cell fraction, the top 30 included 27 apoptosis signaling-related genes (Table 3). They included upregulation in MS of intercellular adhesion molecule-1 (ICAM1; No. 1, Table 3), cell division cycle 42 (CDC42; No. 2), receptor-interacting serine/threonine kinase 2 (RIPK2; No. 3), Max dimerization protein (MAD; No. 5), chemokine, CXC motif, ligand 2 (CXCL2; No. 6), silencer of death domains (SODD; No. 7), and topoisomerase 2 alpha (TOP2A; No. 8). They also included downregulation in MS of SMARCA3 (No. 9), RGS14 (No. 10), COX15 (No. 11), A-kinase anchor protein 11 (AKAP11; No. 12), TCF17 (No. 13), cell division cycle 25B (CDC25B; No. 14), granzyme A (GZMA; No. 15), BCL2 (No. 17), complement component receptor 2 (CR2; No. 18), replication protein A1 (RPA1; No. 19), RNA polymerase II, subunit H (POLR2H; No. 20), E2F transcription factor 5 (E2F5; No. 21), Ras associated protein RAB7-like 1 (RAB7L1; No. 22), nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 3 (NFATC3; No. 23), heat shock 70-kD protein-like 1 (HSPA1L; No. 24), retinoblastoma-binding protein 4 (RBBP4; No. 25), protein kinase, DNA-activated, catalytic subunit (PRKDC; No. 26), Ras association domain family 1

(RASSF1; No. 27), DAXX (No. 29), and epidermal growth factor (EGF; No. 29). The simultaneous upregulation of proapoptotic and antiapoptotic genes, such as RIPK2, MAD and SODD, suggests that non-T cells in MS also show the gene expression pattern which represents a counterbalance between inducing and suppressing apoptosis.

Upregulated genes in MS were expressed in cultured PBMC in an activation-dependent manner

To identify the stimuli that regulate the expression of apoptosis signaling-related genes in lymphocytes, PBMC were *in vitro* exposed to three different stimuli, such as PMA plus IOM, anti-CD3 mAb, or IFNγ. PBMC exposed to PMA plus IOM or anti-CD3 mAb showed marked upregulation of CD69, a marker for early activation of lymphocytes, while PBMC treated with IFNγ exhibited the highest level of IFN-induced 15-kDa protein (ISG15) (Fig. 3a and 3c, lanes 2-4). IFN regulatory factor 1 (IRF-1) expression was elevated following exposure of PBMC to all these stimuli, indicating that PBMC well responded to PMA plus IOM, anti-CD3 mAb, and IFNγ. (Fig. 3b, lanes 2-4). The expression of NR4A2, ICAM1, RIPK2, and CXCL2 was the highest in PBMC exposed to PMA plus IOM (Fig. 3e, 3g, 3i, and 3l, lane 2). CDC42, SODD and TOP2A were upregulated more markedly after treatment with anti-CD3 mAb (Fig. 3h, 3m and 3n, lane 3). In contrast, IL1R2, and MAD levels were reduced in PBMC after exposure to PMA plus IOM (Fig. 3j and 3k, lane 2). NR4A2, TCF8, IL1R2, MAD, CXCL2, and TOP2A levels were not elevated in PBMC by treatment with IFNγ (Fig. 3e, 3f, 3j, 3k, 3l, and 3n, lane 4). CYP1A2 mRNA was undetectable in PBMC incubated under any culture conditions (not shown). These results suggest that the genes upregulated in MS were expressed in cultured PBMC, mostly in an activation- and stimulation-dependent manner.

Discussion

In the present study, we have investigated the comprehensive gene expression profile of T cells and non-T cells of 72 MS patients and 22 CN subjects. Among 1,258 genes on a cDNA microarray, 173 genes in T cells and 50 genes in non-T cells were expressed differentially between MS and CN groups. The great majority of top 30 genes were able to be categorized into apoptosis signaling-related genes of both proapoptotic and antiapoptotic classes. The upregulated genes in MS were expressed in cultured PBMC in an activation-dependent manner. These observations indicate that PBMC of MS show the gene expression pattern that represents a counterbalance between promoting and preventing apoptosis, and suggest that MS lymphocytes might be ceaselessly exposed to exogenous and endogenous apoptosis-inducing stimuli and stresses (Fig. 4). Because the elimination of pathogenic autoreactive T cells is pivotal for the homeostasis of the immune system, dysregulation of apoptosis might contribute to the autoimmune pathogenesis of MS. Furthermore, our results propose that the use of apoptosis-modulating agents would be a promising approach to treatment of MS. Therefore, it is important to characterize how the genes deregulated in MS are involved in the mechanisms underlying lymphocyte apoptosis.

