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Gene Expression Profile in Microglia following Ischemia-Reperfusion Injury

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Microglial activation is thought to play a role in the pathogenesis of many brain disorders. Therefore, understanding the response of microglia to noxious stimuli may provide insights into their role in disorders such as stroke and neurodegeneration. Many genes involved in this response have been identified individually, but not systematically. In this regards, the microarray system permitted to screen a large number of genes in biological or pathological processes. Therefore, we used microarray technology to evaluate the effect of oxygen glucose deprivation (OGD) and reperfusion on gene expression in microglia under ischemia-like and activating conditions. Primary microglial cultures were prepared from postnatal mice brain. The cells were exposed to 4 hrs of OGD and 1 h of reperfusion at 37°C. Isolated mRNA were run on GeneChips. After OGD and reperfusion, >2-fold increases of 90 genes and >2-fold decrease of 41 genes were found. Among the genes differentially increased by OGD and reperfusion in microglia were inflammatory and immune related genes such as prostaglandin E synthase, IL-1 β, and TNF-α. Microarray analysis of gene expression may be useful for elucidating novel molecular mediators of microglial reaction to reperfusion injury and provide insights into the molecular basis of brain disorders.

Key Words: Gene expression, Microglia, Ischemia-reperfusion, Microarray

INTRODUCTION

Microglia are brain macrophages. Activation of macrophages is a biological process necessary for the defense of the central nervous system (CNS) against microorganisms, for the removal of tissue debris in neurodegenerative diseases or normal development, and in inflammatory autoimmune disorders of the brain. Many molecules, such as cytokines and lipid metabolites, are known to trigger macrophage activation. However, the biological pathways that regulate macrophage activation are poorly understood, nevertheless, of great interest. When tissue macrophages of the CNS, such as parenchymal microglia and perivascular macrophages, are activated, a wide array of neurotoxins are released, including proteins, peptides, lipids, and metabolites (Persidsky & Gendelman, 2003), even though some of which might not yet be discovered. These molecules are also known to recruit and activate more microglia/macrophages at the site of pathology.

Microglial activation is a characteristic of many degenerative CNS disorders, including HIV-associated dementia, Alzheimer's disease, and Parkinson's diseases, as well as the degenerative stage of inflammatory diseases such as multiple sclerosis and ischemic diseases including stroke. Since microglial activation was shown to play a role in the pathogenesis of CNS disorders, the understanding of the molecular mechanisms involved in microglial activation may lead to new treatment strategies for CNS disorders.

The completion of the mapping and sequencing of the genome made it possible to analyse gene expression in individual cell types and organs. In recent years, DNA microarray technology has gained increasing popularity for gene expression studies. Oligonucleotide microarrays are now capable of probing the expression of more than 30,000 genes, thus allowing simultaneous detection and quantification of the differential expression of thousands of genes in a single experiment. Combined together with the advancements in computing software and statistical algorithms that support the analysis of microarray data, these advances can provide the background for sophisticated studies of the transcriptome. Another advantage of microarray study is that many different molecular pathways can globally be investigated. The power of microarray technology was demonstrated initially in cancer research, and this technology is now being extended to many research areas, including studies of cerebral ischemia (Raghavendra Rao et al, 2002; Stenzel-Poore et al, 2003).

Approximately 20% of the mouse genome has been characterized, therefore, many genes are yet to be discovered. Some of these genes are undoubtedly involved in the process of microglial activation and in the mediation of CNS

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ABBREVIATIONS: OGD, oxygen glucose deprivation; IL, (MMPs) interleukin; MMPs, matrix metalloproteinases; TIMP, tissue inhibitor of MMP.

disorders. Therefore, understanding of transcriptional changes in microglia after ischemia is expected to provide a strong basis to researchers and therapeutic targets for treating stroke to clinicians. This experiment was performed to study genomic changes of cultured primary murine microglia after they were subjected to combined OGD condition and reperfusion.

METHODS

Cell culture

Murine microglial cultures were prepared from newborn mouse pups as previously described (Yenari & Giffard, 2001). Whole brains from postnatal day 1~3 Swiss Webster mice were plated in 75 cm^2 primaria coated flasks in Eagle's minimal essential medium (Gibco BRL, USA) supplemented with 10% equine serum, 10% fetal bovine serum, recombinant epidermal growth factor (total 1 mg/dl), glutamine (total 2 mM), glucose (total 21 mM) and bicarbonate (total 26 mM). The cultures were maintained in a humidified incubator at 37°C with 5% CO_2 and balanced air. Media were changed every 2~3 days for the first 10 days. Microglial cultures were prepared using the method previously described (Yenari & Giffard, 2001). After 10~14 days in vitro, the flasks were inspected for microglia growing on top of a confluent cell layer and shaken at 160 rpm for 30 min at 37°C. The supernatant was collected and spun at 800 g for 5 min. The resulting pellets were resuspended in plating media and plated at a density of $2\sim3\times10^5$ cells/ml in uncoated 24-well plates. The plates were returned to the incubator for 1 h to allow the microglia to attach, then washed and returned to the incubator with fresh media and antibiotics. Cultures 24 hrs after plating were used for experiments. Histochemical staining with Griffonia simplicifolia B4-isolectin (IB4, Sigma, USA) confirmed that the majority of these cells were indeed microglia. The experiments were repeated three times using cells isolated from three different dissections.

