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내독소로 활성화된 뇌소교세포의 유전자 발현에 대한 유전자칩분석

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Gene Expression Analysis of Murine Primary Microglia Stimulated with LPS using Microarray

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Background: Since heightened microglial activation was shown to play a role in the pathogenesis of many brain disorders, understanding the molecular mechanisms of microglial activation may lead to new treatment strategies. The microarray system permitted screening of large numbers of genes in biological or pathological processes. Therefore, we evaluated the gene expression pattern during microglial activation using microarray analysis.
Methods: Primary microglial cultures were prepared from postnatal Swiss Webster mice. The cells were activated by lipopolysaccharide (LPS, 10 μg/ml) for 5 hours prior to cell harvesting. From the cultured cells, we isolated mRNA, synthesized cDNA, converted to biotinylated cRNA and then reacted with GeneChips (Affymetrix MU74A-v2). The data were normalized and analyzed.
Results: After microglial activation with LPS, we found >4 fold increases in the expression of 139 genes and >4 fold decreases of 16 genes expression compared with control. Most of the induced or suppressed genes were known to regulate inflammation, immune reactions, injury responses, cell death or survival related mechanisms.
Conclusions: These results suggest that microarray analysis of gene expression may be useful for screening novel molecular mediators of microglial activation and making profound understanding of the cellular mechanisms as a whole. Such screening techniques should provide insights into the molecular basis of brain disorders and help to identify potential targets for therapy.

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INTRODUCTION

Microglial cells are brain macrophages, which serve specific functions in the defense of the central nervous system (CNS) against microorganisms, the removal of tissue debris in neurodegenerative diseases or during normal development, and in autoimmune inflammatory disorders of the brain. In cultured microglial cells, several soluble inflammatory mediators such as cytokines and bacterial products like lipopolysaccharide (LPS) were demonstrated to induce a wide range of microglial

activities, e.g. increased phagocytosis, chemotaxis, secretion of cytokines, activation of the respiratory burst and induction of nitric oxide synthase.[1-4]

Inflammation has been widely perceived as participating in the etiology of acute and chronic neurodegenerative conditions.[5] Accordingly, in the context of acute or chronic diseases in the CNS, activated microglia have been viewed as detrimental and attempts have been made to treat both conditions. Recent studies have suggested that microglia act as standby cells in the service of both the immune and the nervous systems. In the healthy CNS, microglia are quiescent, but in the event of injury they perform their function by buffering harmful compounds and clearing debris from the damaged site, and provide immune-related requirement for recovery.[2,4] In addition, inflammation has been implicated as a secondary injury mechanism following ischemia and stroke.[5] The vascular endothelium promotes inflammation through the upregulation of adhesion molecules, binding of circulating leukocytes and their migration into the CNS. Once in the CNS, the production of cytotoxic molecules may facilitate cell death. The microglial response to injury may either be beneficial by scavenging necrotic debris or detrimental by facilitating cell death in neurons that would otherwise recover. While many studies have tested these hypotheses, the importance of inflammation in these models is inconclusive.

Since microglial activation was shown to play a role in the pathogenesis of CNS disorders, understanding the molecular mechanisms of microglial activation may lead to new treatment strategies for CNS diseases. The microarray system allowed evaluation of large numbers of genes. In this study, we evaluated the usefulness of the microarray analysis in the model of activated microglia.

MATERIALS and METHODS

1. Cell Culture

Murine microglial cultures were prepared from newborn mouse pups as previously described.[6] Whole brains from postnatal day 1~3 Swiss Webster mice were plated in 75 cm^2 primaria coated flasks in Eagle's Minimum Essential Medium (Gibco BRL) supplemented with 10% equine serum, 10% fetal bovine serum, recombinant epidermal growth factor (total 1 mg/100 ml), glutamine (total 2 mM), glucose (total 21 mM) and bicarbonate (total 26 mM). The cultures were maintained in a 37℃ humidified incubator with a 5% CO_2 atmosphere. Media was changed every 2~3 days for the first 10 days. Microglial cultures were prepared using methods previously described.[3] After 10~14 days in vitro, flasks were inspected for microglia growing on top of a confluent cell layer and shaken at 160 rpm for 30 min at 37℃. The supernatant was collected and spun for 5 min at 800 g. The resulting pellet was 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 were used for experiments 24 h after plating.

Histochemical staining with *Griffonia simplicifolia* B4-isolectin (IB4, Sigma) confirmed that the majority of these cells were indeed microglia. Experiments were repeated three times using cells isolated from three different dissections. Cultured microglia were activated by exposure to LPS (E. Coli serotype 055:B5; Sigma). Dose of LPS was 10 g/ml diluted in plating media and exposed 5 h before harvesting. Control cultures were in only plating media.

