

# Lipopolysaccharide, Interleukin (IL)-12/IL-2 Interferon Gamma T Cell Chemokine

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## T Cell Chemokine Inducibility of Lipopolysaccharide, Interleukin (IL)-12/IL-2 and Interferon Gamma in Mouse Brain

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**Background** : Interferon gamma (IFN- $\gamma$ )-inducible protein 10 (IP-10) and monokine induced by IFN- $\gamma$  (Mig) are members of CXC-chemokine family, that are produced from macrophage/monocyte activated by IFN- $\gamma$ . These chemokines especially recruit activated T cell into inflammatory site. Here, we studied effect of lipopolysaccharide (LPS), interleukin (IL)-12/IL-2 or IFN- $\gamma$  on induction of MIG and IP-10 in mouse brain. **Methods** : In order to evaluate Mig and IP-10 gene expression in brain, we injected LPS, IFN- $\gamma$  or IL-12/IL-2 into mice intraperitoneally, and measured chemokine message in brain by RT-PCR. **Results** : In vivo injection of LPS induced Mig and IP-10 gene expression in brain of normal mice and IFN- $\gamma$  knockout mouse, however, in vivo injection of IL-12/IL-2 induced Mig and IP-10 gene expression in only the brain of normal mice. IFN- $\gamma$  induced chemokine expression in cultured brain cells, but anti-inflammatory drugs did not block IFN- $\gamma$  effects. **Conclusions** : Immune stimulating agents, LPS or IL-12/IL-2 or IFN- $\gamma$ , can induce T cell chemokine gene expression in brain cells, and these chemokines may play a role in T cell infiltration in various brain diseases.

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**Key Words** : Chemokine, Lipopolysaccharide, Interleukin-12, Interleukin-2, Interferon gamma, Brain

(cytokine) (multiple sclerosis, MS),  
90 interleukin(IL)-1, IL-6 IL-1, IL-6 TNF 가 ,<sup>3-5</sup> trans-  
tumor necrosis factor(TNF)가 - - forming growth factor(TGF)-<sup>6</sup> TGF- super-  
(hypothalamus-pituitary-adrenal axis, family bone morphogenetic proteins,<sup>7</sup>  
HPA axis) <sup>8</sup>  
IL-1, IL-6 TNF HPA axis 가

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T 가  
<sup>9</sup> MS <sup>10,11</sup> T  
(chemokine) 가 가

CXC CC  
<sup>12</sup>  
 가 T  
 (chemotaxis) mo-  
 nokine induced by interferon gamma(Mig) in-  
 terferon gamma inducible protein 10(IP-10)  
 glutamate-leucine-arginine(ERL)  
 CXC , T  
<sup>13</sup> ,<sup>14</sup>  
<sup>15</sup>  
 T 가  
 LPS IL-12  
<sup>16</sup> IL-12 T  
 T IFN-  
<sup>17</sup> LPS IL-12 IFN-  
 가  
 IFN- T  
<sup>18</sup>  
 가 , MS 가  
 T 가  
 가  
 Mig IP-10 LPS, IL-12 IFN- 가  
 IFN-  
 가  
 1. 6~8 BALB/c  
 IFN- (gamma-IFN knockout,  
 GKO) (BALB/c origin) . GKO  
 Dr. Robert H. Wiltrout  
 10~15  
 2.  
 1) LPS LPS 50 µg  
 1 µg/ml  
 IL-2 300,000  
 U 7 , IL-12  
 0.5 µg 4 7 11  
 IFN- 1 4 11  
 U 50,000 U , 50,000  
 U 2 4

RNA - 70  
 2)  
 (mixed brain cell culture)  
 10 ~15  
 70% ethanol  
 가  
 trypsin(0.25%) DNase(0.5%)가 가 Hank's  
 balanced salt solution(HBSS) 37 , 5%  
 CO<sub>2</sub> 30 HBSS 가  
 (1200 rpm, 5 )  
 HBSS 가  
 HBSS (Pasteur pipet-  
 tet) 20 , 10 ml  
 HBSS 가 5  
 10% fetal bovine  
 serum(FBS) 1 × - (GIBCO BRL)가  
 가 Dulbecco's Modified Eagle's Medium(DMEM,  
 Gibco-BRL) 가  
 1 × 10<sup>6</sup>  
 cell/ml poly-D-lysine coating  
 plate 37 , 5% CO<sub>2</sub> 2~3  
 3)  
 Neuro 2A  
 IFN- (100 U/ml)  
 LPS(1 µg/ml) 가 6 24  
 PBS 2  
 RNA mRNA  
 4) (reverse transcriptase-poly-  
 merase chain reaction, RT-PCR)  
 RNA  
 PBS 3  
 1.5 ml RNAzolB cell scraper  
 2 ml  
 RNAzolB 가  
 RNA  
 RNAzolB 1/10 chloro-  
 form 가 12,000 rpm 15  
 RNA  
 RNA 1.5 ml  
 RNA 100% isopropanol 가  
 - 20 16 RNA  
 RNA-isopropanol 12,000 rpm  
 RNA (pellet)

