班員共同執筆論文 Microarray analysis identifies IFNβ-regulated genes in MS（投稿中）

3. Results

3.1. Identification of IFNβ-regulated genes in T cell and non-T cell fractions of MS patients treated with IFNβ

Among 1,263 genes examined, 21 genes showed a statistically significant change in the level of expression in the T cell fraction, non-T cell fraction, or both derived from the 13 MS patients at 3 or 6 months after starting IFNβ treatment (Table 1 and Fig. 1). Of note, 9 of the 21 genes contained an IFN-responsive promoter element that has been characterized previously (No. 1～5, 8～10, and 17 in Table 1). Other 5 genes (No. 6, 7, 11,12, and 18) were known to respond to IFNs, although a regulatory promoter element is not described in the literature . Northern blot analysis indicated that a 4 to 24 hour-exposure of unfractionated PMBC to 100 ng/ml IFNβ markedly up-regulated the expression of IFN regulatory factor 7A (IRF7; No.1 in Table 1, 2.0 kb and 2.6 kb transcripts), IFN-induced 15-kDa protein (ISG15; No.2, 0.7 kb transcript), and IFNα-inducible cDNA 6-16 (IFI6-16; No.3, 0.9 kb transcript) (Fig. 2, panels a-c, lanes 2,6), supporting the validity of cDNA microarray analysis for screening of differential gene expression. A 4 to 24 hour-exposure of PBMC to IFNγ also elevated the mRNA levels of IRF7, ISG15, and IFI6-16, but to a smaller extent for the latter two genes (Fig. 2, panels a-c, lanes 3,7). The similar results were obtained from a set of the experiments performed at a different time (data not shown). As such, the 21 genes identified were enriched in those known to respond to IFNβ (14 out of 21 genes; 67%). Although the remaining 7 genes (No. 13～16, 19～21) were not previously shown to be IFN-responsive, they could be also regarded as novel IRGs. Although 16 of the 21 IRGs (No. 1～16) were up-regulated, five (No. 17～21) were down-regulated in the patients treated with IFNβ.

Among the up-regulated genes, IFN-induced protein 60 (IFI60; No.5), IFNγ-inducible protein 30 (IFI30; No.6), activating transcription factor 3 (ATF3; No.12) and toll-like receptor 5 (TLR5; No.13) were elevated exclusively in T cells. In contrast, IFNα-inducible protein 27 (IFI27; No.7), IFN-induced protein 17 (IFI17; No.8), ATP-binding cassette transporter TAP1 (TAP1; No.9), TNFα-induced protein 6 (TNFAIP6; No.10), TGFβ-stimulated protein TSC-22 (TSC22; No. 11), sulfotransferase family 1C member 1 (SULT1C1; No.14), 39-kDa RNA polymerase III-specific subunit (RPC39; No. 15), and ras family member Rab11a (RAB11A; No. 16) were up-regulated only in non-T cells. Regarding the timing for the up-regulation of the gene, although two

of the four T cell-exclusive genes were significantly elevated at 6 (but not at 3) months after IFNβ treatment, all the eight non-T cell-exclusive genes were significantly elevated at 3 months (Table 1). Three of them were persistently elevated until at 6 months, but five had declined by 6 months. The expression of IRF7 (No.1), ISG15 (No.2), IFI6-16 (No.3), and IFN-induced 56-kDa protein (IFI56; No.4) was elevated in both T and non-T cell fractions. This group of the genes was induced at 3 months and the significant up-regulation could be detected also at 6 months except for IRF7 in non-T cells and ISG15 in T cells.

The remaining five genes had been down-regulated after starting IFNβ therapy. Among these, interleukin-3 (IL-3; No.19) was repressed in both T and non-T cells, but the others were suppressed only in T cells. Whereas protein kinase A-anchor protein 4 (AKAP4; No.20) and guanine nucleotide-binding protein α13 (GNA13; No.21) were suppressed at 3 and 6 months, the others were found to be down-regulated at 6 months, but not at 3 months. Representative panels of the gene expression profile are shown in figure.

3.2. Cytokine gene expression profile of RRMS patients did not support a Th2 shift after IFNβ treatment

Several previous reports suggested that IFNβ exerts preventive effects against MS via inducing a shift of the Th1-biased immune balance towards Th2 (Hall et al., 1997; Karp et al., 2000). However, the IRGs disclosed in the present study did not include the genes encoding the cytokine markers characteristic of either Th1 or Th2 cells or those potentially involved in induction of either population. We investigated the possibility that T cells from the IFNβ-treated patients might show a trend for Th2 polarization, even though the Th2 shift is not large enough to be statistically significant. To verify this possibility, we carefully analyzed the microarray data of additional 31 genes that could be regarded as Th1- or Th2-associated genes. Contrary to our expectations, we did not find a trend for a Th2 shift, but rather observed a trend for down-regulation of Th2 cytokines such as IL-4 and IL-10 predominantly in T cell fraction (data not shown), consistent with a trend for Th1 bias. There was also a trend for up-regulation of CCR5 in both T and non-T cells (data not shown), a putative marker for Th1 cells. Thus, the cytokine gene expression profile of T cell samples did not support a Th2-shifted immune response, but rather suggested a partially Th1-shifted profile. However, the study of a larger number of samples is required to make conclusive remarks on the Th1/Th2 balance altered by IFNβ therapy, because all of the changes

observed in the expression of Th1- or Th2-associated genes did not reach the levels of statistical significance.