2. 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). Briefly, 20 ug 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 (Ambion, Megascript T7 high yield transcription kit, #1334) and then fragmented.

3. Microarray analysis

The fragmented cRNA was hybridized to duplicate Affymetrix Murine Genome U74v2, GeneChip A (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 then the data were analyzed with dChip7 and GenMAPP tools.[8]

4. RNA isolation and reverse transcription-polymerase chain reaction assay

From the cultured microglial samples, total RNA was isolated with RNeasy Midi kit (Qiagen, #75144) by the supplier's instructions. The concentration of total RNA isolated was quantified by ultraviolet spectrophotometry at 260/280 nm. Reverse transcription?polymerase chain reaction (RT-PCR) assays were performed with Thermo-Script RT-PCR system (11146-016; Invitrogen, Carlsbad, CA, U.S.A.). One microgram of total RNA from each sample was reverse-transcribed into complementary DNA (cDNA), and PCR was performed following the manufacturer' s protocol. The following primers were used: TNF-alpha sense: 5 '-GCTGCCCCGACTATGTGCTCC TCA -3-, antisense: 5' -ACGCCCCGGCCTTCCAAATAAAT -3 '; GAPDH, sense: 5' -TGAAGGTCGGTGTG AACGGATT TGG-3 ', antisense: 5' -ACGACATACTCAGC ACCAGCATCA C-3 '; SOD2 sense: 5' -GCG ACCTACGTGAACAATCTGAAC G-3 ', antisense 5' -TCAATCCCCAGCAGTGGAATAAGGC-3 '. Amplification cycles were performed in a thermal cycler (Techne Inc., Princeton, NJ, U.S.A.). Before the cycle started, the sample was preheated at 94℃ for 2 minutes. Each cycle consisted of 25 cycles of 45 seconds at 94℃, 30 seconds at 63℃, 45 seconds at 72℃ (TNF-alpha), 26 cycles of 45 seconds at 94℃, 30 seconds at 55℃, 45 seconds at 72℃ (GAPDH), 25 cycles of 1 minutes at 94℃, 45 minutes at 60℃, and 1 minutes at 72℃ (SOD2) followed by 10 minutes at 72℃. Negative control reactions were performed with each batch of cDNA synthesis without reverse transcriptase. The PCR products were analyzed by electrophoresis on 1% agarose gels (Agarose; Gibco BRL, Carlsbad, CA, U.S.A.) with 1× Tris/borate/EDTA buffer (Gibco BRL). The gels were stained with ethidium bromide and densitometric measurements were made from the film using a GS-700 imaging densitometer (Bio-Rad) and then quantified using Multi-Analyst (Bio-Rad). The mRNA level of constitutively expressed GAPDH was determined to control for differences in cDNA synthesis efficiency.

RESULTS

1. Data processing

Array images are shown to demonstrate signal intensities from the DNA chips after hybridization of the samples and probes on the chips (Fig. 1-A, 1-B). After acquisition of the signals, we transformed them into digital numbers and performed absolute analysis. To test the reproducibility of each microarray analysis, we compared the absolute data from each chips (Fig. 1-C). Normalization and comparison of the data was made and hierachical clustering was performed (Fig. 2). The genes showing similar gene expression patterns are placed in the same group or clustered.

2. RT-PCR analysis

We performed RT-PCR to validate the results of microarray analysis. We selected 2 representative genes involved in LPS induced microglial activation such as tumor necrosis factor-alpha (TNF-alpha) and superoxide dismutase 2 (SOD2). They showed dramatic increase after LPS treatment, which is consistent with the microarray results. We used the same RNA samples isolated for the microarray experiments to perform the RT-PCR (Fig. 3).