Oxygen-glucose deprivation (OGD) and reperfusion

Cultures were transferred to an anaerobic chamber (Coy Laboratory Products Inc. Grass Lake, MI, USA) with an atmosphere of 5% CO_2, 5% H_2 and balanced N_2. Culture medium was replaced three times with deoxygenated, glucose-free balanced salt solution (BSS_0), pH 7.4, containing phenol red (10 mg/l) and the followings (in mM): NaCl 116, $CaCl_2$ 1.8, $MgSO_4$ 0.8, KCl 5.4, NaH_2PO_4 1, $NaHCO_3$ 14.7, HEPES 10. The cultures were placed for 4 hrs in a humidified 37°C incubator within the anaerobic chamber. Oxygen tension was monitored with an oxygen electrode meter and kept under 0.02%. OGD was ended by adding glucose ($BSS_{5.5}$) to the culture medium and returning the cultures to normoxia (reperfusion). $BSS_{5.5}$ contains 5.5 mM glucose in BSS. The cultured cells and media were harvested 1 h after reperfusion initiation.

Cytotoxicity assay

Cytotoxicity of microglia was quantitated using enzyme assays - lactate dehydrogenase (LDH) activity in culture medium. LDH activity was assayed by a colorimetric method using a LDH assay kit (Sigma, USA).

RNA isolation, cDNA and cRNA synthesis

From the cultured microglial samples, total RNA was isolated with RNeasy Midi kit (Qiagen, #75144) by the supplier's instructions. mRNA was purified from the total RNA samples (Qiagen, Oligotex Midi kit, #70042). Synthesis of cDNA and biotin-labeled cRNA, fragmentation, and hybridization were performed according to the Affymetrix Genechip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA, USA). Briefly, 20 μg of total RNA was used for cDNA synthesis using the Superscript II cDNA synthesis kit (Invitrogen, Superscript choice system for cDNA synthesis, #18090); the cDNA was then cleaned by Phase Lock Gel Centrifugation (Eppendorf). Biotin-labeled cRNA was synthesized by in vitro transcription of the cDNA (Ambio, Megascript T7 high yield transcription kit, #1334) and then fragmented.

Microarray analysis

The fragmented cRNA was hybridized to duplicate Affymetrix Murine Genome U74v2 (Affymetrix, #900249). The chip contains probe sets for 12,000 full-length mouse genes and EST clusters. Hybridization and scanning were done by the Stanford University core facility, with the Affymetrix Fluidic Station 400 and Gene Array scanner. Data acquisition was performed using the Affymetrix Microarray Suite version 4.0, and the data were then analyzed with dChip (Li and Wong, 2001a & b; Available from http://biosun1.harvard.edu/complab/dchip/) and GenMAPP tools (Dahlquist et al., 2002; Available from http://www.genmapp.org/). All the analysis processes are performed following the instruction of the software providers' manual as previously published in other papers (Li & Wong, 2001a & b).

RESULTS

Cytotoxicity

LDH level in the media obtained from cultured microglia showed an increase following 4 h of OGD and 1 h of reperfusion (Fig. 1).

Data processing

Array images were observed to check general signal intensities from the DNA chips after hybridization of the samples and probes on the chips. After acquisition of the signals from the images, they were transformed into digital numbers and absolute analysis was performed. Since scanned images may have different overall brightness, generally the normalization is needed to adjust the brightness of the arrays to comparable level. dChip needs to normalize arrays at probe level before computing model-based expression levels. After normalization of data, the reproducibility of the chips with same experimental conditions was tested using correlation analysis. All the compared pairs showed significant correlations ($R > 0.95$). One of the representative comparisons was demonstrated in Fig. 2.

Overall gene expression pattern by reperfusion injur

All the analysis from this step were performed with the

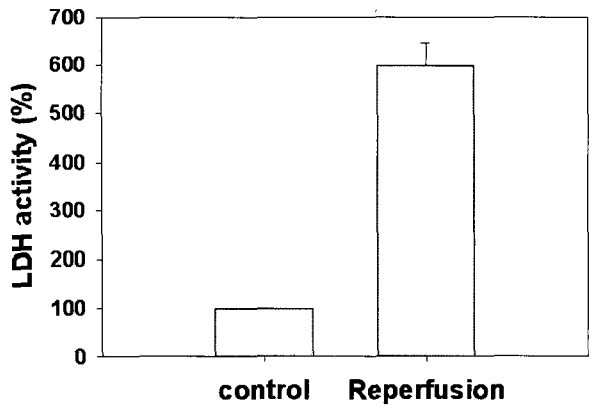

Fig. 1. Cell death of microglia was quantitated using enzyme assay - lactate dehydrogenase (LDH) activity in culture medium. LDH activity was assayed by a colorimetric method using a LDH assay kit. OGD and reperfusion resulted in increased LDH release in microglia cultures.