The genes involved in thymic T cell development

A battery of the genes we identified act as a key regulator of T cell development in thymus. NR4A2 designated Nurr1, identified as the most significantly upregulated gene in MS T cells, is an orphan member of the steroid-thyroid hormone receptor superfamily. A previous study showed that Nurr1 is induced in human T cells during apoptosis (Okabe et al., 1995). The members of this family positively regulate clonal deletion of self-reactive T cells in thymus (Zhou et al., 1996). TCF8 upregulated in MS T cells encodes a transcriptional repressor for the IL-2 gene (Williams et al., 1991).

Thymocyte development is impaired in mice expressing the mutant TCF8 (Higashi et al., 1997). CREB1 downregulated in MS T cells is a leucine zipper-containing transcription factor. CREB1 phosphorylated by protein kinase A (PKA) binds as a homodimer to the cAMP-responsive element (CRE) located in the promoter of various genes essential for T cell function (Barton et al., 1996). Thymocytes and T cells of transgenic mice expressing a dominant-negative mutant CREB show a profound proliferative defect, accompanied by apoptotic death following activation (Barton et al., 1996). TRAIL downregulated in MS T cells is a type II membrane protein of the TNF family that induces apoptosis preferentially in transformed cells via the death receptors DR4 and DR5. A previous study by using RT-PCR analysis showed that TRAIL mRNA levels are elevated in PBMC of MS (Huang et al., 2000), being apparently inconsistent with our observations. This discrepancy might be derived from the different study populations and methods employed. A recent study showed that serum soluble TRAIL levels are reduced in RRMS, supporting our findings (Wandinger et al., 2003). TRAIL-deficient mice are hypersensitive to induction of autoimmune diseases, associated with a severe defect in thymocyte apoptosis (Lamhamedi-Cherradi et al., 2003). NFATC3 downregulated in MS non-T cells is expressed chiefly in double positive thymocytes during development. Development of CD4 and CD8 single positive thymocytes and peripheral T cells is impaired in mice lacking NFATC3, accompanied by increased apoptosis of double positive thymocytes (Oukka et al., 1998). All of these observations suggest the hypothesis that thymic T cell development might be aberrantly regulated in MS.

The genes involved in oxidative stress in mitochondria

Several genes we identified are involved in the regulation of oxidative stress in mitochondria. CYP1A2 upregulated in MS T cells belongs to the cytochrome P450 superfamily of monooxygenases that regulate the metabolism of drugs, toxic chemicals

and carcinogens. CYP1A2 located in mitochondria is involved in oxidative stress-induced apoptosis (Nebert et al., 2000). Interestingly, cigarette smoking which increases the amount of CYP1A2 in human liver microsomes (Nakajima et al., 1999) is one of risk factors for MS (Riise et al., 2003). COX15 downregulated in both T and non-T cells of MS, located in the mitochondrial inner membrane, promotes the biogenesis of COX. COX is the terminal component of the mitochondrial respiratory chain that provides an antioxidant defense in mitochondria. GABPB1 upregulated in MS T cells regulates transcription of the COX gene. Persistent inhibition of COX by nitric oxide induces the formation of peroxynitrite, a potent inducer of apoptotic cell death (Moncada and Erusalimsky, 2002). These observations suggest that MS lymphocytes might be continuously exposed to a certain level of oxidative stress.

The genes involved in lymphocyte recruitment in the CNS

A panel of the regulatory molecules for lymphocyte recruitment are aberrantly expressed in MS. ICAM-1, the most significantly upregulated gene in MS non-T cells, is a ligand for lymphocyte-function associated antigens LFA-1 and Mac-1. ICAM-1 expresssed on activated endothelial cells, T cells, B cells and monocytes regulates the lymphocyte trafficking into the central nervous system (CNS). Furthermore, a costimulatory signal through ICAM-1 protects T cells from apoptosis by upregulating the expression of BCL2 (Kohlmeier et al., 2003). A previous study showing that serum soluble ICAM-1 levels are elevated in active MS support our observations (Khoury et al., 2000). ITGB1 downregulated in MS T cells encodes a common beta chain of the very late activation (VLA) protein family. The interaction of VLA4 on T cells with VCAM-1 on endothelial cells is a pivotal step for the recruitment of activated T cells into the CNS through the blood-brain barrier in MS (Calabresi et al., 1997). Again, the activation of ITGB1 inhibits apoptosis of $CD4^+$ T cells (Stallmach et al., 2001). CCR5 downregulated in MS T cells is a receptor specific for RANTES, MIP1α, MIP1β,

MCP2 and macrophage-tropic HIV virus. CCR5 is expressed predominantly in polarized Th1 T cells (Bonecchi et al., 1998). The interaction of CCR5 with a HIV Env protein upregulates FasL expression, leading to a Fas-dependent apoptotic death of HIV-uninfected $CD4^+$ T cells (Algeciras-Schimnich et al., 2002). A previous study showed that the number of $CCR5^+$ T cells producing high levels of IFNγ is elevated in progressive MS but not RRMS, suggesting that $CCR5^+$ T cells might be involved in the conversion of two distinct clinical phases of MS (Balashov et al., 1999). CXCL2 downregulated in MS non-T cells is a member of the CXC subfamily of chemokines produced chiefly by macrophages and monocytes. CXCL2 acts as a chemotactic factor for polymophonuclear leukocytes and natural killer (NK) T cells by binding to CXCR2, the receptor shared with IL-8. Macrophages phagocytizing apoptotic cells produce a large amount of CXCL2 during the late stage of apoptosis (Kurosaka et al., 2003). This raises the possibility that the apoptotic cell-phagocytotic activity of macrophages might be reduced in MS.