4. Discussion

By using cDNA microarray, we here analyzed gene expression profiles of $CD3^+$ T cells and $CD3^-$ non-T cells derived from 13 RRMS patients receiving IFNβ1b. This systemic analysis has identified 21 IRGs, whose expression is either stimulated or repressed by IFNβ treatment *in vivo*. Although previous studies have mainly analyzed the total PBMC, we here used isolated T cells and non-T cells, which enabled us to reveal that the IRGs are differentially expressed in distinct fractions. Among these IRGs, IRF7, ISG15, IFI56, IFI6-16, IFI60, IFI17, TAP1, TNFAIP and MIG are regarded as prototype ISGs, since previous studies showed that the promoter regions of these genes have IFN-responsive elements (Reich et al., 1987; Porter et al., 1988; Reid et al., 1989; Beck et al., 1992; Lee et al., 1993; Bluyssen et al., 1994; Wong et al., 1994; de Veer et al., 1998; Taniguchi and Takaoka, 2002), and furthermore, Northern blot analysis verified IFNβ-stimulated expression of a set of the genes described above. Although IFN-responsive promoter elements have not yet been characterized, an IFN-induced expression of IFI30, IFI27, TSC22, ATF3, or SLC7A1 in cell lines or primary cells was reported previously (Luster et al., 1988; Rasmussen et al., 1993; Drysdale et al., 1996; Ohta et al., 1996; Simmons et al., 1996). The reliability and relevance of our observations are further supported by a previous study that human fibrosarcoma cell line exposed to IFNβ up-regulates the expression of 6 of the 21 genes (ISG15, IFI56, IFI6-16, IFI60, IFI17 and TAP1) assessed on microarray (Der et al., 1998). Of note, the 21 genes that we have identified included a set of heretofore unreported IRGs. Among these, TLR5, SULT1C1, RPC39 and RAB11A were up-regulated, whereas IL-3, AKAP4 and GNA13 were down-regulated in the specific lymphocyte fractions. Since detailed information on these genes is available from various sources, we focus on the changes that might be relevant for understanding the therapeutic effects of IFNβ.

4.1. Up-regulation of known IRGs possibly accounting for the effect of IFN-β

Among the known IRGs (table), TNFAIP rewards special attention. This gene encodes a 39-kDa secreted glycoprotein designated as tumor necrosis factor-α-stimulated gene-6 (TSG-6), that is highly homologous to CD44 (Lee et al., 1993; Wisniewski et al., 1996; Bardos et al., 2001). Proinflammatory cytokines such as TNFα and IL-1 can rapidly induce expression of TSG-6 *in vitro* (Lee et al., 1993). Consistently, TSG-6 expression is up-regulated in the inflamed tissues

such as arthritic joint (Bardos et al., 2001). TSG-6 forms a stable complex with inter-α-inhibitor (IαI) that has a serine protease inhibitory activity (Wisniewski et al., 1996). The protease inhibitory activity of IαI is elevated more than 100-fold after making the TSG-6/IαI complex, which then blocks serine proteases involved in the plasmin/plasminogen activation. Recombinant TSG-6 protein exhibits a potent anti-inflammatory effect in murine models of inflammation or experimental arthritis (Wisniewski et al., 1996; Bardos et al., 2001). Of note, the plasmin system is tightly linked with activation of matrix metalloprotease (MMP)-9 (Bardos et al., 2001 Legrand et al., 2001), and IFNβ treatment reduces MMP-9 and MMP-7 mRNA expression in PMBC of RRMS patients (Yong et al., 1998; Galboiz et al., 2001). Therefore, the possiblilty exists that IFNβ suppresses the disease activity of MS by up-regulating TSG-6 levels in non-T cells such as macrophages/microglia, leading to the blockade of the protease cascade involved in progression of inflammatory demyelination. Another interesting gene is IFI17, which encodes a membrane protein implicated in the control of cell growth (Deblandre et al., 1995) Although it remains an open question if the function of autoimmune T cells is significantly modulated by IFI17, this might somehow contribute to the anti-proliferative effects mediated by IFNβ.

4.2. Down-regulation of known IRGs possibly accounting for the effect of IFN-β

The present observations suggest that down-regulation of monokine induced by IFNγ (MIG; No.17) in the T cell samples might have significant implications. MIG encodes a chemokine of the CXC subfamily produced chiefly by monocytes/macrophages, acting as a chemoattractant for memory T cells by interacting with a receptor CXCR3 exclusively expressed on activated T cells (Liao et al., 1995). Notably, MIG and the receptor CXCR3 are over-expressed on macrophages, T cells and reactive astrocytes in active demyelinating lesions of MS (Simpson et al., 2000). These results suggest that down-regulation of MIG by IFNβ would lead to ameriolation of the inflammatory process in MS.

Down-regulation of SLC7A1 in the T cell samples may be also relevant. SLC7A1 encodes a 68-kDa membrane cationic amino acid transporter termed CAT-1, homologous to the murine ecotropic retroviral receptor (Yoshimoto et al., 1991). Up-regulated expression of CAT-1 in rat cardiac myocytes by exposure to IFNγ increases uptake of L-arginine and stimulates production of nitric oxide (NO) (Simmons et al., 1996), whose involvement in inflammatory demyelination been suggested (Liu et al., 2001). On the other hand, IFNβ inhibits IFNγ-induced expression of

the inducible NO synthase (iNOS) in cultured human astrocytes (Hua et al., 1998). If CAT-1 regulates NO production in human T cells, reduced expression of CAT-1 might represent a mechanism for IFNβ-mediated down-regulation of NO production in inflammatory demyelinating lesions of MS.

4.3. Down-regulation of newly identified IRGs possibly accounting for the effect of IFN-β

Down-regulation of IL-3, AKAP4 or GNA13 needs further evaluation with regard to the therapeutic effects of IFNβ on MS. Overexpression of IL-3 in astrocytes induces multifocal demyelination, accompanied with accumulation of activated macrophages/microglia, suggesting a role of IL-3 as a mediator of inflammatory demyelination (Chiang et al., 1996). These observations, together with our own, suggest that IL-3 could provide a promising target for therapeutic intervention in MS.