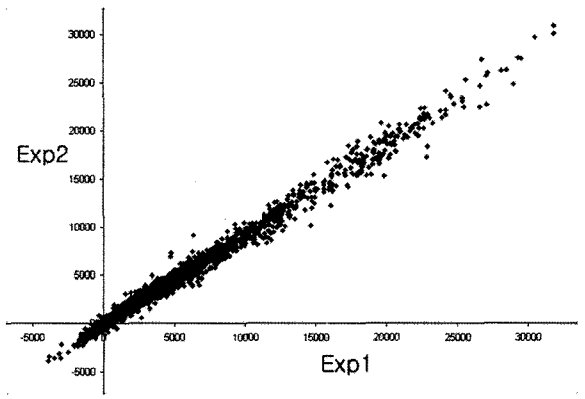

Fig. 2. Distribution of the signal intensity from each gene set in the OGD and reperfusion injury group is compared between 2 repeated chips, Exp1 and Exp2, to show the reproducibility of the experiments. One of the representative comparison of paired chips in the same condition was shown in this figure. The data used in this figure are normalized data from each chip.

Table 1. Number of genes showing increased or decreased expression after oxygen-glucose deprivation (OGD) and reperfusion exposure compared to control at various threshold (fold change)

Fold change	Number of increase	Number of decrease	Total number
1.2	201	204	405
1.5	141	118	259
2.0	90	41	131

genes which passed the filter criteria provided by the dChip software to decrease the possibility of false positive results. OGD and reperfusion exposure caused marked alterations of the expression levels of a large number of genes. To observe the global transcriptional activity after reperfusion mediated insult, we counted the number of genes showing

Table 2. Functional classification of genes whose expressions were increased more than 2 folds after OGD and reperfusion exposure

Function gene	Fold change
Inflammatory & immune response	
CD14 antigen	2.72
Chemokine (C-C motif) ligand 2	5.74
Chemokine (C-C motif) ligand 3	6.78
Chemokine (C-C motif) ligand 4	6.62
Chemokine (C-C motif) ligand 5	12.71
Chemokine (C-C motif) ligand 7	3.32
Chemokine (C-C motif) receptor-like 2	2.95
Chemokine (C-X-C motif) ligand 1	7.46
Chemokine (C-X-C motif) ligand 2	4.03
Expressed sequence AA408868	3.46
Interferon, alpha-inducible protein	5.96
Interferon-induced protein with tetratricopeptide repeats 1	2.95
Interleukin 1 alpha	20.55
Interleukin 1 beta	10.99
Interleukin 1 receptor antagonist	4.3
Prostaglandin E synthase	2.43
Prostaglandin-endoperoxide synthase 2	7.35
Serum amyloid A 3	3.31
Tumor necrosis factor	5.57
Tumor necrosis factor (ligand) superfamily, member 9	2.32
Response to stress	
Heat shock 70kD protein 5 (glucose-regulated protein)	2.98
Heat shock protein 1A	5.68
HLA-B-associated transcript 9	4.56
Lymphocyte antigen 6 complex, locus E	2.24
Metallothionein 1	2.14
Metallothionein 2	3.45
Pentaxin related gene	3.14
Prion protein	2.33
Enzymatic activity	
Aldolase 3, C isoform	2.74
Carboxypeptidase E	3.45
Cholesterol 25-hydroxylase	5.1
Lysyl oxidase	2.79
Purine-nucleoside phosphorylase	2.33
RIKEN cDNA 2510004L01 gene	2.26
Serine (or cysteine) proteinase inhibitor, clade B, member 2	4.98
Transglutaminase 2, C polypeptide	3.75
Transporter activity	
Aquaporin 4	2.4
ERO1-like (S. cerevisiae)	2.53
lipocalin 2	3.02
Solute carrier family 2 (facilitated glucose transporter), member 1	2.35
Tubulin, alpha 1	2.45
Carbohydrate binding	
C-type (calcium dependent, carbohydrate recognition domain) lectin, superfamily member 9	3.07
Cell death	
Clusterin	2.17
Cell proliferation & growth	
Schlafen 2	2.83
Insulin-like growth factor binding protein 3	4.29

Table 2. Functional classification of genes whose expressions were increased more than 2 folds after OGD and reperfusion exposure (Continued)

Function gene	Fold change
Regulation of transcription	
CCAAT/enhancer binding protein (C/EBP), delta	4.27
FBJ osteosarcoma oncogene	9.41
Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	2.78
RNA polymerase 1-2	3.09
Zinc finger protein 275	4.46
Signal transduction	
Guanylate nucleotide binding protein 2	2.93
Phosphatidic acid phosphatase type 2B	2.61
Ras homolog gene family, member B	2.56
Regulator of G-protein signaling 16	4.77
Suppressor of cytokine signaling 3	6.75
Cytoskeleton & its binding protein	
Calponin 3, acidic	3.17
Tubulin, alpha 1	2.45
Extracellular matrix	
Fibronectin 1	2.25
Tissue inhibitor of metalloproteinase 1	2.41
Tissue inhibitor of metalloproteinase 3	3.35
Vascular cell adhesion molecule 1	3.74