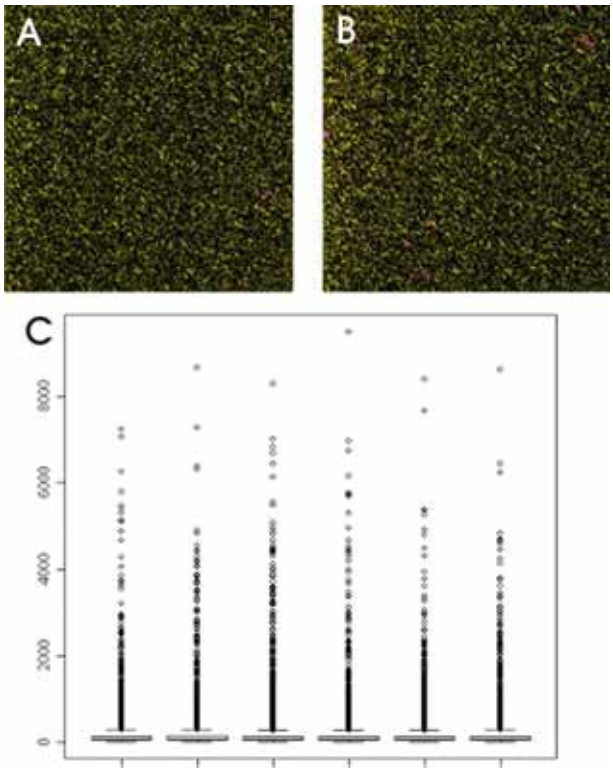

Figure 1. Representative scanned microarray chip images of control (A) and LPS treated groups (B). Distribution of the signal intensities from each gene sets are compared between chips to show the reproducibility of the experiments (C).

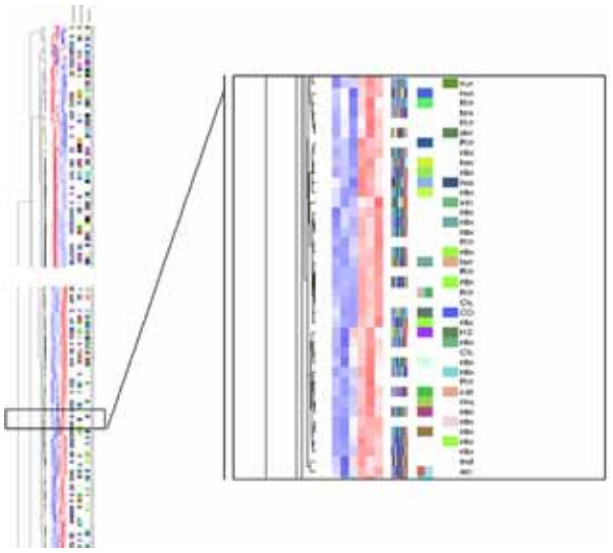

Figure 2. Hierarchical clustering. Genes showing similar patterns are clustered in the same groups and data from chips can be compared with each other. Left column shows data from thousands of genes and figure in the inset are enlarged to show the detail.

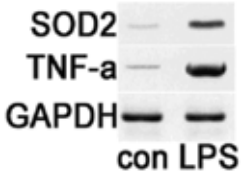

Figure 3. Gel electrophoresis of polymerase chain reaction products. Gene expression level of superoxide dismutase 2 (SOD2) and tumor necrosis factor-alpha (TNF-a) in cultured microglia are observed. Total RNA is isolated from the microglial cells harvested before (con) or 5 h after LPS treatment (LPS). GAPDH is used as an internal control.

Table 1. Number of genes showing increased or decreased expression compared to control at various threshold (fold change)

Fold change	Total number of altered genes	Number of increased genes	Number of decreased genes
1.2	416	283	133
1.3	396	273	123
1.4	370	266	104
1.5	350	259	91
1.7	323	245	78
2	287	231	56
3	216	189	27
4	155	139	16
5	125	117	8
6	112	105	7

3. Overall expression patterns of genes during microglial activation

Cultured cells were stained positive for Griffonia simplicifolia B4-isolectin, a marker for microglia, and the activation of the microglia was confirmed morphologically. LPS treatment caused marked alterations of the expression levels of a large number of genes. To observe the global transcriptional activity after LPS treatment, we counted the numbers of genes showing altered transcriptional activities compared to control group using various criteria (Table 1). With the increase of the threshold in criteria, the number of genes that passed the criteria diminished. The majority of genes showing altered transcriptional activity were attributed to the increased gene expression. After comparing the data from 3 repeated experiments, we found out that some genes

Table 2. Functional classification of genes whose expression were changed more than 4 fold after LPS exposure