Apoptosis-regulatory genes whose involvement is upredicted in MS

The microarray analysis highlighted an aberrant expression of key apoptosis regulators in MS, including those previously unreported. RIPK2 upregulated in MS non-T cells is a RIP-related protein kinase having an N-terminal kinase domain and a C-terminal caspase activation and recruitment domain (CARD), a homophilic interaction motif that mediates the recruitment of caspases (Inohara et al., 1998). RIPK2 interacts with CLARP, a caspase-like molecule known to bind to Fas-associated protein with death domain (FADD) and caspase-8. Overexpression of RIPK2 potentiates Fas-mediated apoptosis following activation of nuclear factor-κB (NF-κB), Jun NH_2-terminal kinase (JNK) and caspase-8 (Inohara et al., 1998). Importantly, Th1 differentiation and cytokine production are profoundly impaired in RIPK2-deficient mice (Kobayashi et al., 2002). DAXX downregulated in both T and non-T cells of MS,

by binding to the death domain (DD) of Fas, enhances Fas-induced apoptosis following activation of apoptosis signal-regulating kinase 1 (ASK1) and the JNK pathway (Yang et al., 1997). Mad upregulated in MS non-T cells, by forming a heterodimer with Max, acts as a transcriptional repressor that mediates antiapoptotic activities (Zhou and Hurlin, 2001), while Myc downregulated in MS T cells increases cell susceptibility to TNF-mediated apoptosis by inhibiting NF-κB activation (You et al., 2002). SODD upregulated in MS non-T cells, by binding to the DD of TNFR1 and death receptor DR3, blocks the post-receptor signal transduction (Jiang et al., 1999). SODD has a BAG domain that targets the heat shock protein HSP70 at the cytoplasmic domain of TNFR1 (Tschopp et al., 1999). The HSP70 family proteins protect the cells against apoptosis by sequestering apoptotic protease activating factor-1 (Apaf-1) (Beere and Green, 2001). HSP70 induced in MS brain lesions facilitates processing of myelin basic protein by antigen-presenting cells (Cwiklinska et al., 2003). However, we found that the expression of two members of this family, such as HSPA1A and HSPA1L was substantially reduced in either T cells or non-T cells of MS. BCL2 downregulated in MS non-T cells is an integral mitochondrial inner membrane protein that blocks the apoptotic cell death. BAG1 downregulated in MS T cells binds to BCL2 and enhances the antiapoptotic activity of BCL2 (Takayama et al., 1995). By contrast to our findings of reduction of the two mitochondrial antiapoptotic regulators in MS lymphocytes, a previous study showed that the ratio of proapoptotic to antiapoptotic BCL2 family members is decreased in monocyte-depleted PBMC of clinically active MS patients (Sharief et al., 2003). CR2 termed CD21 downregulated in MS non-T cells is the membrane receptor specific for the C3d fragment of activated C3. CR2 is expressed mainly on B cells and follicular dendritic cells where it is upregulated by NF-κB activation (Fearon and Carrol, 2000). A trimolecular complex composed of CD21, CD19 and CD81 enhances signaling through B cell antigen receptor, accompanied by upregulation of BCL2 expression (Roberts and Snow, 1999).

The genes involved in DNA repair, replication and chromatin remodeling

The microarray analysis proposed a novel view that the expression of various genes involved in DNA repair, replication and chromatin remodeling is aberrantly regulated in MS. DNA topoisomerase (TOP) is a nuclear enzyme that alters the topologic states of DNA. TOP1 downregulated in MS T cells cuts and rejoins a single-stranded DNA, while TOP2A upregulated in MS non-T cells catalyzes a double-stranded DNA and mediates the caspase-independent excision of DNA loop domains during apoptosis (Solovyan et al., 2002). SMARCA3 downregulated in both T and non-T cells of MS belongs to a member of the SWI/SNF family of chromatin remodeling enzymes with DNA helicase activity (Sheridan et al., 1995). The SWI/SNF family proteins, by interacting with Myc, facilitate transcriptional activation of several apoptosis-regulatory genes (Klochendler-Yeivin et al., 2002). RBBP4 downregulated in MS non-T cells is a component of the retinoblastoma (Rb) protein-associated histone deacetylase complex that regulates transcriptional repression of E2F-responsive proapoptotic genes (Nicolas et al., 2000). E2F5 downregulated in MS non-T cells acts as a Smad cofactor that transduces the TGFβ receptor signal to repress transcription of Myc (Chen et al, 2002). PARP downregulated in MS T cells is a chromatin-associated enzyme that modifies various nuclear proteins by polyADP-ribosylation, involved in maintenance of genomic stability. PARP is cleaved by caspase-3 at the onset of apoptosis (Nicholson et al., 1995). RPA1 downregulated in MS non-T cells is a single-stranded DNA-binding protein associated with a large RNA polymerase II (POLR2) complex that regulates gene transcription, DNA replication and repair. The expression of POLR2H, the H subunit of POLR2, was downregulated in non-T cells of MS. RPA1 is phosphorylated by DNA-dependent protein kinase (DNA-PK) following DNA damage (Wold, 1997). DNA-PK is a nuclear serine/threonine protein kinase activated upon binding to double-stranded DNA brakes. DNA-PK plays a pivotal role in the V(D)J recombination, maintenance of chromatin and telomere structure, regulation of