Table 3. Functional classification of genes whose expressions were decreased more than 2 folds after OGD and reperfusion exposure

Function gene	Fold change
Cell growth and/or maintenance	
Alanyl (membrane) aminopeptidase	−2.48
Growth arrest specific 6	−3.03
Insulin-like growth factor 2 receptor	−3.59
SET translocation	−3.5
Transporter activity	
Adaptor-related protein complex 3, delta subunit	−2.1
Purinergic receptor P2X, ligand-gated ion channel 4	−2.37
RIKEN cDNA 1500005G05 gene	−20.83
Solute carrier family 6 (neurotransmitter transporter, taurine), member 6	−2.85
Solute carrier family 12, member 2	−2.61
Membrane	
Dehydrogenase/reductase (SDR family) member 3	−2.47
Transmembrane 7 superfamily member 1	−2.18
Cytoskeleton	
Spindlin	−3.12
Actin, beta, cytoplasmic	−5.33
Inflammatory & immune response	
Chemokine (C-X-C motif) receptor 4	−14.02
Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	−2.24
Antioxidant	
Cathepsin B	−2.26
Glutathione peroxidase 3	−2.78
Receptor	
Mannose receptor, C type 1	−3.79
Signal transduction	
IQ motif containing GTPase activating protein 1	−2.41
Nuclear protein	
LIM domain only 2	−4.07
Zinc finger RNA binding protein	−3.51
Enzymatic activity	
Dehydrogenase/reductase (SDR family) member 3	−2.47
Glutamate-cysteine ligase, modifier subunit	−2.24
Glycogenin 1	−4.04
Lipoprotein lipase	−2.23
Plasma glutamate carboxypeptidase	−3.32

altered transcriptional activities by various criteria, compared to control group (Table 1). With the increase of the threshold in criteria, the number of genes that satisfied the criteria diminished. When the threshold criteria were put at 1.2 fold, 405 genes passed the criteria. Of the 405 genes, 201 genes showed increased expression level and 204 showed decreased expression level. At 1.5 fold criteria, 141 genes of 259 altered genes were increased. At 2 fold criteria, 41 genes out of 131 altered genes were decreased. After comparing the data from 3 repeated experiments, we found out that some genes showed high degree of deviation from average, indicating that the result of one experiment is extremely different from those of the other two experiments. Because these data can cause false positive or negative results, these data were excluded. By increasing the criteria threshold, we could successfully eliminate those unreliable data and decided to use threshold criteria of 2 fold change in the following analysis.

Functional classification of genes with altered expression by reperfusion injury

After the general evaluation was done, we investigated the identities of individual genes which passed the criteria of 2 fold change. Then, those genes were classified into functionally related groups (Table 2 and 3). The major upregulated genes were found to be involved in inflammation, immune and stress responses. Genes belonging to signal transduction, gene transcription, transporter, enzymatic process, structural protein, cell death and growth related functional groups were upregulated or downregulated depending on their own properties.

Regulation of gene expression in some pathways

Apoptotic pathways: Since OGD and reperfusion injury are well known causes of cell death and apoptosis is a major pathway of ischemic cell death, we attempted to closely observe the changes of the genes in the apoptotic pathway. As shown in Fig. 3, only fibroblast-associated cell surface (Fas) ligand was markedly upregulated, whereas the other genes belonging to pro- or anti-apoptotic groups showed subtle changes.

Inflammatory and immune responses: Microglia is known as the major immune cell of the brain. Following the reperfusion injury, microglia were transformed into activated inflammatory form. Both interleukin (IL)-1 alpha and beta, tumor necrosis factor (TNF)-α, IL-6, and fibronectin were significantly upregulated. Some cytokine receptors such as IL-5 and TNF receptor 2 showed increased expression (Fig. 4).

Eicosanoid synthesis: Increase of inducible type of cyclooxygenase, COX-2, was prominent, while constitutive COX-1 was not significantly changed by reperfusion injury. Enzymes involved in thromboxane and leukotriene synthesis