Gene function	Probe set	Name of gene	Accession	Fold change
eicosanoid metabolism				
	104647_at	prostaglandin-endoperoxide synthase 2	M88242	92.03
	104406_at	prostaglandin E synthase	AI060798	22.76
	92546_r_at	prostaglandin D2 synthase (21 kDa, brain)	AB006361	19.58
superoxide metabolism				
	96042_at	superoxide dismutase 2, mitochondrial	L35528	10.31
	103909_at	copper chaperone for superoxide dismutase	AI839702	6.46
cell motility				
	103448_at	S100 calcium binding protein A8 (calgranulin A)	M83218	6.86
	99387_at	formyl peptide receptor 1	L22181	9.4
cell death				
	92488_at	caspase 12	Y13090	34.98
	161666_f_at	growth arrest and DNA-damage-inducible 45 beta	AV138783	11.6
	102905_at	caspase 4, apoptosis-related cysteine protease	Y13089	6.79
	93931_at	perforin 1 (pore forming protein)	X12760	5.83
	98427_s_at	nuclear factor of kappa light chain gene enhancer in B-cells 1, p105	M57999	4.84
signaling pathway				
	96764_at	interferon-inducible GTPase	AJ007971	8.15
	101457_at	Janus kinase 2	L16956	6.54
receptor binding activity				
	160873_at	ghrelin	AV206059	10
matrix metalloendopeptidase activity				
	100484_at	matrix metalloproteinase 13	X66473	59.66
response to stress				
	160879_at	telomeric repeat binding factor 1	AA692742	13.92
cytokine and chemokine activity				
	102218_at	interleukin 6	X54542	141.46
	94755_at	interleukin 1 alpha	M14639	122.79
	103486_at	interleukin 1 beta	M15131	126.59
	95348_at	chemokine (C-X-C motif) ligand 1	J04596	67.22
	98406_at	chemokine (C-C motif) ligand 5	AF065947	57.76
	101160_at	chemokine (C-X-C motif) ligand 2	X53798	45.49
	94761_at	chemokine (C-C motif) ligand 7	X70058	42.23
	93871_at	interleukin 1 receptor antagonist	L32838	27.5
	102629_at	tumor necrosis factor	D84196	26.58
	102424_at	chemokine (C-C motif) ligand 3	J04491	19.75
	94146_at	chemokine (C-C motif) ligand 4	X62502	16.63
	93858_at	chemokine (C-X-C motif) ligand 10	M33266	14.39
	102736_at	chemokine (C-C motif) ligand 2	M19681	13.29
	93717_at	chemokine (C-C motif) ligand 12	U50712	11.54
inflammatory and immune response				
	94224_s_at	interferon activated gene 205	M74123	76.81
	103639_at	interferon-induced protein with tetratricopeptide repeats 2	U43085	75.23
	98822_at	interferon-stimulated protein	X56602	44.79
	100981_at	interferon-induced protein with tetratricopeptide repeats 1	U43084	43.44
	93956_at	interferon-induced protein with tetratricopeptide repeats 3	U43086	33.54
	102712_at	serum amyloid A 3	X03505	25.3
	98417_at	myxovirus (influenza virus) resistance 1	M21038	21.48
	103465_f_at	serum amyloid A 2	U60438	16.57

Table 2. Continued

Gene function	Probe set	Name of gene	Accession	Fold change
	98465_f_at	interferon activated gene 204	M31419	11.68
	98988_at	molecule possessing ankyrin-repeats induced by lipopolysaccharide	AA614971	11.47
	93865_s_at	histocompatibility 2, T region locus 10	M35244	9.89
	98472_at	histocompatibility 2, T region locus 23	Y00629	9.59
	94774_at	interferon activated gene 202A	M31418	8.94
	103080_at	SAM domain and HD domain, 1	U15635	8.39
	103035_at	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	U60020	6.34
	93497_at	complement component 3	K02782	5.26
	94928_at	tumor necrosis factor receptor superfamily, member 1b	X87128	7.6
	98088_at	CD14 antigen	X13333	5.06
	102791_at	proteosome subunit, beta type 8 (large multifunctional protease 7)	U22033	4.62
	97540_f_at	histocompatibility 2, D region locus 1	M69069	4.55
	94142_at	colony stimulating factor 3 (granulocyte)	M13926	4.04
physiological processes				
	95024_at	ubiquitin specific protease 18	AW047653	35.16
	104509_at	cholesterol 25-hydroxylase	AF059213	21.97
	92903_at	transcription factor AP-2 beta	X78197	21.73
	92232_at	suppressor of cytokine signaling 3	U88328	10.16
	102313_at	GTP cyclohydrolase 1	L09737	10.1
	104669_at	interferon regulatory factor 7	U73037	8.02
	94186_at	Tnf receptor-associated factor 1	L35302	6.21
	92471_i_at	schlafen 2	AF099973	6.06
	162288_f_at	pyruvate carboxylase	AV374281	6.01
	94214_at	fatty acid binding protein 3, muscle and heart	X14961	5.46
	160564_at	lipocalin 2	X81627	5.21
	161964_r_at	protein kinase C, zeta	AV367375	5.19
	94255_g_at	chloride intracellular channel 4 (mitochondrial)	AI845237	4.59
	101554_at	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	U57524	4.37
	102737_at	endothelin 1	U35233	4.65
	93483_at	hemopoietic cell kinase	J03023	4.16
	94246_at	E26 avian leukemia oncogene 2, 3' domain	J04103	4.03
	94881_at	cyclin-dependent kinase inhibitor 1A (P21)	AW048937	4.02

Probe set; Affymetrix probe set number, Accession; Genebank accession code number, Fold change; expression level vs. control

showed high degree deviation from average, which means the datum of one experiment is extremely different from those of the other 2 experiments. Because these data can make false positive results, we decided to exclude these data. By increasing the criteria threshold, we could eliminate those data and decided to use threshold criteria of 4 fold. At this threshold, all the data were significantly consistent between the repeated experiments. When we put the threshold criteria at 4 fold, 155 genes passed the criteria. From 155 genes, 139 genes showed increased expression and only 16 showed decreased expression level. The increase of the transcriptional activity after LPS stimulation was consistent all through the different threshold criteria. Thus we concluded that LPS exposure mainly induced gene expression with the minor exceptions.