AKAP4 encodes a 82-kDa phosphoglycoprotein expressed exclusively in sperm. This molecule is suggested to regulate sperm motility by anchoring protein kinase A to the sperm fibrous sheath (Mohapatra et al., 1998). It remains unknown whether AKAP4 is also involved in the control of T cell motility. It is worthy to note that the male transgenic mice overexpressing IFNβ gene are sterile because of degeneration of sperm cells (Iwakura et al., 1988). These observations suggest that IFNβ acts as a regulator of sperm function during spermatogenesis and raise the question as to if IFNβ therapy may induce male infertility.

GNA13 encodes a component of G-protein α subunit ($G\alpha_{13}$). $G\alpha_{13}$-deficient mice are lethal during embryogenesis because of defective angiogenesis (Offermanns et al., 1997). The fibroblasts isolated from these mice show an impaired migratory response to thrombin, suggesting a functional role of $G\alpha_{13}$ in cellular movement to specific ligands. These observations raise the possibility that $G\alpha_{13}$ is involved in transmigration of activated T cells across the BBB.

4.4. Cytokine and chemokine gene expression

The cytokine gene expression profile showed a trend for down-regulation of both IL-4 and IL-10 genes, and concomitant upregulation of IFNγ, TNFα and CCR5 genes in specific lymphocyte fractions after IFNβ treatment, suggesting a partially Th1-shifted profile in the IFNβ-treated patients. Consistent with our observations, a previous study showed a transient

increase in numbers of IFNγ-secreting PBMC in MS patients during the first two months of IFNβ treatment (Dayal et al., 1995). The expression of CCR5 gene is elevated in unfractionated lymphocytes of MS patients receiving IFNβ (Wandinger et al., 2001). A microarray analysis identified up-regulation of IFNγ, MIG, TAP1 and CCR5 genes in purified human Th1 cells (Rogge et al., 2000). All of these observations suggest that IFNβ might reset an immunological imbalance between T cells and non-T cells of MS patients via more complex mechanisms than a simple Th2-shifted immune response. It does not necessarily contradict the previous documentation that MBP-reactive autoimmune T cell lines selected from the IFNβ-treated patients are Th2 biased. However, our results would indicate that primary targets of IFNβ are a battery of the genes with the specific machinery for responding to IFNs.

In summary, we found that less than 2% of the examined genes (21 out of 1,263 genes) showed a statistically significant change in expression in the T or non-T samples obtained from IFNβ-treated MS patients and that the IRGs are dominated by the genes with IFN responsive promoter element. Some of these changes is relevant for understanding the therapeutic effects or possible side effects of IFNβ treatment. It remains to be further investigated whether the IRGs provide a clinically useful marker to monitor and predict the effectiveness of IFNβ in MS patients.

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References

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.

Bardos,T., Kamath, R.V., Mikecz, K., Glant, T.T., 2001.
Anti-inflammatory and chondroprotective effect of TSG-6 (tumor necrosis factor-α-stimulated gene-6) in murine models of experimental arthritis. Am. J. Pathol. 159, 1711-1721.

Beck, S. Kelly, A., Radley, E, Khurshid, F., Alderton, R.P., Trowsdale, J. 1992.
DNA sequence analysis of 66 kb of the human MHC class II region encoding a cluster of genes for antigen processing. J. Mol. Biol. 228, 433-441.

Bluyssen, H.A.R., Vlietstra, R.J., Faber, P.W., Smit, E.M.E., Hagemeijer, A., Trapman, J., 1994. Structure, chromosome localization, and regulation of expression of the interferon-regulated mouse *Ifi54/Ifi56* gene family. Genomics 24,137-148.

Chabas, D., Baranzini, S.E., Mitchell, D., Bernard, C.C.A., Rittling, S.R., Denhardt, D.T., Sobel, R.A., Lock, C., Karpuj, M., Pedotti R., Heller, R., Oksenberg, J.R., Steinman, L., 2001. The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science 294, 1731-1735.

Chiang, C.-S., Powell, H.C., Gold, L.H., Samimi, A., Campbell, I.L., 1996.
Macrophage/microglial-mediated primary demyelination and motor disease induced by the central nervous system production of interleukin-3 in transgenic mice. J. Clin. Invest. 97, 1512-1524.

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

Darnell, J.E.Jr, Kerr, I.M., Stark, G.R., 1994.
Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415-1421.

Dayal, A.S., Jensen, M.A., Lledo, A., Arnason, B.G.A., 1995.
Interferon-gamma-secreting cells in multiple sclerosis patients treated with interferon beta-1b. Neurology 1995, 2173-2177.

Deblandre, G.A., Marinx, O.P., Evans, S.S., Majjaj, S., Leo, O., Caput, D., Huez, G.A., Wathelet, M.G., 1995. Expression cloning of an interferon-inducible 17-kDa membrane protein implicated in the control of cell growth. J. Biol. Chem. 1995, 23860-

23866.

De Maeyer, E., De Maeyer-Guignard , J., 1994. Interferons. In: Thomson, A.W. (Ed.), The cytokine handbook, 2nd ed., Academic Press, San Diego, pp. 265-288.

Der, S.D., Zhou, A., Williams, B.R.G., Silverman, R.H., 1998. Identification of genes differentlally regulated by interferon α, β, or γ using oligonucleotide arrays. Proc. Natl. Acad. Sci. U.S.A. 95, 15623-15628.

de Veer, M.J., Sim, H., Whisstock, J.C., Devenish, R.J., Ralph, S.J., 1998. IFI60/ISG60/IFIT4, a new member of the human IFI54/IFIT2 family of interferon-stimulated genes. Genomics 54, 267-277.