transcription and apoptosis (Smith and Jackson, 1999). A nonsense mutation in the PRKDC gene encoding the catalytic subunit of DNA-PK causes the phenotype of severe combined immunodeficiency (SCID) mouse devoid of mature T and B lymphocytes. The expression of PRKDC was also downregulated in non-T cells of MS. GZMA downregulated in MS non-T cells is a cytotoxic T lymphocyte- and NK cell-specific serine protease that mediates caspase-independent apoptosis of target cells by creating single-stranded DNA breakes, followed by a cleavage of apurinic endonuclease-1, the rate-limiting enzyme of DNA base excision repair (Fan et al., 2003).

Transcription factors and signal transducers involved in regulation of apoptosis

Finally, we found that the expression of various transcription factors and signal transducers involved in regulation of apoptosis is dysregulated in MS. MAPK1 downregulated in MS T cells is a member of the MAP kinase family serine/threonine kinases involved in cell proliferation, differentiation, and protection of the cells from apoptosis (Allan et al., 2003). The RGS protein family is a group of GTPase-activating proteins that downregulate signal transduction through heterotrimeric G protein-coupled receptors. RGS14 downregulated in both T and non-T cells of MS attenuates IL-8 receptor-mediated MAPK activation (Cho et al., 2000). TCF17 downregulated in both T and non-T cells of MS is a zinc finger-containing transcriptional repressor that induces the nucleolar fragmentation in overexpressing cells (Huang et al, 1999). TCF21 downreglated in MS T cells is a member of the basic helix-loop-helix family transcription factors. TCF21-deficient mice show rapid apoptosis of splenic precursor cells during development (Lu et al., 2000). TSC22 downregulated in MS T cells is a TGFβ-inducible transcription factor. Overexpression of TSC22 in gastric cancer cells induces apoptotic death following activation of caspase-3 (Ohta et al., 1997). RASSF1 downregulated in MS non-T cells is a tumor suppressor gene having a Ras association

domain. RASSF1 expression is frequently repressed in lung and ovarian tumor cells, while overexpression of RASSF1 in HEK293 cells induces apoptotic death (Vos et al., 2000). CDC42 upregulated in MS non-T cells is a central member of the small GTPase of Rho-subfamily that regulates cell morphology, migration, endocytosis, cell cycle progression and apoptosis (Aspenström, 1999). CDC42 is a substrate for caspases in the Fas-signaling pathway (Tu and Cerione, 2001). Rab7L1 downregulated in non-T cells of MS belongs to a family of Ras-related small GTP-binding proteins that regulate vesicular transport in specific intracellular compartments. Rab7L1 shows sequence similarity to Rab7 located in the late endosome that plays a role in ingestion of apoptotic cells by phagocytes. ATP6V1B2, a subunit of vacuolar H^+-ATPase (V-ATPase) downregulated in MS T cells, mediates acidification of endosomal and lysosomal compartments critical for protein transport and receptor-mediated endocytosis. Concanamycin A, a specific V-ATPase inhibitor, induces apoptosis of B cells (Akifusa et al., 1998). CDC25B downregulated in MS non-T cells regulates G_2-M progression in the cell cycle following activation of CDC2 protein kinase by dephosphorylation. Overexpression of CDC25B enhances apoptosis in cancer cells (Miyata et al., 2001). CDC16 downregulated in MS T cells is a component of the anaphase-promoting complex that acts as a ubiquitin ligase essential for cyclin A and B degradation. It is inactivated during Fas-induced apoptosis in Jurkat cells (Zhou et al., 1998). PTPN6 named SHP-1 downregulated in MS T cells is a cytoplasmic protein-tyrosine phosphatase with two SH2 domains. PTPN6 inactivates a variety of receptor and non-receptor tyrosine kinases by dephosphorylation, and plays a role in induction of apoptosis upstream BCL2 (Thangaraju et al., 1999). AKAP11 downregulated in MS non-T cells belongs to a family of scaffolding molecules that regulate spatial and temporal location of PKA. AKAP11, by forming a complex with the regulatory subunit of PKA and type 1 protein phosphatase, inhibits glycogen synthase kinase-3β, an enzyme involved in regulation of apoptosis (Tanji et al., 2002). EGF downregulated in MS non-T cells induces apoptosis of A431 epidermoid carcinoma cells, accompanied by

induction of caspase-1 in a STAT-dependent manner (Chin et al., 1997). ERBB4 downregulated in MS T cells is a member of EGF receptor-related receptor tyrosine kinase family that interacts with neuregulins. The neuregulin signaling activates an antiapoptotic serine/threonine kinase Akt in oligodendrocytes (Flores et al., 2000).