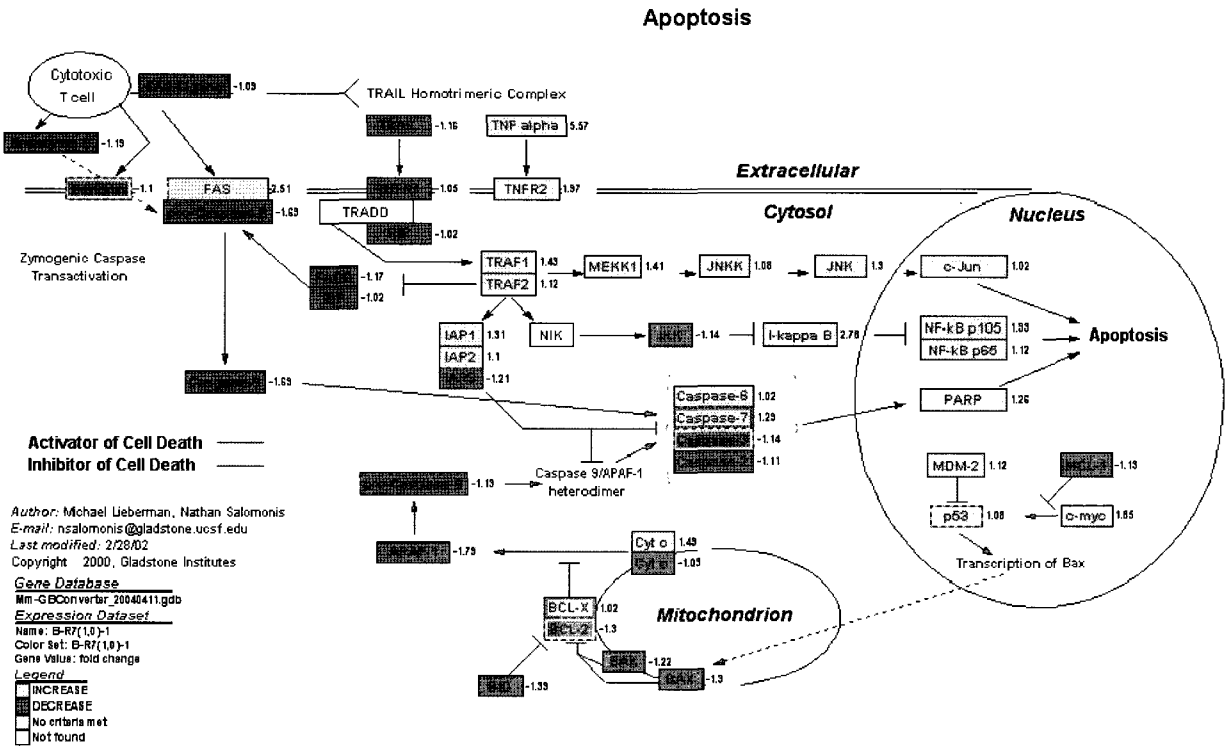

Fig. 3. Genes involved in apoptotic pathway. Expression of the genes in pink is up-regulated and that of the genes in blue is down-regulated.

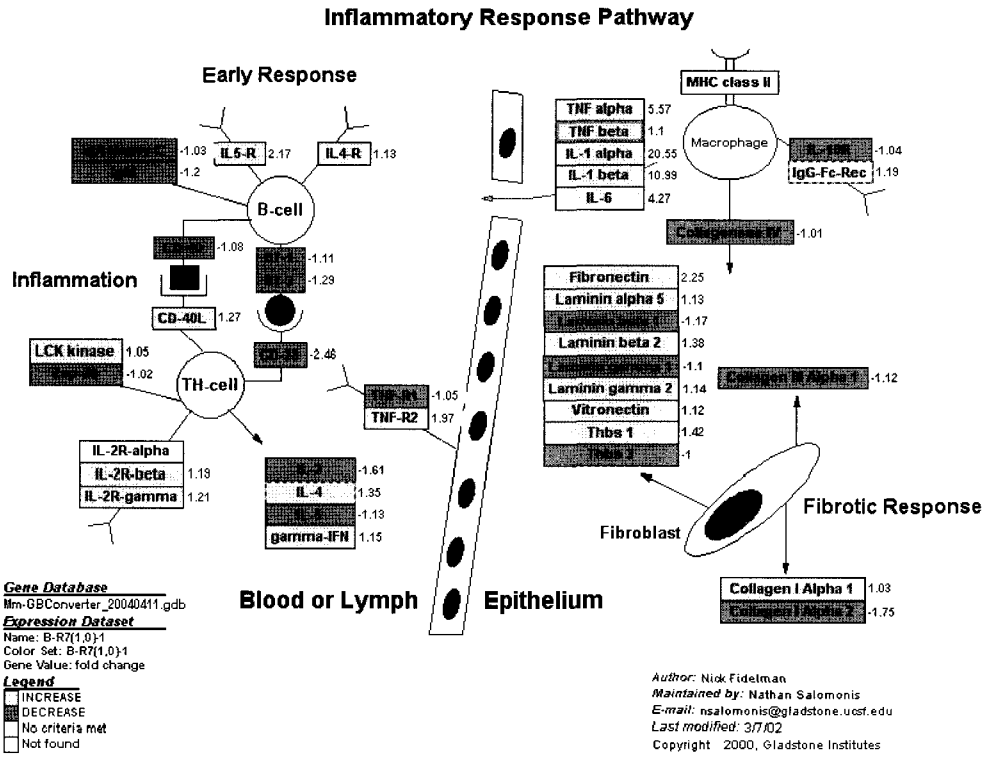

Fig. 4. Genes involved in inflammatory response pathway. Expression of the genes in pink is up-regulated and that of the genes in blue is down-regulated.