3. Functional classification of genes expressed during microglial activation

After the general estimation was done, we started to investigate the identities of individual genes. We focused on the genes which passed the criteria of 4 fold change to reduce the number and then we classified those genes into different functional subgroups (Table 2). LPS induced expression of a large number of genes associated with inflammation or immune responses such as prostaglandin synthases, cytokines, chemokines and cytokine

induced genes. The other genes with diverse functions were also induced. They included transcription factors, cell signaling components, proteases, receptor binding proteins, enzymes, ion channels, local hormone and so on. From the genes whose expression were down-regulated, matrix metalloproteinase (MMP) 7 & 12 and ameloblastin were the representative genes.

4. Observation of some important pathways

Chemotaxis related genes showed a variety of responses to LPS exposure. Small inducible cytokine precursors (Ccls) such as Ccl-2, 3, 4, 5, 7, 8, 9, 12 and 17 increased. Another macrophage related inflammatory protein Cxcl-2 & -10, Fpr1, and S100a8 also increased the transcriptional level. Some of the chemokine receptors such as Ccr5, Cxcr4 and Cmkor1 showed slight decrease (Fig. 4). Among mitogen activated protein kinase (MAPK) related pathway members, B-raf, MAPK kinase kinase (MEKK)-1 & -2, MAPK kinase (MEK)-1 and dual specificity mitogen-activated protein kinase kinase 3 (MKK3) increased while MEKK3 and p38 decreased. One of the target protein of MAPK pathway, microtubule associated protein (MAP)-2, showed increased gene expression (Fig. 5). Proinflammatory cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-6 showed elevated gene levels (Fig. 6). Signal transducers and activators of the transcription family of proteins (STAT)-1 and -3 increased. Nuclear factor-kappaB (NF-kappaB), a main inflammation regulating transcription factor, also increased gene expression markedly. The expression of tumor growth factor (TGF)-beta receptors slightly increased their transcriptional level. Smad-2 and -4, the transcriptional modulator of TGF-beta, showed minor increase of gene expression while the other Smads did not (Fig. 7).

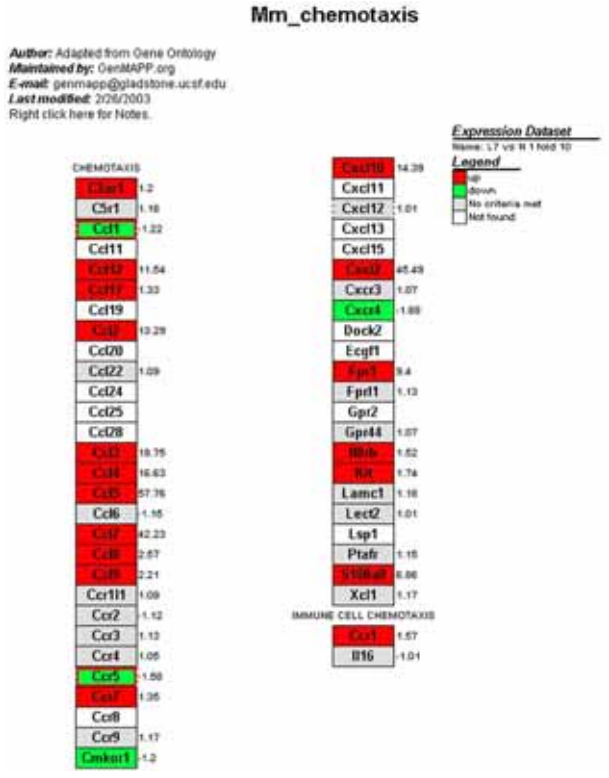

Figure 4. Chemotaxis related gene expression. Expression of the genes in dark gray is up-regulated and that of the genes in light gray is down-regulated.

DISCUSSION

In the present study, we investigated the gene expression patterns of cultured microglia following LPS treatment using microarray analysis. There have been reports that applied microarray analysis technology in the field of CNS[9-10] and ours may be the first report showing the mechanism of microglial activation using microarray in the culture system.