Thus, the microarray analysis identified an aberrant expression of various apoptosis signaling-related genes, including those heretofore unreported, in T cells and non-T cells of MS. These observations suggest that PBMC of MS show the complex gene expression pattern representing a counterbalance between promoting and preventing lymphocyte apoptosis and DNA damage.

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References

1. Akifusa, S., Ohguchi, M., Koseki, T., Nara, K., Semba, I., Yamato, K., Okahashi, N., Merino, R., Núñez, G., Hanada, N., Takehara, T., Nishihar, T., 1998. Increase in Bcl-2 level promoted by CD40 ligation correlates with inhibition of B cell apoptosis induced by vacuolar type H^+-ATPase inhibitor. Exp. Cell Res. 238, 82-89.
2. Algeciras-Schimnich, A., Vlahakis, S.R., Villasis-Keever, A., Gomez, T., Heppelmann, C.J., Bou, G., Paya, C.V., 2002. CCR5 mediates Fas- and caspase-8 dependent apoptosis of both uninfected and HIV infected primary human CD4 T cells. AIDS 16, 1467-1478.
3. Allan, L.A., Morrice, N., Brady, S., Mageee, G., Pathak, S., Clarke, P.R., 2003. Inhibition of caspase-9 through phosphorylation at Thr 125 by ERK MAPK. Nature Cell Biol. 5, 647-654.
4. Aspenström, P., 1999. Effectors for the Rho GTPases. Curr. Opin. Cell Biol. 11, 95-102.
5. Balashov, K.E., Rottman, J.B., Weiner, H.L., Hancock, W.W., 1999. $CCR5^+$ and $CXCR3^+$ T cells are increased in multiple sclerosis and their ligands MIP-1α and IP-10 are expressed in demyelinating brain lesions. Proc. Natl. Acad. Sci. U.S.A. 96, 6873-6878.
6. Baldi, P., Long, A.D., 2001. A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inferences of gene changes. Bioinformatics 17, 509-519.
7. Barton, K., Muthusamy, N., Chanyangam, M., Fischer, C., Clendenin, C., Leiden, J.M., 1996. Defective thymocyte proliferation and IL-2 production in transgenic mice expressing a dominant-negative form of CREB. Nature 379, 81-85.
8. Beere, H.M., Green, D.R., 2001. Stress management-heat shock protein-70 and the regulation of apoptosis. Trends Cell Biol. 11, 6-10.

9. Bonecchi, R., Bianchi, G., Bordignon, P.P., D'Ambrosio, D., Lang, R., Borsatti, A., Sozzani, S., Allavena, P., Gray, P.A., Mantovani, A., Sinigaglia, F., 1998. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J. Exp. Med. 187, 129-134.

10. Bonetti, B., Stegagno, C., Cannella, B., Rizzuto, N., Moretto, G., Raine, C.S., 1999. Activation of NF-κB and c-*jun* transcription factors in multiple sclerosis lesions. Implications for oligodendrocyte pathology. Am. J. Pathol. 155, 1433-1438.

11. Calabresi, P.A., Pelfrey, C.M., Tranquill, L.R., Maloni, H., McFarland H.F. 1997. VLA-4 expression on peripheral blood lymphocytes is downregulated after treatment of multiple sclerosis with interferon beta. Neurology 49, 1111-1116.

12. Chen, C.-R., Kang, Y., Siegel, P.M., Massagué, J., 2002. E2F4/5 and p107 as Smad cofactors linking the TGFβ receptor to c-*myc* repression. Cell 110, 19-32.

13. Chin, Y.E., Kitagawa, M., Kuida, K., Flavell, R.A., Fu, X.-Y., 1997. Activation of the STAT signaling pathway can cause expression of caspase 1 and apoptosis. Mol. Cell. Biol. 17, 5328-5337.

14. Cho, H., Kozasa, T., Takekoshi, K., De Gunzburg, J., Kegrl, J.H., 2000. RGS14, a GTPase-activating protein for Giα, attenuates Giα- and G13α-mediated signaling pathways. Mol. Pharmacol. 58, 569-576.