Drysdale, B.E., Howard, D.L., Johnson, R.J., 1996. Identification of a lipopolysaccharide inducible transcription factor in murine macropahges. Mol. Immunol. 33, 989-998.

Galboiz, Y., Shapiro, S., Lahat, N., Rawashdeh, H., Miller, A., 2001. Matrix metalloproteinases and their tissue inhibitors as markers of disease subtype and response to interferon-β therapy in relapsing and secondary-progressive multiple sclerosis patients. Ann. Neurol. 50, 443-451.

Greenberg, S.A., 2001. DNA microarray gene expression analysis technology and its application to neurological disorders. Neurology 57, 755-761.

Hall, G.L., Compston, A., Scolding, N.J., 1997. Beta-interferon and multiple sclerosis. Trends Neurosci. 20, 63-67.

Hua, L.L., Liu, J.S., Brosnan, C.F., Lee, S.C., 1998. Selective inhibition of human glial inducible nitric oxide synthase by interferon-beta: implications for multiple sclerosis. Ann. Neurol. 43, 384-387.

Iwakura, Y., Asano, M., Nishimune, Y., Kawade, Y., 1988. Male sterility of transgenic mice carrying exogeneous mouse interferon-β gene under the control of the metallothionein enhancer-promoter. EMBO J. 7, 3757-3762.

Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Granger, C.V., Simon, J.H., Alam, J.J., Bartoszak, D.M., Bourdette, D.N., Braiman, J., Brownscheidle, C.M., Coats, M.E., Cohan, S.L., Dougherty, D.S., Kinkel, R.P., Mass, M.K., Munschauer, F.E.III., Priore, R.L., Pullicino, P.M., Scherokman, B.J., Weinstock-Guttman, B., Whitham, R.H., The Multiple Sclerosis

Collaborative Research Group, 1996. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. Ann. Neurol. 39, 285-294.

Jiang, H., Milo, R., Swoveland, P., Johnson, K.P., Panitch, H., Dhib-Jalbut, S., 1995. Interferon β-1b reduces interferon γ-induced antigen-presenting capacity of human glial and B cells. J. Neuroimmunol. 61, 17-25.

Karp, C.L., Biron, C.A., Irani, D.N., 2000. Interferon β in multiple sclerosis: is IL-12 suppression the key? Immunol. Today 21, 24-28.

Keegan, B.M., Noseworthy, J.H., 2002. Multiple sclerosis. Annu. Rev. Med. 53, 285-302.

Lee, T.H., Klampfer, L., Shows, T.B., Vilcek, J., 1993. Transcriptional regulation of TSG6, a tumor necrosis factor- and interleukin-1-inducible primary response gene coding for a secreted hyaluronan-binding protein. J. Biol. Chem. 268, 6154-6160.

Legrand, C., Polette, M., Tournier, J.-M., de Bentzmann, S., Huet, E., Monteau, M., Birembaut, P., 2001. uPA/plasmin system-mediated MMP-9 activation is implicated in bronchial epithelial cell migration. Exp. Cell Res. 264, 326-336.

Liao, F., Rabin, R.L., Yannelli, J.R., Koniaris, L.G., Vanguri, P., Farber, J.M., 1995. Human mig chemokine: biochemical and functional characterization. J. Exp. Med. 182, 1301-1314.

Liu, J.S.-H., Zhao, M.-L., Brosnan, C.F., Lee, S.C., 2001. Expression of inducible nitric oxide synthase and nitrotyrosine in multiple sclerosis. Am . J. Pathol. 158, 2057-2066.

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.

Luster, A.D., Weinshank, R.L., Feinman, R., Ravetch, J.V., 1988. Molecular and biochemical characterization of a novel γ-interferon-inducible protein. J. Biol. Chem. 263, 12036-12043.

Mamane, Y., Heylbroeck, C., Génin, P., Algarté, M., Servant, M.J., LePage, C., DeLuca, C., Kwon, H., Lin, R., Hiscott, J., 1999. Interferon regulatory factors: the next

generation. Gene 237, 1-14.

Mohapatra, B., Verma, S., Shankar, S., Suri, A., 1998.
Molecular cloning of human testis mRNA specifically expressed in haploid germ cells, having structural homology with the A-kinase anchoring proteins. Biochem. Biophys. Res. Commun. 244, 540-545.

Offermanns, S., Mancino, V., Revel, J.-P., Simon, M.I., 1997.
Vascular system defects and impaired cell chemokinesis as a result of $G\alpha_{13}$ deficiency. Science 275, 533-536.

Ohta, S., Shimekake, Y., Nagata, K., 1996.
Molecular cloning and characterization of a transcription factor for the C-type natriuretic peptide gene promoter. Eur. J. Biochem. 242, 460-466.

Panitch, H.S., Hirsch, R.L., Schindler, J., Johnson, K.P., 1987.
Treatment of multiple sclerosis with gamma interferon: Exacerbations associated with activation of the immune function. Neurology 37, 1097-1102.

Paty, D.W., Li, D.K.B., MS/MRI Study Group, IFNB MS Study Group, 1993.
Interferon beta-1b is effective in relapsing-remitting multiple sclerosis II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 43, 662-667.

Phillips, J., Eberwine, J.H., 1996.
Antisense RNA amlification: a linear amplification method for analyzing the mRNA population from single living cells. Methods 10, 283-288.

Porter, A.C., Chernajovsky, Y., Dale, T.C., Gilbert, C.S., Stark, G.R., Kerr, I.M., 1988.
Interferon response element of the human gene 6-16. EMBO J. 7, 85-92.