We found that LPS, a strong stimulator of microglial activation, evoked expression changes of hundreds of genes 5 h after treatment. LPS exposure caused induction of a large numbers of genes and suppression of very small number of genes. This result coincided with our expectation that microglial activation would accompany up-regulation of genes. Even though the microarray analysis could provide enormous data, it was not easy to deal with all the genes. So we tried to reduce the number of genes by screening the genes with minimal transcriptional activity out. Then, we classified those genes into functionally related groups.

Up-regulated expression was easily observed in cytokines, chemokines and inflammation and immune response related genes. Superoxide dismutase, a key

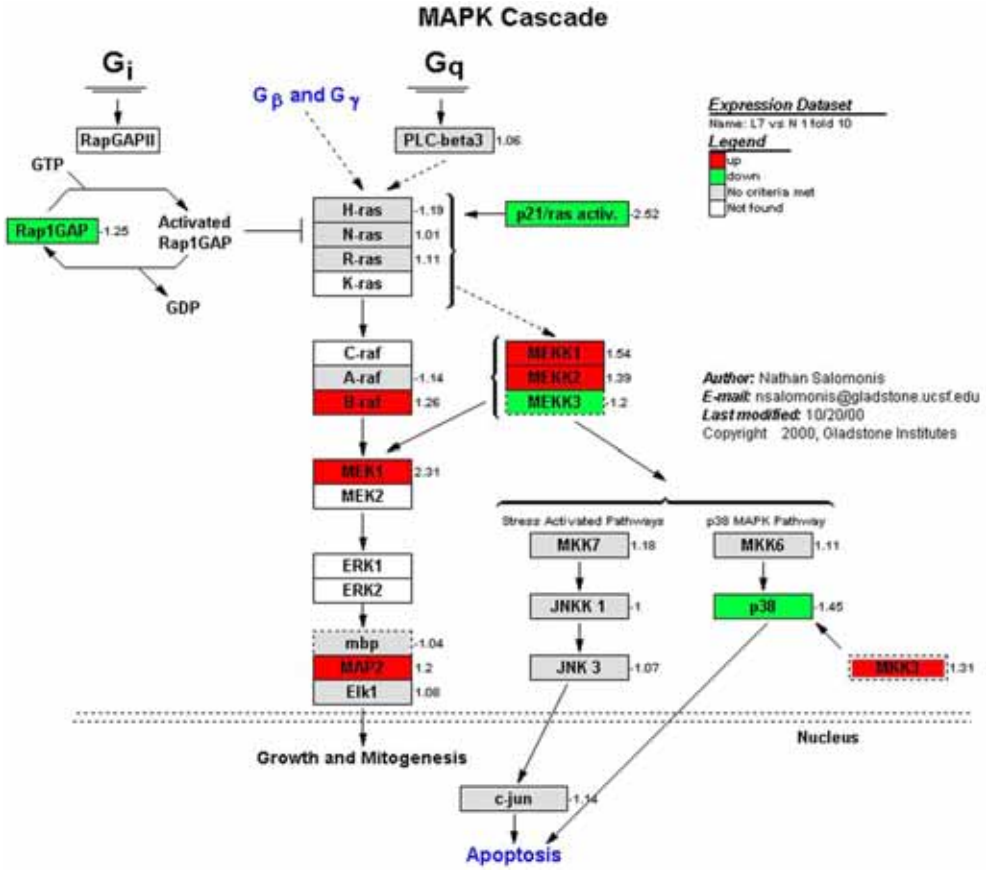

Figure 5. Genes of the MAPK pathway. Expression of the genes in dark gray is up-regulated and that of the genes in light gray is down-regulated.

enzyme in superoxide metabolism, and various types of prostaglandin synthases were activated following LPS treatment. Some transcription regulating factors such as transcription factor AP-2 beta and nuclear factor kappa B inhibitors, also showed high level of gene expression. Even though we could not detect any cell death from the cultured microglia, we observed high level of expression of some death related genes. While we observed hundreds of elevated gene expressions, suppressed gene expression was rare. Matrix metalloproteinases (MMPs) showed interesting pattern of expression. While MMP 13 was up-regulated, MMP 7 & 12 were down-regulated. Extra-cellular matrix (ECM) macromolecules are important for creating the cellular environments. MMP, collectively called matrixins, are proteinases that participate in ECM degradation.[11,12] Under normal physiological conditions, the activities of MMPs are precisely regulated at the level of transcription, activation of the precursor zymogens, interaction with specific ECM components, and inhibition by endogenous inhibitors.[11,12]