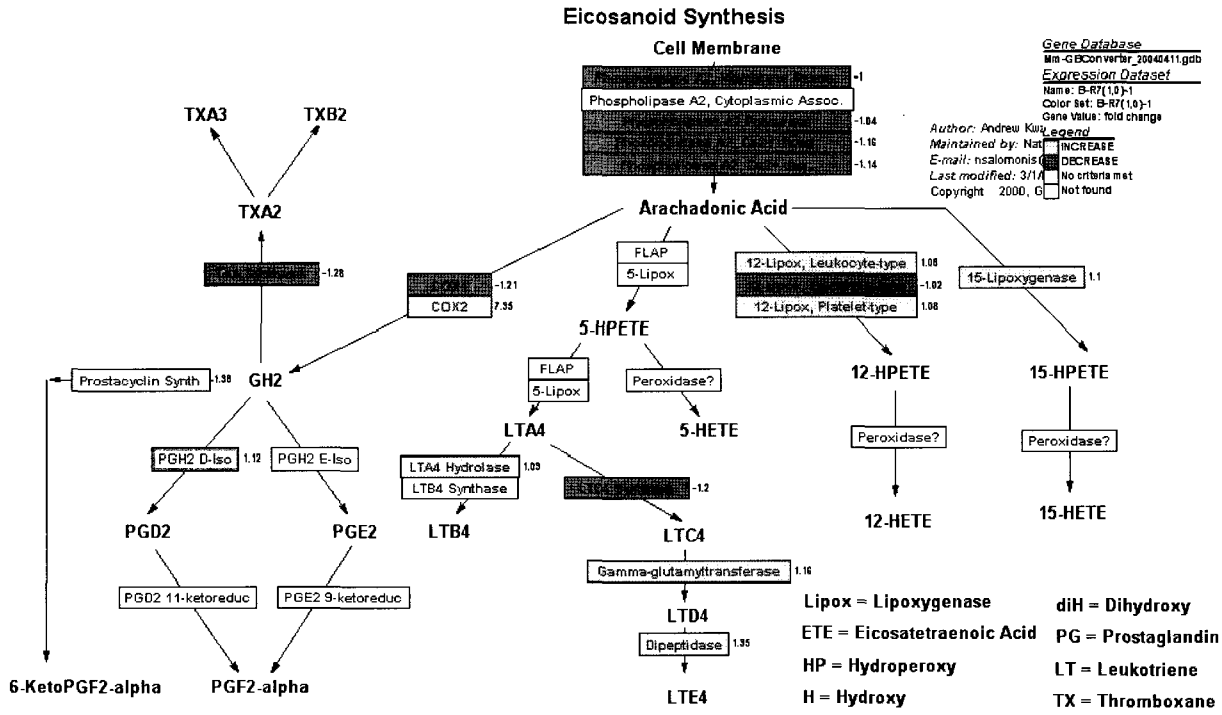

Fig. 5. Genes involved in eicosanoid synthesis. Expression of the genes in pink is up-regulated and that of the genes in blue is down-regulated