Poser, C.M., Paty, D.W., Scheinberg, L., McDonald, W.I., Davis, F.A., Ebers, G.C., Johnson, K.P., Sibley, W.A., Silberberg, D.H., tourtellotte, W.W., 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann. Neurol. 13, 227-231.

Reich, N., Evans, B., Levy, D., Fahey, D., Knight, E.Jr., Darnell, J.E.Jr., 1987.
Interferon-induced transcription of a gene encoding a 15-kDa protein depends on an upstream enhancer element. Proc. Natl. Acad. Sci. U.S.A. 84, 6394-6398.

Rasmussen, U.B., Wolf, C., Mattei, M.-G., Chenard, M.-P., Bellocq, J.-P., Chambon, P., Rio, M.-C., Basset, P., 1993. Identification of a new interferon-α-inducible gene (*p27*) on human chromosome 14q32 and its expression in breast carcinoma. Cancer Res. 53, 4096-4101.

Reid, L.E., Brasnett, A.H., Gilbert, C.S., Porter, A.C.G., Gewert, D.R., Stark, G.R., Kerr, I.M., 1989. A single DNA response element can confer inducibility by both α- and γ-interferons. Proc. Natl. Acad. Sci. U.S.A. 86, 840-844.

Rogge, L., Bianchi, E., Biffi, M., Bono, E., Chang, S.-Y.P., Alexander, H., Santini, C., Ferrari, G., Sinigaglia L., Seiler, M., Neeb, M., Mous, J., Sinigaglia, F., Certa, U., 2000. Transcriptional imaging of the development of human T helper cells using oligonucleotide arrays. Nature Genet. 25, 96-101.

Satoh, J.-I., Paty, D.W., Kim, S.U., 1995.
Differential effects of beta and gamma interferons on expression of major histocompatibility complex antigens and intercellular adhesion molecule-1 in cultured fetal human astrocytes. Neurology 45, 367-373.

Simmons, W.W., Closs, E.I., Cunningham, J.M., Smith, T.W., Kelly, R.A., 1996.
Cytokines and insulin induce cationic amino acid transporter (CAT) expression in cardiac myocytes. J. Biol. Chem. 271, 11694-11702.

Simpson, J.E., Newcombe, J., Cuzner, M.L., Woodroofe, M.N., 2000.
Expression of the interferon-γ-inducible chemokines IP-10 and Mig and their receptor, CXCR3, in multiple sclerosis lesions. Neuropathol. Appl. Neurobiol. 26, 133-142.

Staudt, L.M., 2001.
Gene expression physiology and pathophysiology of the immune system. Trends Immunol. 22:35-40.

Stone, L.A., Frank, J.A., Albert, P.S., Bash, C., Smith, M.E., Maloni, H., McFarland, H.F., 1995. The effects of interferon-β on blood-brain barrier disruptions demonstrated by contrast-enhanced magnetic resonance imaging in relapsing-remitting multiple sclerosis. Ann. Neurol. 37, 611-619.

Taniguchi, T., Takaoka, A., 2002.
The interferon-α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. Curr. Opin. Immunol. 14, 111-116.

The IFNB Multiple Sclerosis Study Group, 1993.
Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 43, 655-661.

Wandinger, K.-P., Stürzebecher, C.-S., Bielekova, B., Detore, G., Rosenwald, A., Staudt, L.M., McFarland, H.F., Martin, R., 2001. Complex immunomodulatory effects of

interferon-β in multiple sclerosis include the upregulation of T helper 1-associated marker genes. Ann. Neurol. 50, 349-357.

Whitney, L.W., Ludwin, S.K., McFarland, H.F., Biddison, W.E., 2001.
Microarray analysis of gene expression in multiple sclerosis and EAE identifies 5-lipoxygenase as a component of inflammatory lesions. J. Neuroimmunol. 121, 40-48.

Wisniewski, H.-G., Hua, J.-C., Poppers, D.M., Naime, D., Vilcek, J.,
Cronstein, B.N., 1996. TNF/IL-1-inducible protein TSG-6 potentiates plasmin inhibition by inter-α-inhibitor and exerts a strong anti-inflammatory effect in vivo. J. Immunol. 156, 1609-1615.

Wong, P., Severns, C.W., Guyer, N.B., Wright, T.M., 1994.
A unique palindromic element mediates gamma interferon induction of mig gene expression. Mol. Cell. Biol. 14, 914-922.

Yong, V.W., Chabot, S., Stuve, O., Williams, G., 1998.
Interferon beta in the treatment of multiple sclerosis. Mechanisms of action. Neurology 51, 682-689.

Yoshimoto, T., Yoshimoto, E., Meruelo, D., 1991.
Molecular cloning and characterization of a novel human gene homologous to the murine ecotropic retroviral receptor. Virology 185, 10-17.

Figure Legends

Fig. 1. Gene expression profile of IFNβ-regulated genes whose expression is significantly changed in T cell and non-T cell fractions of MS patients during a 6 month-treatment with IFNβ. By using a cDNA microarray containing 2,881 genes, the gene expression profile was studied in both $CD3^{+}$ T cell fraction and $CD3^{-}$ non-T cell fraction isolated from 13 patients with RRMS, before starting IFNβ treatment designated Pre, at 3 months and at 6 months after the treatment. The results expressed as box and whisker plots represent the following genes: (a) IFN regulatory factor 7A (IRF7) in T cells, (b) IFN-induced 15-kDa protein (ISG15) in non-T cells, (c) IFNα-inducible cDNA 6-16 (IFI6-16) in T cells, (d) TGFβ-stimulated protein (TSC22) in non-T cells, (e) toll-like receptor 5 (TLR5) in T cells, (f) sulfotransferase family 1C member 1 (SULT1C1) in non-T cells, (g) monokine induced by IFNγ (MIG) in T cells, and (h) interleukin-3 (IL-3) in T cells. The longitudinal axis indicates the gene expression level (GEL) as described in the Methods section. The *p* value of the gene regulation index (GRI) for individual genes is listed in Table 1.