**Table 1.** Primers used in RT-PCR

Name	Type*	Primer sequence
G3PDH	S	GCCACCCAGAAGACTGTGGATGGC
	AS	CATGTAGGCCATGAGGTCCACCAC
IP-10	S	ACCATGAACCCAAGTGCTGCCGTC
	AS	GCTTCACTCCAGTTAAGGAGCCCT
MIG	S	GATCAAACCTGCCTAGATCC
	AS	GGCTGTGTAGAACACAGAGT

\* S, sense primer; AS, antisense primer

ice-cold 70% 1 ml 가  
 RNA DEPC-  
 DW RNA  
 RT-PCR  
 RT-PCR RNA  
 70 ~75 10 RNA  
 5X RT buffer 2 µl, 10 mM  
 dATP 0.25 µl, 10 mM dGTP 0.25 µl, 10 mM dTTP  
 0.25 µl, 10 mM dCTP 0.25 µl, MMLV reverse trans-  
 criptase(200 U/µl) 0.25 µl, RNase inhibitor(28 U/µl)  
 0.25 µl, 50 µM oligo dT primer 0.5 µl, DEPC-DW 4 µl  
 PCR RT-  
 RNA (1 µg/µl) 2 µl 가  
 (mineral oil) 1 10  
 PCR (Cetus 2400, Perkin Elmer Co.)  
 42 60 , 94 3  
 1:1  
 PCR PCR 10X PCR  
 buffer 3 µl, 25 mM MgCl<sub>2</sub> 1.8 µl, 10 mM dATP 0.3  
 µl, 10 mM dGTP 0.3 µl, 10 mM dTTP 0.3 µl, 10  
 mM dCTP 0.3 µl, 50 µM sense antisense primer  
 0.25 µl, Taq polymerase(5 U/µl, Promega) 0.25 µl  
 25 µl  
 PCR PCR PCR  
 1 PCR 5 µl  
 (Cetus 2400,  
 Perkin Elmer) PCR  
 94 5 가 94 30 , 57 45  
 , 72 45 1 (cycle) 18~35  
 DNA 72 5  
 PCR . 1% agarose PCR  
 UV (transilluminater)  
 DNA band DNA  
 Mig IP-10 DNA band Gel Doc  
 2000(Biorad ) G3PDH  
 (relative intensity, RI)  
 RT-PCR primer  
 Table 1

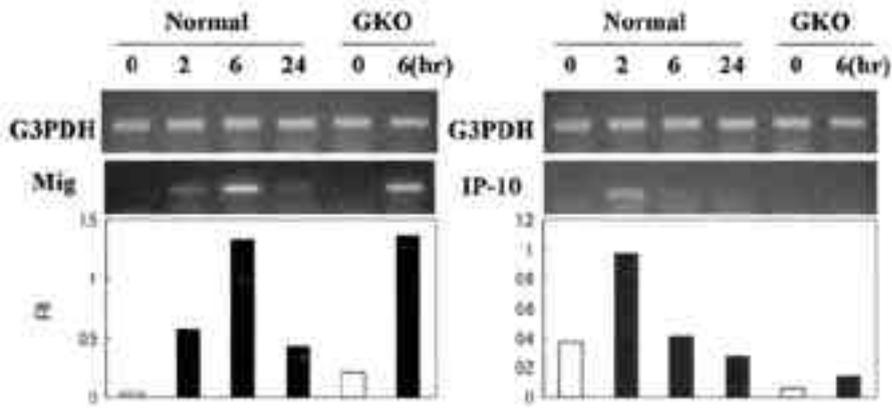
4 24 . TPBS  
 BSA-TPBS (1:500) (Anti-mouse Mig  
 Antibody, R & D system Cat. No.:AF-492-NA)  
 2 . TPBS 3 BSA-  
 TPBS (1:500) 2 (Anti-goat IgG-POD,  
 Sigma: No. A 8150) 2  
 TPBS 3 Amersham phar-  
 macia biotec ECL detection kit (RPN 2109)