15. Comi, C., Leone, M., Bonissoni, S., DeFranco, S., Bottarel, F., Mezzatesta, C., Chiocchetti, A., Perla, F., Monaco, F., Dianzani, U., 2000. Defective T cell Fas function in patients with multiple sclerosis. Neurology 55, 921-927.

16. Compston, A., Coles, A., 2002. Multiple sclerosis. Lancet 359, 1221-1231.

17. Cwiklinska, H., Mycko, M.P., Luvsannorov, O., Walkowiak, B., Brosnan, C.F., Raine, C.S., Selmaj, K.W. 2003. Heat shock protein 70 associations with mylein basic protein and proteolipid protein in multiple sclerosis brains. Int. Immunol. 15, 241-249.

18. D'Souza, S.D., Bonetti, B., Balasingam, V., Cashman, N.R., Barker, P.A., Troutt, A.B., Raine, C.S., Antel, J.P., 1996. Multiple sclerosis; Fas signaling in

oligodendrocyte cell death. J. Exp. Med. 184, 2361-2370.

19. Fan, Z., Beresford, P.J., Zhang D., Xu, Z., Novina, C.D., Yoshida, A., Pommier Y., Lieberman, J., 2003. Cleaving the oxidative repair protein Apel enhances cell death mediated by granzyme A. Nature Immunol. 4, 145-153.
20. Fearon, D.T., Carroll, M.C., 2000. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. Annu. Rev. Immunol. 18, 393-422.
21. Flores, A. I., Mallon, B.S., Matsui, T., Ogawa, W., Rosenzweig, A., Okamoto, T., Macklin, W.B., 2000. Akt-mediated survival of oligodendrocytes induced by neuregulins. J. Neurosci. 20, 7622-7630.
22. Gomes, A.C., Jönsson, G., Mjörnheim, S., Olsson, T., Hillert, J., Grandien, A., 2003. Upregulation of the apoptosis regulators cFLIP, CD95 and D95 ligand in peripheral blood mononuclear cells in relapsing-remitting multiple sclerosis. J. Neuroimmunol. 135, 126-134.
23. Higashi, Y., Moribe, H., Takagi, T., Sekido, R., Kawakami, K., Kikutani, H., Kondoh, H., 1997. Impairment of T cell development in δEF1 mutant mice. J. Exp. Med. 185, 1467-1480.
24. Huang, W.-X., Huang, P., Gomes, A., Hillert, J., 2000. Apoptosis mediators FasL and TRAIL are upregulated in peripheral blood mononuclear cells in MS. Neurology 55, 928-934.
25. Huang, Z., Philippin, B., O'Leary, E., Bonventre, J.V., Kriz, W., Witzgall, R., 1999. Expression of the transcriptional repressor protein Kid-1 leads to the disintegration of the nucleolus. J. Biol. Chem. 274, 7640-7648.
26. Inohara, N., de Peso, L., Koseki, T., Chen, S., Núñez, G., 1998. RICK, a novel protein kinase containing a caspase recruitment domain, interacts with CLARP and regulates CD95-mediated apoptosis. J. Biol. Chem. 273, 12296-12300.
27. Jiang, Y., Woronicz, J.D., Liu, W., Goeddel, D.V., 1999. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science 283, 543-546.

28. Khoury, S.J., Orav, E.J., Guttmann, C.R.G., Kikinis, R., Jolesz, F.A., Weiner, H.L., 2000. Changes in serum levels of ICAM and TNF-R correlate with disease activity in multiple sclerosis. Neurology 53, 758-764.

29. Klochendler-Yeivin, A., Muchardt, C., Yaniv, M., 2002. SWI/SNF chromatin remodeling and cancer. Curr. Opin. Genet. Dev. 12, 73-79.

30. Kobayashi, K., Inohara, N., Hernandez, L.D., Galán, J.E., Núñez, G., Janeway, C.A., Medzhitov, R., Flavell, R.A., 2002. RICK/Rip2/CARDIAK mediates signaling for receptors of the innate and adaptive immune systems. Nature 416, 194-199.

31. Kohlmeier, J.E., Rumsey, L.M., Chan, M.A., Benedict, S.H., 2003. The outcome of T-cell costimulation through intercellular adhesion molecule-1 differs from costimulation through leukocyte function-associated antigen-1. Immunology 108, 152-157.

32. Koike, F., Satoh, J., Miyake, S., Yamamoto, T., Kawai, M., Kikuchi, S., Nomura, K., Yokoyama, K., Ota, K., Kanda, T., Fukazawa, T., Yamamura, T., 2003. Microarray analysis identifies interferon β-regulated genes in multiple sclerosis. J. Neuroimmunol. 139, 109-118.

33. Kurosaka, K., Takahashi, M., Kobayashi, Y., 2003. Activation of extracellular signal-regulated kinase 1/2 is involved in production of CXC-chemokine by macrophages during phagocytosis of late apoptotic cells. Biochem. Biophys. Res. Commun. 306, 1070-1074.