Fig. 2. Northern blot analysis of IRF7, ISG15, and IFI6-16 mRNA expression in peripheral blood mononuclear cells exposed to IFNβ or IFNγ. Unfractionated PBMC isolated from a healthy subject were incubated for 4 to 24 hours in the culture medium supplemented with 100 ng/ml of IFNβ, IFNγ, or both, and then processed for RNA preparation. Four μg of total RNA was separated on a 1.5% agarose-6% formaldehyde gel and transferred onto a nylon membrane. The membranes were hybridized with the DIG-labeled DNA probe specific for IRF7 (panel a), and rehybridized with the probe specific for ISG15 (panel b) or the probe specific for IFI6-16 (panel c). The ethidium bromide staining of the corresponding gel is shown in the panel d. The lanes (1-8) represent the following; the cells (1) untreated for 4 hours, treated with (2) IFNβ for 4 hours, (3) IFNγ for 4 hours, (4) IFNβ plus IFNγ for 4 hours, (5) untreated for 24 hours, treated with (6) IFNβ for 24 hours, (7) IFNγ for 24 hours, and (8) IFNβ plus IFNγ for 24 hours.

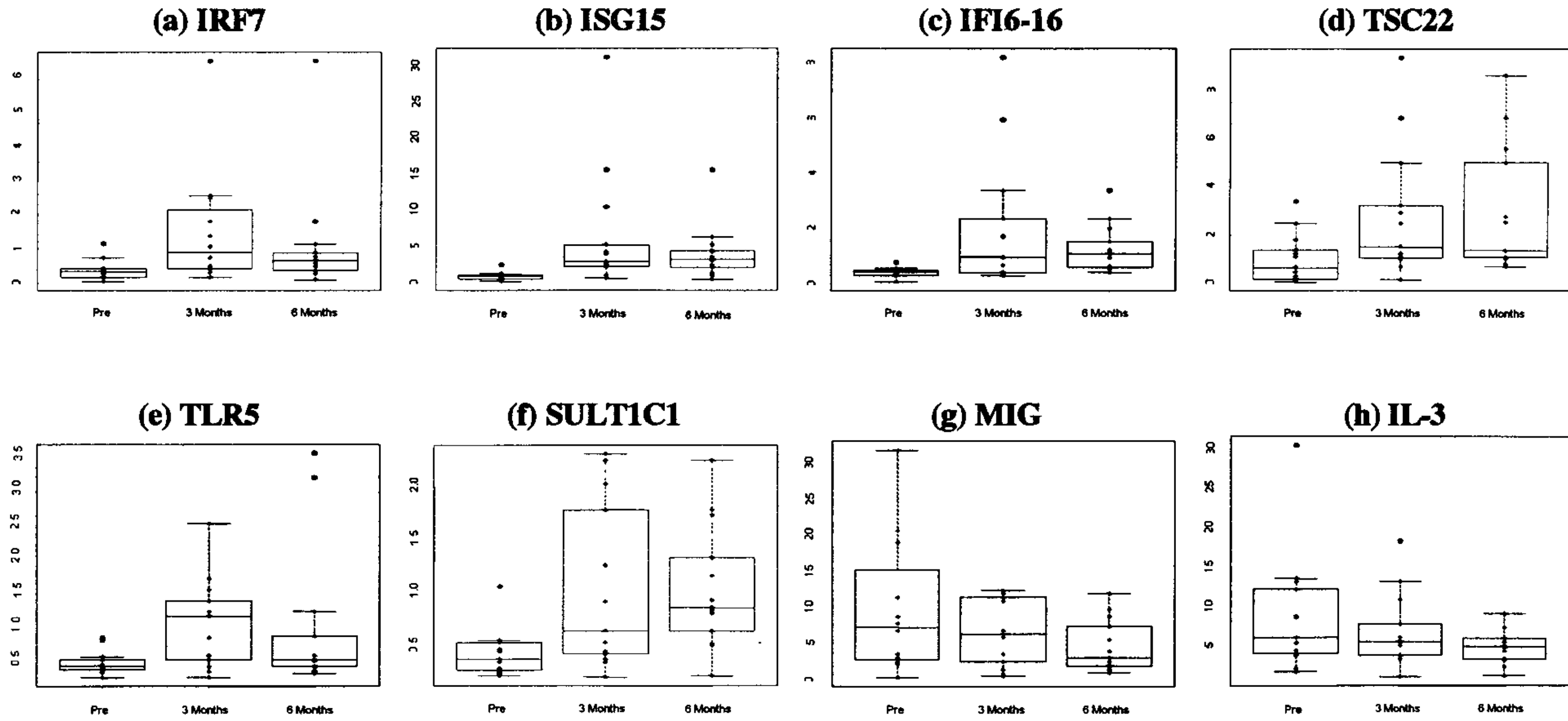

Fig. 1 Koike et al.