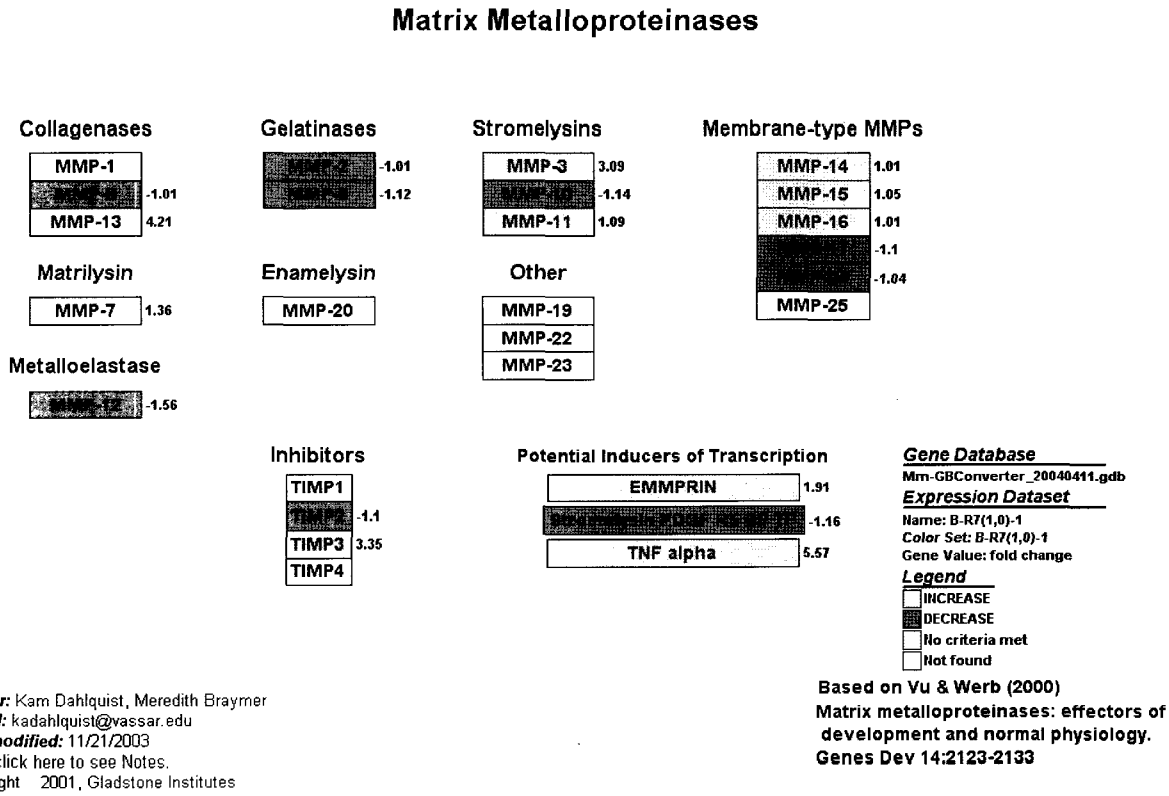

Fig. 6. Matrix metalloproteinases. Expression of the genes in pink is up-regulated and that of the genes in blue is down-regulated.

were not altered (Fig. 5).

Matric metalloproteinases: Matrix metalloproteinases (MMPs) showed interesting pattern of expression. Among the various types of MMPs, MMP 3 and 13 were upregulated, whereas MMP 12 was slightly down-regulated. Tissue inhibitor of MMP (TIMP)-3 was increased (Fig. 6).

DISCUSSION

Microglia are important, because they can control the severity of reperfusion injury in the ischemic penumbra after stroke attack. In the present study, the gene expression patterns of primary cultured microglia following OGD and reperfusion were investigated using microarray analysis.

The ischemic penumbra has been documented in the laboratory animals as severely hypoperfused, nonfunctional, but still viable brain tissue surrounding the irreversibly damaged ischemic core. Saving the penumbra is the main target of acute stroke therapy, and is the theoretical basis behind the reperfusion concept. In experimental focal ischemia, early reperfusion has been shown to both prevent infarct growth and aggravate edema formation and hemorrhage, depending on the severity and duration of prior ischemia and the efficiency of reperfusion, whereas neuronal damage with or without enlarged infarction may result also from reperfusion (so-called reperfusion injury). Activated microglia contribute to reperfusion injury. To reduce potential neurologic morbidity, a sufficient understanding of the pathophysiologic mechanisms involved in reperfusion injury by microglia is needed to design clinically effective therapeutical options. Microglial effects on brain injury are divided into those with immediate influences and those with delayed or long-term effects. In this study, we focused on the immediate effect by limiting the experimental condition to 1 h of reperfusion following 4 h of OGD exposure.

We found that reperfusion injury, a strong stimulator of microglial activation, evoked transcriptional changes of hundreds of genes in a model of 4 hrs of OGD and 1 h of reperfusion. Reperfusion caused the induction of many genes as well as the suppression of other large number of genes. These results were highly expected, because microglial activation would accompany up-regulation of genes, even though ischemic insult generally would suppress gene transcription. Most of genes activated by reperfusion injury are inflammation and immune related genes, whereas death related genes were hardly changed.

Cell death, especially apoptosis, may not be the major response in this experimental condition, because microglia have previously been reported to be very resistant to the ischemia and reperfusion injury (Yenari & Giffard, 2001). It has been reported that microglia display resistance to ischemia-like insults, and that microglial activation in some settings renders them capable of generating factors that enhance their own survival, enhancing their resistance to an apoptotic, but not to a necrotic insult (Yenari & Giffard, 2001). In the present study, since cell death measured by LDH assay was not significant and anti-apoptotic genes in addition to pro-apoptotic genes were not altered, reperfusion under the present experimental condition was not expected to cause damaging effect to microglia. However, this reperfusion condition caused almost complete cell death of neuron or astrocyte at our hands (data not shown).

Phagocytic cells in ischemic brain lesions are derived from distinct sources, including perivascular monocytes, infiltrating macrophages derived from blood monocytes, and parenchymal microglia. Microglia, the major glial component of the CNS, play a critical role as resident immunocompetent and phagocytic cells in the CNS, and serve as scavenger cells in the event of infection, inflammation, trauma, ischemia and neurodegeneration in the CNS (Thomas, 1992; El Khoury et al, 1998). Upon activation as a part of the inflammatory response, microglia are transformed into phagocytes, which are capable of releasing potentially cytotoxic substances such as oxygen free radicals, nitric oxide (NO), proteases, and cytokines (Colton & Gilbert, 1987; Banati et al, 1993) including interleukin (IL)-1, IL-6 and TNF-α (Dickson et al., 1993). Following the formation of ischemic brain lesions, endogenous stimulators activate monocytic cells via CD14, a receptor that mediates inflammatory events (Asea et al, 2000). In this experiment, reperfusion injury increased many of the inflammatory cytokines such as TNF-α, IL-1 and IL-6. This result strongly supports the recent theory that inflammation caused by microglia contributes mainly to the delayed damage in the stroke brain.