34. Lamhamedi-Cherradi, S.-E., Zheng, S.-J., Maguschak, K.A., Peschon, J., Chen, Y.H., 2003. Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL$^{-/-}$ mice. Nature Immunol. 4, 255-260.

35. Lock, C. Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S.J., Oksenberg, J.R., Raine, C.S., Heller, R., Steinman, L., 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets

validated in autoimmune encephalomyelitis. Nature Med. 8, 500-508.

36. Lu, J., Chang, P., Richardson, J.A., Gan, L., Weiler, H., Olson, E.N., 2000. The basic helix-loop-helix transcription factor capsulin controls spleen organogenesis. Proc. Natl. Acad. Sci. U.S.A. 97, 9525-9530.

37. Maas, K., Chan, S., Parker, J., Slater, A., Moore, J., Olsen, N., Aune, T.M., 2002. Cutting edge: molecular portrait of human autoimmune disease. J. Immunol. 169, 5-9.

38. McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.-P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., Reingold, S.C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., van der Noort, S., Weinshenker, B.Y., Wolinsky, J.S., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann. Neurol. 50, 121-127.

39. Miyata, H., Doki, Y., Yamamoto, H., Kishi, K., Takemoto, H., Fujiwara, Y., Yasuda, T., Yano, M., Inoue, M., Shiozaki, H., Weinstein, B., Monden, M., 2001. Overexpression of CDC25B overrides radiation-induced G_2-M arrest and results in increased apoptosis in esophageal cancer cells. Cancer Res. 61, 3188-3193.

40. Moncada, S., Erusalimsky, J.D., 2002. Does nitric oxide modulate mitochondrial energy generation and apoptosis? Nature Rev. Mol. Cell Biol. 3, 214-220.

41. Nakajima, M., Yokoi, T., Mizutani, M., Kinoshita, M., Funayama, M., Kamataki, T., 1999. Genetic polymorphism in the 5'-flanking region of human *CYP1A2* gene: effects on the CYP1A2 inducibility in humans. J. Biochem. 125, 803-808.

42. Nebert, D.W., Roe, A.L., Dieter, M.Z., Solis, W.A., Yang Y., Dalton, T.P., 2000. Role of the aromatic hydrocarbon receptor and [*Ah*] gene battery in the oxidative stress response cell cycle control, and apoptosis. Biochem. Pharmacol. 59, 65-88.

43. Nicholson, D.W., Ali, A., Thornberry, N.A., Vaillancourt, J.P., Ding, C.K., Gallant, M., Gareau, Y., Griffin, P.R., Labelle, M., Lazebnik, Y.A., Munday N.A., Raju, S.M., Smulson, M.E., Yamin, T.-T., Yu, V.L., Miller, D.K., 1995. Identification and

inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 376, 37-43.

44. Nicolas, E., Morales, V., Magnaghi-Jaulin, L., Harel-Bellan, A., Richard-Foy, H., Trouche, D., 2000. RbAp48 belongs to the histone deacetylase complex that associates in the retinoblastoma protein. J. Biol. Chem. 275, 9797-9804.

45. Ohta, S., Yanagihara, K., Nagata, K., 1997. Mechanism of apoptotic cell death of human gastric carcinoma cells mediated by transforming growth factor β. Biochem J. 324, 777-782.

46. Okabe, T., Takayanagi, R., Imasaki, K., Haji, M., Nawata, H., Watanabe, R., 1995. cDNA cloning of a NGFi-B/nur77-related transcription factor from an apoptotic human T cell line. J. Immunol. 154, 3871-3879.

47. Oukka, M., Ho, I.-C., de la Brousse, F.C., Hoey, T., Grusby, M.J., Glimcher, L.H., 1998. The transcription factor NAFT4 is involved in the generation and survival of T cells. Immunity 9, 295-304.

48. Riise, T., Nortvedt, M.W., Acherio, A., 2003. Smoking is a risk factor for multiple sclerosis. Neurology 61, 1122-1124.

49. Roberts, T., Snow, E.C., 1999. Cutting edge: recruitment of the CD19/CD21 coreceptor to B cell antigen receptor is required for antigen-mediated expression of Bcl-2 by resting and cycling hen egg lysozyme transgenic B cells. J. Immunol. 162, 4377-4380.

50. Satoh, J., Kuroda, Y., 2001. Differing effects of IFNβ vs IFNγ in MS. Gene expression in cultured astrocytes. Neurology 57, 681-685.

51. Schwab, B.L., Leist, M., Knippers, R., Nicotera, P., 1998. Selective proteolysis of the nuclear replication factor MCM3 during apoptosis. Exp. Cell Res. 238, 415-421.

52. Sharief, M.K., Matthews, H., Noori, M., 2003. Expression ratios of the Bcl-2 family proteins and disease activity in multiple sclerosis. J. Neuroimmunol. 134, 158-165.