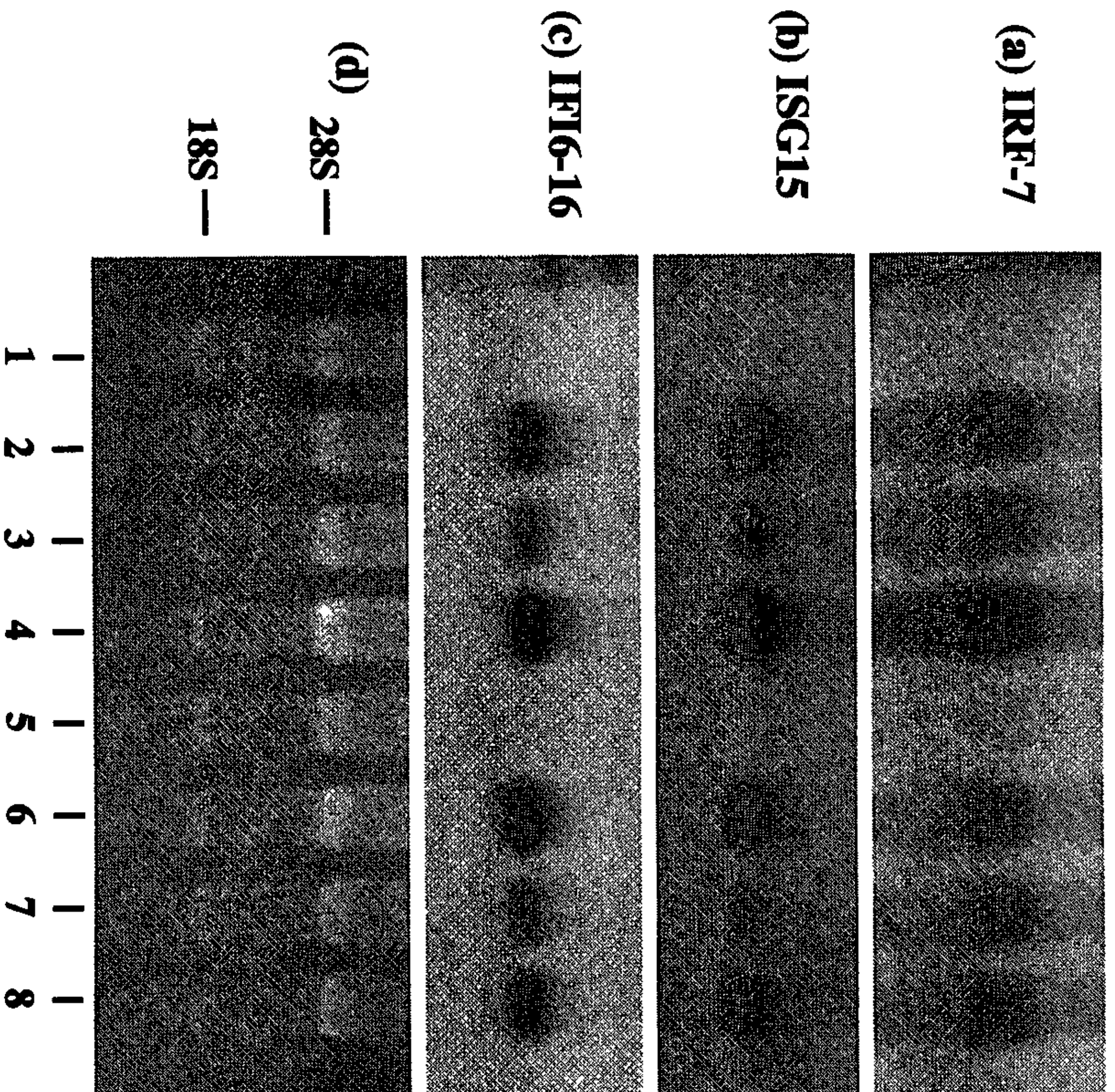

Fig. 2 Koike et al.