Western blotting immunoblotting  
 SDS-polyacrylamide  
 . 15% well 5  
 20mA  
 3 . transfer buffer  
 10 ECL Hybond membrane  
 Mini Blot Module 180mA

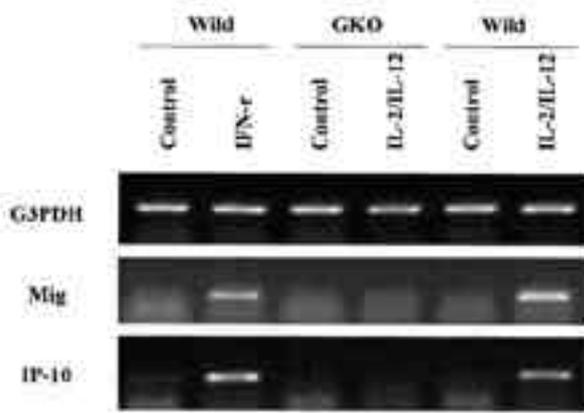
1 Amido-  
 Black dye  
 Mig band immunoblot-  
 ting  
 6) 가

IFN-  
 IFN- (100 U/ml)  
 cyclohexamide(CHX, 2 µg/ml), cyclosporin  
 A(CSA, 3 µg/ml) sodium salicylate(SS, 5 mM),  
 wortmanin(WM, 10 ng/ml) piperazine( PZ, 20  
 µM), dexamethasone(1 µM) 가 6  
 PBS 3 RNAzoIB

5) Immunoblotting western blotting  
 Immunoblotting  
 (1×10<sup>6</sup>/ml) IFN- (10<sup>2</sup> U/ml) 24  
 Amicon microcon-10  
 (3000×g, 30min)  
 ECL Hybond membrane(Amersham Life  
 Science, RPN 303D) blotting 3% BSA  
 0.05% tween 20 가 PBS(BSA-TPBS)



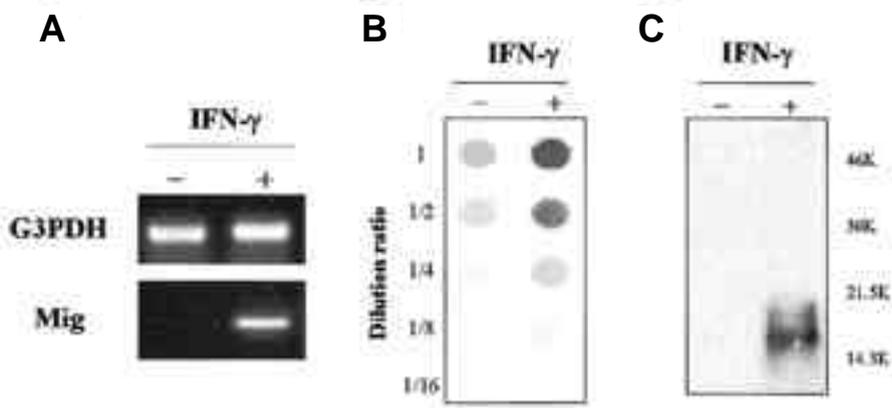
**Figure 1.** Effect of LPS on Mig and IP-10 gene expression in brain of normal and GKO mice. Normal or GKO mice are treated with LPS (50 ug/mouse) peritoneally, and brain tissue is harvested 2, 6, or 24 hours after LPS injection. Mig and IP-10 message are detected by RT-PCR. LPS ; lipopolysaccharide, GKO ; gamma-IFN knockout, RT-PCR ; reverse transcriptase-polymerase chain reaction.



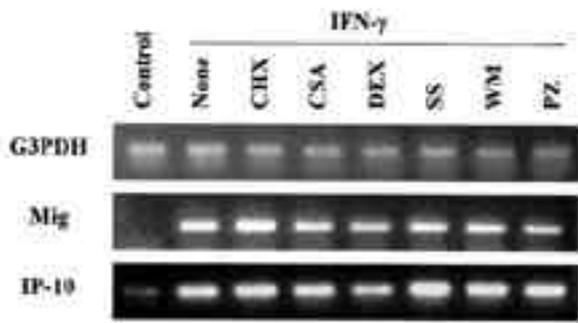
**Figure 2.** Effect of IFN- $\gamma$  and IL-12/IL-2 on Mig and IP-10 gene expression in brain of normal or GKO mice. IFN- $\gamma$  (50,000 U per dose) is given intraperitoneally and intravenously on day 0 and 1, and brain is harvested on day 3. IL-2 (300,000 IU per dose) is administered intraperitoneally twice on day 0 and IL-12 (0.5 g per dose) is given intraperitoneally on day 0-3. Brains of mice treated with IL-12/IL-2 are harvested on day 4. Mig and IP-10 message of brain are detected by RT-PCR. GKO ; gamma-IFN knockout, RT-PCR ; reverse transcriptase-polymerase chain reaction.