Since simple description of highly expressed genes is not meaningful and microglial actions on inflammation in CNS disorders are relatively well known, we made close observations on a couple of well known microglia related pathways. This pathway finding method included most of the known components of each mechanism and could provide the precise and overall explanation. Following LPS exposure, small inducible cytokine precursors (Ccls) and other inflammatory proteins involved in chemotaxis increased. These results suggested that microglial activation induced production of chemoattractants for different cell types.[13] But most of the chemokine

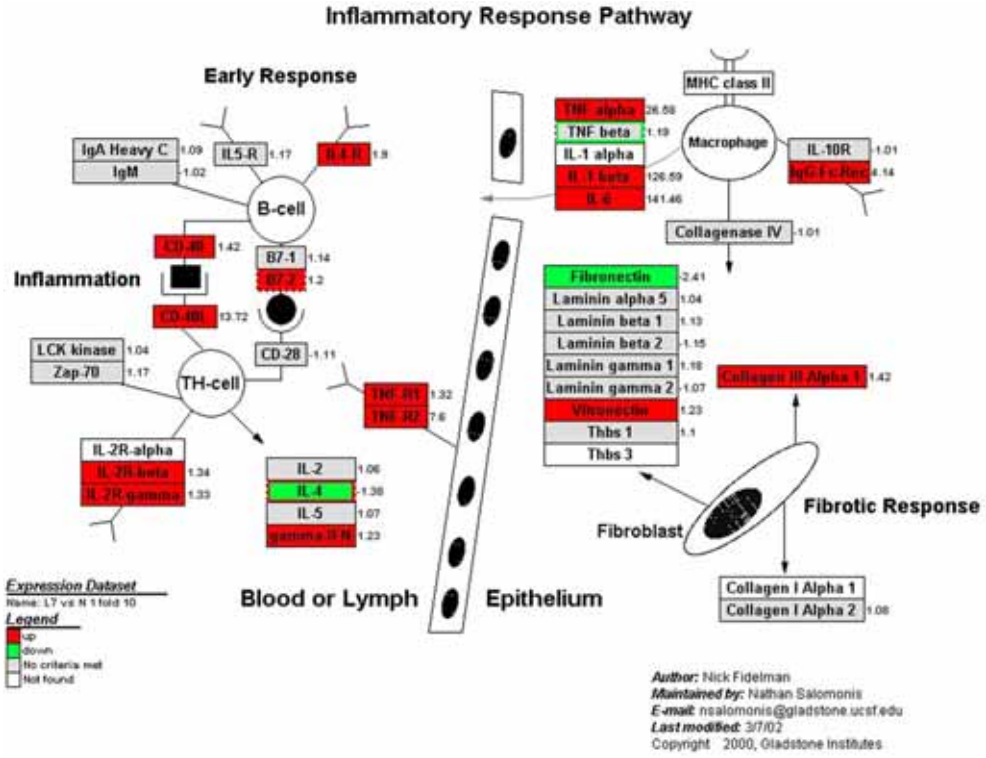

Figure 6. Genes involved in inflammatory responses. Expression of the genes in dark gray is up-regulated and that of the genes in light gray is down-regulated.