Table 1 The genes regulated by IFN β in T cells and non-T cells of MS patients

No	Gene	Entrez definition	Genbank accession number	Cell fractions	Average of the gene regulation index (GRI) at 3 months (3 mo; p value) or 6 months (6 mo, p value) vs at Pre	IFN-responsive promoter elements	Induction preferentially by type I or type II IFN	Presumed function
	The upregulated genes							
1	**IRF7**	IFN regulatory factor 7A	U53830	T	+4.7 (3 mo; 1.69E-05), +3.6 (6 mo; 2.03E-06)	(+)	type I	a transcription factor that regulates the IFN-stimulated genes
				non-T	+3.9 (3 mo; 2.63E-06)			
2	**ISG15**	IFN-induced 15-kDa protein	M13755	T	+4.3 (3 mo, 2.06E-06)	(+)	type I > type II	a secretory protein homologous to ubiquitin
				non-T	+6 3 (3 mo; 1.12E-11), +2.9 (6 mo; 3.09E-04)			
3	**IFI56**	IFN-induced 56-kDa protein	X03557	T	+4.5 (3 mo; 1.92E-05), +3.8 (6 mo; 1 20E-04)	(+)	type I, type II	a cytoplasmic protein of unknown function
				non-T	+7 3 (3 mo, 3.63E-07), +8.1 (6 mo; 3.87E-05)			
4	**IFI6-16**	IFNα-inducible cDNA 6-16	X02492	T	+5 1 (3 mo, 1.99E-05), +3.5 (6 mo; 7.65E-07)	(+)	type I	a protein of unknown function
				non-T	+5 5 (3 mo, 3.09E-06), +4.5 (6 mo; 7.27E-05)			
5	**IFI60**	IFN-induced protein 60	AF083470	T	+4 3 (6 mo, 4.04E-07)	(+)	type I	a protein of unknown function
6	**IFI30**	IFNγ-inducible protein 30	J03909	T	+1 5 (3 mo, 5.92E-02#)	ND	type I < type II	a lysosomal thiol reductase involved in class II MHC-restricted antigen presentation
7	**IFI27**	IFNα-inducible protein 27	X67325	non-T	+5.4 (3 mo; 5.55E-15), +7.0 (6 mo; 4.34E-09)	ND	type I	a nuclear membrane protein of unknown function
8	**IFI17**	IFN-induced protein 17	J04164	non-T	+2.3 (3 mo; 3.84E-04)	(+)	type I > type II	a membrane protein involved in inhibition of cell growth
9	**TAP1**	ATP-binding cassette transporter TAP1	X57522	non-T	+2.5 (3 mo, 2.73E-04)	(+)	type I, type II	an ATP-binding cassette transporter involved in class I MHC-restricted antigen presentation
10	**TNFAIP6**	TNFα-induced protein 6	M31165	non-T	+3.4 (3 mo; 2.45E-04)	(+)	ND	a secretory protein homologous to CD44 presenting with an anti-inflammatory activity
11	**TSC22**	TGFβ-stimulated protein TSC-22	U35048	non T	+2.7 (3 mo; 1 92E-03), +2.6 (6 mo; 6.86E-04)	ND	type II	a transcription factor that regulates the C-type natriuretic peptide gene
12	**ATF3**	activating transcription factor 3	L19871	T	+2.4 (3 mo; 5.77E-04)	ND	type II	an ATF/CREB family of transcription factor
13	**TLR5**	toll-like receptor 5	U88881	T	+2.6 (6 mo, 4.13E-05)	ND	ND	a receptor for bacterial flagellin signaling through activation of NF-κB
14	**SULT1C1**	sulfotransferase family 1C member 1	U66036	non-T	+2.8 (3 mo; 9.34E-06), +2.4 (6 mo; 2.02E-04)	ND	ND	a cytoplasmic sulfotransferase
15	**RPC39**	39-kDa RNA polymerase III-specific subunit	U93869	non-T	+3 4 (3 mo, 1.04E-05)	ND	ND	a subunit of RNA polymerase III involved in transcriptional initiation
16	**RAB11A**	ras family member Rab11a	AF000231	non-T	+2.4 (3 mo; 1.89E-04)	ND	ND	a member of RAS family small GTP-binding protein involved in membrane trafficking
	The downregulated genes							
17	**MIG**	monokine induced by IFN γ	X72755	T	-2.3 (6 mo; 8.48E-07)	(+)	type II	a member of CXC family chemokine presenting with a T-cell chemoattractant activity
18	**SLC7A1**	solute carrier family 7 member 1	NM_003045	T	-3.2 (6 mo; 1.55E-04)	ND	type II	a cationic amino acid transporter homologous to the mouse ecotropic retroviral receptor
19	**IL-3**	interleukin-3	M17115	T	-1.9 (6 mo; 1.75E-04)	ND	ND	a multi-colony stimulating factor
				non-T	2.1 (6 mo, 1.25E-05)			
20	**AKAP4**	protein kinase A-anchor protein 4	NM_003886	T	-6 0 (3 mo; 2.07E-05), -5.3 (6 mo; 1.86E-05)	ND	ND	a testis-specific protein involved in sperm motility
21	**GNA13**	guanine nucleotide-binding protein α13	L22075	T	-6.5 (3 mo; 1.06E-06), -6.5 (6 mo, 1.09E-08)	ND	ND	a G-protein α subunit involved in regulation of cell movement and developmental angiogenesis

The genes whose mRNA expression levels changed significantly in T cells or non-T cells of MS patients after IFN β treatment are listed. ND; not described previously
The p value of GRI (the numberE-x) represents the number X 10-x #The p value of logGRI for IFI30 at 3 mo is 1.34E-04.

Ⅱ．　分担研究報告

厚生科学研究費補助金(特定疾患対策研究事業)
分担研究報告書

再発寛解型多発性硬化症患者末梢血リンパ球における IFNβ-responsive genes (IRG)：DNA microarray による解析

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研究要旨　近年欧米および本邦の臨床試験により、インターフェロンベータ(interferon-beta; IFNβ)が多発性硬化症(multiple sclerosis; MS)で再発抑制に有効であることが立証されたが、また同時に IFNβ高応答群(responder)・低応答群(nonresponder)の存在が明らかになった。現在まで MS における IFNβ治療効果の分子細胞生物学的機序は十分解明されていない。IFNβは第一に IFNγによるクラス II 主要適合性抗原発現誘導に拮抗して抗原提示能を抑制し、第二に抗原提示細胞による IL-12 産生を抑制して Th1 シフトを是正し、第三に活性化自己反応性 T 細胞の血液脳関門通過を阻止することにより、強力な抗炎症作用を呈することが報告されている。本研究で我々は遺伝子アレイを用いて MS における IFNβ治療効果発現に重要な役割を果たすと推測される IFN 応答遺伝子群(IFN-responsive genes; IRG)を同定した。IRG には IFNβ産生における正の制御転写因子 IRF-7 や抗原呈示機構の主要構成因子 IFI30, TAP1 が含まれていた。我々の結果は IRG が MS における IFNβ responder, nonresponder を識別するマーカーとして研究対象となる可能性を示唆する。

A. 研究目的

インターフェロンベータ(IFNβ)は多発性硬化症(MS)で再発・進行を抑制するが、その薬理学的効果発現機序は十分解明されていない。本研究では MS における IFNβ治療効果発現に重要な役割を果たすと推測される IFN 応答遺伝子群(IFN-responsive genes; IRG)を同定するため、IFNβ治療前後で MS 患者末梢血リンパ球における遺伝子発現を包括的に解析した。

B. 研究方法

Poser の診断基準を満たす 13 例の再発寛解型 clinically definite MS 患者(EDSS 2.7 ±1.7)で、インフォームドコンセントを取得後に、IFNβ 治療(Betaferon 800 MIU, sc, on alternate days)開始前(Pre)・投与後 3 カ月(3 mo)・6 カ月(6 mo)時に末梢血を採取し、Ficoll-Paque でリンパ球(peripheral blood mononuclear cells; PBMC)を得た。MACS cell separator で PBMC より $CD3^+$ T 細胞分画お