53. Sheridan, P.L., Schorpp, M., Voz, M.L., Jones, K.A., 1995. Cloning of an SNF2/SWI2-related protein that binds specifically to the SPH motifs of the SV40 enhancer and to the HIV-1 promoter. J. Biol. Chem. 270, 4575-4587.

54. Smith, G.C.M., Jackson, S.P., 1999. The DNA-dependent protein kinase. Genes Dev. 13, 916-934.

55. Solovyan, V.T., Bezvenyuk, Z.A., Salminen, A., Austin, C.A., Courtney, M.J., 2002. The role of topoisomerase II in the excision of DNA loop domains during apoptosis. J. Biol. Chem. 277, 21458-21467.

56. Stallmach, A., Giese, T., Pfister, K., Wittig, B.M., Künne, S., Humphries, M., Zeitz, M., Meuer, S.C., 2001 Activation of β_1 integrins mediates proliferation and inhibits apoptosis of intestinal CD4-positive lymphocytes. Eur. J. Immunol. 31, 1228-1238.

57. Steinman, L., Zamvil, S., 2003. Transcriptional analysis of targets in multiple sclerosis. Nature Rev. Immunol. 3, 483-492.

58. Stürzebecher, S., Wandinger, K.P., Rosenwald, A., Sathyamoorthy, M., Tzou, A., Mattar, P., Frank, J.A., Staudt, L., Martin, R., McFarland, H.F., 2003. Expression profiling identifies responder and non-responder phenotypes to interferon-β in multiple sclerosis. Brain 126, 1419-1429.

59. Takayama, S., Sato, T., Krajewski, S., Kochel, K., Irie, S., Millan, J.A., Reed, J.C., 1995. Cloning and functional analysis of BAG-1: a novel Bc-2-binding protein with anti-cell death activity. Cell 80, 279-284.

60. Tanji, C., Yamamoto, H., Yorioka, N., Kohno, N., Kikuchi, K., Kikuchi, A., 2002. A-kinase anchoring protein AKAP220 binds to glycogen synthase kinase-3β (GSK-3β) and mediates protein kinase A-dependent inhibition of GSK-3β. J. Biol. Chem. 277, 36955-36961.

61. Thangaraju, M., Sharma, K., Leber, B., Andrews, D.W., Shen, S.-H., Srikant, C.B., 1999. Regulation of acidification and apoptosis by SHP-1 and Bcl-2. J. Biol. Chem. 274, 29549-29557.

62. Tschopp, J., Martinon, F., Hofmann, K., 1999. Apoptosis: silencing the death

receptors. Curr. Biol. 9, R381-R384.

63. Tu, S., Cerione, R.A., 2001. Cdc42 is a substrate for caspases and influences Fas-induced apoptosis. J. Biol. Chem. 276, 19656-19663.

64. Vos, M.D., Ellis, C.A., Bell, A., Birrer, M.J., Clark, G.J., 2000. Ras uses the novel tumor suppressor RASSF1 as an effector to mediate apoptosis. J. Biol. Chem. 275, 35669-35672.

65. Wandinger, K.-P., Lünemann, J., Wengert, O., Bellmann-Stroble, J., Aktas, O., Weber, A., Grundström, E., Ehrlich, S., Wernecke K.-D., Volk, H.-D., Zipp, F., 2003. TNF-related apoptosis inducing ligand (TRAIL) as a potential response marker for interferon-beta treatment in multiple sclerosis. Lancet 361 2036-2041.

66. Weinstock-Guttman, B., Badgett, D., Patrick, K., Hartrich, L., Santos, R., Hall, D., Baier, M., Feichter, J., Ramanathan, M., 2003. Genomic effects of IFN-β in multiple sclerosis patients. J. Immunol. 171, 2694-2702.

67. Williams, T.M., Moolten, D., Burlein, J., Romano, J., Bhaerman, R., Godillot, A., Mellon, M., Rauscher III, F.J., Kant, J.A., 1991. Identification of a zinc finger protein that inhibits IL-2 gene expression. Science 254, 1791-1794.

68. Wold, M.S., 1997. Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annu. Rev. Biochem. 66, 61-92.

69. Yang, X., Khosravi-Far, R., Chang, H.Y., Baltimore, D., 1997. Daxx, a novel Fas-binding protein that activates JNK and apoptosis. Cell 89, 1067-1076.

70. You, Z., Madrid, L.V., Saims, D., Sedivy, J., Wang, C.-Y., 2002. c-Myc sensitizes cells to tumor necrosis factor-mediated apoptosis by inhibiting nuclear factor κB transactivation. J. Biol. Chem. 277, 36671-36677.

71. Zhou, B.-B., Li, H., Yuan, J., Kirschner, M.W., 1998. Caspase-dependent activation of cyclin-dependent kinases during Fas-induced apoptosis in Jurkat cells. Proc. Natl. Acad. Sci. U.S.A. 95 6785-6790.

72. Zhou, T., Cheng, J., Yang, P., Wang, Z., Liu, C., Su, X., Blurthmann, H., Mountz,