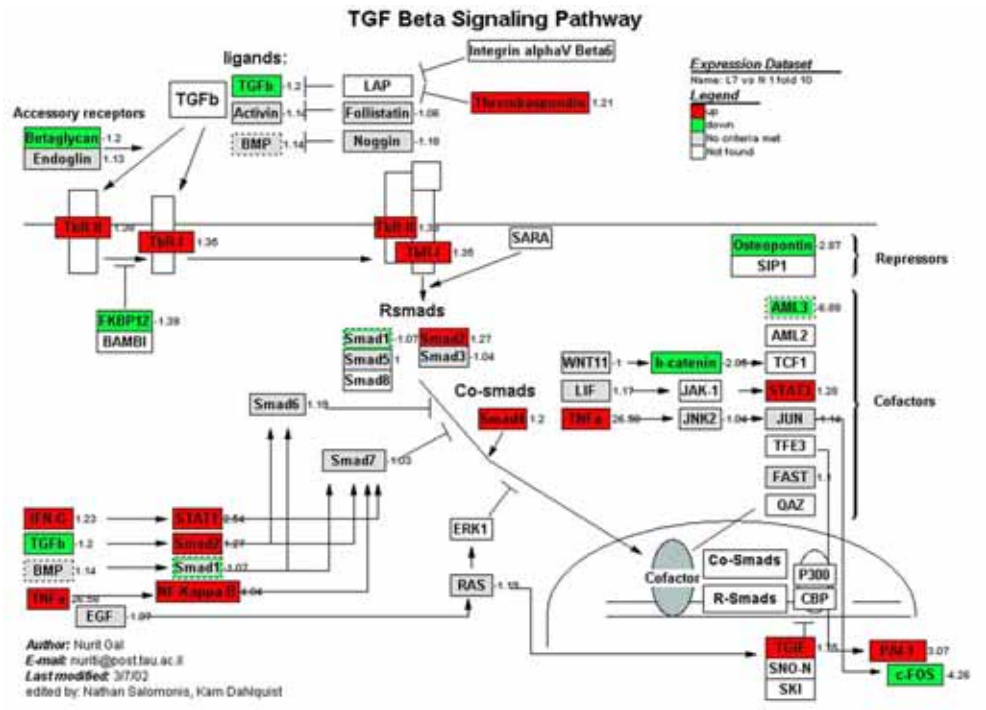

Figure 7. TGF-beta signaling system. Expression of the genes in dark gray is up-regulated and that of the genes in light gray is down-regulated.

receptors did not altered their expressions and some of them showed slight decrease of expression even after microglial activation. This finding is not unexpected because most of chemoattractants are destined to act on surrounding cells.

Mitogen activated protein kinase (MAPK) is one of the major kinases involved in microglial activation.[5] Some of the MAPK pathway members decreased and some others increased while the others made no changes. It seemed that components in the upstream of the MAPK cascade pathway, such as MEKK1 & 2, MKK3, and MEK1, were induced following microglial activation while downstream members, such as c-Jun N-terminal kinase 3 (JNK3) and p38, were suppressed. Since MAPK action is generally regulated by its enzymatic activity not transcriptional level, it is hard to explain the meaning of MAPK pathway alterations based on gene expression study only. But we assume that LPS induce microglial activation by triggering the upstream of MAPK pathway but each specific pathway responds differentially. ERK controls growth and mitogenesis. This part seemed to be activated because one of the its target material MAP2 increased. On the contrary, JNK3 and p38, apoptosis inducing pathway, was suppressed. This is consistent with our culture study because LPS induced cell proliferation and no apoptotic cell death was found at our hand.

It is known that inflammation progresses by the action of pro-inflammatory cytokines, including IL-1, TNF, interferon (IFN) gamma, IL-12, IL-18 and granulocyte-macrophage colony stimulating factor, and is resolved by anti-inflammatory cytokines such as IL-4, IL-10, IL-13, IFN alpha, and TGF-beta.14 Following LPS stimulation, TNF-alpha, IL-1beta and IL-6 showed elevated gene levels. Receptor for TNF also increased. Other inflammatory components which are known to exist in other cell types were not affected. TNF-alpha is mainly secreted by macrophages and microglia and strong inducer of the MMP transcription. This may explain the upregulation of MMP-13.

In some individuals, immune reactivity becomes inappropriate or excessive, and autoimmune or chronic inflammatory diseases ensue. TGF-beta functions as an immuno-suppressive factor or an anti-inflammatory factor.[14] TGF-beta controls the differentiation, proliferation, and activation of immune cells. Therefore, TGF-beta signaling may play an important role in inflammatory diseases. TGF-beta triggered signals are transduced by Smads, a family of proteins that serves as substrates for TGF-beta receptors type I and II, in which the cytoplasmic domain possesses serine/threonine kinase activity.[15,16] The type I receptor recognizes and phosphorylates Smad2 and Smad3 (R-Smad), which associates with Smad4 (Co-Smad), forming complexes that participate in DNA-binding and recruitment of transcription factors. In addition to these agonistic Smads, inhibitory Smads (I-Smad) such as Smad6 and Smad7, which bind to the activated receptors and interfere with Smad2 and Smad3 binding, are present. The expression level of TGF-beta signaling system and Smads did not show marked changes. STAT is one of the substrate of janus-activated kinase (JAK) and plays a role as transcription factor. STAT3 in macrophages apparently plays a negative role in inflammation.[17] NF-kappaB is a key regulator of the inflammatory cascade and many inflammatory mediators such as inflammatory cytokines, adhesion molecules and iNOS have NF-kappaB binding sequences in their promoters.[5] Both STAT, a major pro-inflammatory signal regulators, and NF-kappaB, a main inflammatory transcription factor, increased gene expression markedly.

In this study, we tried to verify that microarray analysis could be a new useful tool to characterize biologic events using microglial activation model. The evidences made at our hand demonstrated that LPS-induced microglial activation was based on the actions of pro-inflammatory transcription factors, intracellular signaling systems and inflammatory cytokines. And these results are consistent with previously reports. Here we suggest that microarray analysis of gene expression may be useful for elucidating novel molecular mediators of microglial activation and making profound understanding of the cellular mechanisms as a whole. Such a screening technique might provide further insights into the molecular basis of brain disorders and identify potential

targets for therapy.

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