PCR Mig IP-10  
 IL12/IL-2 IFN- $\gamma$  Mig IP-10  
 12/IL-2 GKO IL-  
 (Fig. 2).  
 Mig IP-10 IFN-  
 10~15  
 IFN- $\gamma$  6  
 RNA RT-PCR IFN-  
 Mig IFN-  
 Mig 24 Mig immunoblot-  
 ting western blotting IFN-  
 Mig 가 (Fig. 3).  
 Mig IP-10 가 IFN-  
 가  
 IFN-  
 SS, CHX, WM, PZ, DEX 6 CSA,  
 RT-PCR IFN-  
 Mig IP-10 가  
 IFN-  
 IP-10 가 (Fig. 4).  
 Mig

IP-10 2 , Mig 6  
 가 . LPS GKO  
 6  
 Mig 가 (Fig. 1).  
 IL-12/IL-2가  
 IL-2(50,000  
 U/mouse) day 0, IL-12(5 ug/mouse) day 0~3  
 4 . IFN-  
 IFN- (50,000 U/mouse)  
 3  
 RNA RT-



**Figure 3.** Expression of Mig message and protein in IFN- stimulated primary mixed brain cell. Confluent primary mixed brain cells are stimulated with or without IFN- (100 U/ml). Mig message is detected by RT-PCR (A), and Mig protein is detected by immunoblotting (B) and Western blotting (C). RT-PCR ; reverse transcriptase-polymerase chain reaction.



**Figure 4.** Effect of immunosuppressive agents, anti-inflammatory agents and signal blockers on the IFN- induced IP-10 and Mig gene expression of primary mixed brain cell. IFN-stimulated brain cells are treated with drugs for 6 hours, and Mig and IP-10 message are detected by RT-PCR. RT-PCR ; reverse transcriptase-polymerase chain reaction.

IL-2가 IL-12 가 IL-2가 T  
 IL-12 가  
 .<sup>20</sup> Myers <sup>21</sup> IL-12 가  
 T  
 ICAM-1 VCAM-1 CAM  
 가 가 IFN- CAM  
 가 가 .<sup>22</sup> IL-12  
 , CAM 가  
 , T  
 Mig IP-10  
 IL-12  
 IL-12 T NK  
 IFN- ,<sup>23</sup> Mig IP-10  
 IFN-  
 IL-12 Mig IP-10 IL-12  
 IL-12 Mig IP-10 IFN-  
 IFN- Mig IP-10  
 12/IL-2 Mig IP-10  
 GKO Mig IP-10  
 IL-12/IL-2 Mig IP-10  
 IFN- 가  
 IFN- Mig IP-  
 10 가 IFN- 가  
 Mig IP-10  
 IL-12 LPS  
 .<sup>16</sup> LPS  
 Mig IP-10 LPS  
 가  
 RT-PCR IL-12/IL- Mig  
 2 Mig IP-10 가 .  
 GKO LPS LPS Mig  
 LPS IL-12

IFN- $\gamma$  , neurotoxin nitric oxide(NO)

IP-10 LPS 가 가

LPS 2 가 ,<sup>31-33</sup> TNF- NO

LPS GKO IP-10 LPS ,<sup>34</sup> IFN-

6 LPS가 Mig IP-10 가 , ICAM 가

, LPS가 IL-12<sup>16</sup> (neutrophil)<sup>57</sup>

IL-12가 IFN- Mig IFN- 가

IP-10 LPS가 IL-12 IFN- 가

Mig IP-10 가 ,

IP- 가

10 Mig가 가 가 MS So-

RT- rensen<sup>27</sup> MS Mig IP-10

PCR RANTES 가 ,

LPS IFN- 가

Mig IP-10 가 Mig IP-10

가 . 가 Mig IP-10 MS

IFN- LPS , MS

RT-PCR IFN- IFN-

IFN- 가 LPS , LPS IFN-

Mig LPS RT-PCR

LPS IL-12 DEX 가

Mig IP-10 IFN- , LPS

IL-12/IL-2 가 Mig IP-10

Sauty<sup>24</sup> Mig IP-10 IFN- 가 T

<sup>25,26</sup> Mig IP-10 T

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