Cyclooxygenase (COX) is a rate-limiting enzyme in the synthesis of prostaglandins and thromboxanes. Two isoforms have been described: COX-1 and COX-2. COX-1 is constitutively expressed in many cells, where it produces prostanoids that are involved in normal cellular function (Vane et al., 1998). COX-2 is normally expressed at low levels in some cells, such as neurons. However, COX-2 in many cells is upregulated in response to mitogens, inflammatory mediators, and hormones (O'Banion, 1999). In models of inflammation, COX-2 is upregulated and contributes to tissue damage through the production of reactive oxygen species and toxic prostanoids. The catalytic activity of COX-2 is associated with production of reactive oxygen species (O'Banion, 1999). Superoxide produced by active COX-2 may react with NO to form the powerful oxidant peroxynitrite. The present study showed that COX-2 expression was upregulated, but COX-1 remained silent following ischemia and reperfusion. As demonstrated by others, increased COX-2 might play a detrimental role to microglia in many neurological disorders. There is evidence to show that COX-2 participates in the mechanisms of cerebral ischemia. COX-2 mRNA and protein expression were upregulated 12-24 h after cerebral ischemia in rodents (Nogawa et al, 1997; Nogawa et al, 1998), and COX-2 expression was observed in neurons of rodents at the periphery of the infarct, in vascular cells, and possibly in microglia (Nogawa et al, 1998). Recently, COX-2 has also been found to be expressed in the human brain after ischemic stroke (Sairanene et al, 1998, Iadecola et al, 1999), and COX-2 immunoreactivity is observed in ischemic neurons at the border of ischemic territory, and in neutrophils and vascular cells. The upregulation in COX-2 immunoreactivity was confined to the area of damage (Iadecola et al, 1999), and administration of NS-398, a relatively selective COX-2 inhibitor, 6 hrs after ischemia reduced the infarct volume by 20~30% in a model of focal ischemia in rats (Nogawa et al, 1997; Nagayama et al, 1999). The above observation that delayed administration of NS-398 reduces infarct volume supports the hypothesis that COX-2 is involved in the late stages of ischemic injury.

Matrix metalloproteinases (MMPs) are a gene family of neutral proteases that are important in normal develop-

ment, wound healing, and a wide variety of pathological processes, including the spread of metastatic cancer cells, arthritic destruction of joints, atherosclerosis, and neuroinflammation. In the CNS, MMPs have been shown to degrade components of the basal lamina, leading to disruption of the blood-brain barrier (BBB), and to contribute to the neuroinflammatory response in many neurological diseases. MMPs are tightly regulated to avoid unwanted proteolysis. Secreted as inactive enzymes, the MMPs require activation by other proteases and free radicals. The MMPs are part of a larger class of metalloproteinases (MPs), which includes the recently discovered ADAMs (a disintegrin and metalloproteinase domain) and ADAMTS (a disintegrin and metalloproteinase thrombospondin) families. MMPs have complex roles at the cell surface and within the extracellular matrix. At the cell surface, they act as sheddases, releasing growth factors, death receptors, and death-inducing ligands, making them important in cell survival and death (Rosenberg, 2002). In the present study, expression of MMP-3 and -13 were found to increase. These MMPs have common targets such as collagenases, thus leading to destruction of similar extracellular structures. MMP-3 was reported to be localized to macrophage-like cells (Maeda and Sobel, 1996), but the localization of MMP-13 has not previously been reported. In this study, tissue inhibitors of MMP-3 (TIMP-3) were found to be increased. Until now, four endogenously produced TIMPs have been described; they inhibit both the MMPs and the ADAMs families (Brew et al., 2000). There is considerable homology in TIMP molecules: TIMP-1 is a 28-kDa molecule that forms a strong bond with MMP-3 and -9, and TIMP-2 is a 21-kDa molecule that binds most strongly with MMP-2, facilitating activation by MT-MMP. At low concentrations, TIMP-2 facilitates the activation of MMP-2, but it is inhibitory at higher concentrations. TIMP-3 is unique among the TIMPs in that it is bound to ECM (Rosenberg, 2002).

In this study, using microglia and reperfusion injury model, we tried to verify that microarray analysis could be a new useful tool to characterize biologic events. Evidences demonstrated that reperfusion injury induced microglial activation, mainly through the actions of inflammatory and immune related genes. These results are consistent with previous reports in many aspects. Therefore, we suggest that microarray analysis of gene expression may be useful for elucidating novel molecular mechanisms of microglial activation and can make a profound impact on the understanding of the cellular mechanisms as a whole. Such a methodolgy could provide deeper insights into the molecular basis of other brain disorders and identify potential targets for therapy.

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