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A Possible Role of Autogenous IFN-β for Cytokine Productions in Human Fibroblasts

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Abstract It has been already known that human diploid fibroblasts are able to produce not only high levels of IFN-β but also various kinds of cytokines by poly rI: poly rC, and some inflammatory cytokines are induced by IFN-β gene activation. We also obtained similar results. However, in our system, cytokine productions were extremely enhanced by treating the cells with a low dose of type 1 IFN and the priming effects on cytokine productions were blocked by cycloheximide similar to those on IFN-β productions. Most of cytokines were produced later than IFN-β and synthesis patterns of their mRNA showed the same phenomena. We made clear that cytokine productions by poly rI: poly rC are mediated by secreted IFN-β at a protein level using a monoclonal antibody against human IFN-β. Further, it was shown that intra-cellular IFN-β which is not secreted might also participate in cytokine productions. Meanwhile, IL-1β induced various kinds of cytokines in human fibroblasts and production time courses of these cytokines were similar to those of poly rI: poly rC induced cytokines. Although secreted IFN-β was not detected in IL-1β stimulated culture, expression of IFN-β mRNA was augmented. These results showed that priming effects of type 1 IFN on cytokine productions by poly rI: poly rC might not be the direct action, but successive IFN-β production might be essential in the production processes of other cytokines. Further, it was suggested that inducible IFN-β might also take part in IL-1β-induced cytokine productions.
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Key words: interferon-β; cytokine; interleukin-1; human fibroblasts

It has been known that human diploid fibroblasts produce interferon (IFN)-β by viral infection or stimulation with double-stranded RNA such as polyriboinosinic-ribocytidylic acid (poly rI: poly rC) [Cavalieri et al., 1977a; Havell et al., 1978]. Later, it has been reported that some cytokines such as interleukin (IL)-6 [Kohase et al., 1987; Van Damme et al., 1987a,b], IL-8 [Van Damme et al., 1989a], and colony stimulating factors (CSFs) [Fibbe et al., 1988; Van Damme et al., 1989b] are also produced in the fibroblasts stimulated with poly rI: poly rC or IL-1β. Recently, it was shown that some inflammatory cytokine productions are mediated by IFN-β gene activation caused by poly rI: poly rC. To activate IFN-β gene, a lot of molecules such as toll-like receptor (TLR) family which has been reported to participate in innate immunity by detecting invading pathogens [Aderem et al., 2000; Akira et al., 2001; Janeway and Medzhitov, 2002] -3 [Matsumoto et al., 2002], retinoid-inducible gene 1 (RIG-1) [McWhirter et al., 2005] which is an intracellular receptor for viral RNA, interferon regulatory factors (IRFs) [Seth et al., 2005], signal transducers and activators of transcription (STAT) [Darnell et al., 1994], and so on have been reported. IRFs were found to play an important role in IFN induction [Mamane et al., 1999; Sato et al., 2000, 2001; Taniguchi et al., 2001], and it was clarified that IRF-3 and IRF-7 are crucial to induce maximally the expression of IFN-α and -β [Yoneyama et al., 1998; Yeow et al., 2000; Iwamura et al., 2001; Taniguchi and Takaoka, 2001]. Meanwhile, it has been shown that TLRs take part in IFN-β gene activation and subsequent inductions of IFN-β inducible genes containing some cytokines such as RANTES, γ-IFN-induced protein (IP)-10, and monocyte chemotactic and activating factor/

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monocyte chemoattractant protein-1 (MCAF/MCP-1) owing to IRF-3 activation [Kawai et al., 1999, 2001; Toshchakov et al., 2002]. In particular, TLR-3 and TLR-4 were crucial for cytokine productions by double-stranded RNA or lipopolysaccharide (LPS) [Yamamoto et al., 2002, 2003]. Thereafter, it was shown that TLR-3 is selectively expressed on the cell surface of human fibroblasts and specifically recognized by double-stranded RNA [Matsumoto et al., 2002]. Although there is remarkable development of molecular studies related to IFN-β gene activation, biological study or molecular research about cytokine productions in human fibroblasts including some IFN-β-inducible cytokine productions resulted from IFN-β gene activation have not almost investigated until now.

Recently, we have found that human diploid fibroblasts primed with a low dose of IFN-β and treated with poly rI: poly rC could induce a variety of cytokines, chemokines, and CSFs such as IL-1α, IL-1β, IL-6, IL-8, IL-11, tumor necrosis factor (TNF)-α, G-CSF, GM-CSF, stem cell factor, melanoma growth stimulatory activity/growth related oncogene (MGSA/GRO)-α and -β, platelet factor 4 (PF4), IP-10, RANTES, MCAF/MCP-1, epithelial neutrophil-activating protein (ENA)-78, and several others as well as IFN-β. In our production system by poly rI: poly rC, some differences in the production profile between IFN-β and cytokines have been observed. The most distinctive feature among them was different production time courses. Contrary to a rapid production of IFN-β, most of other cytokines appeared more slowly suggesting IFN-β production might be involved in cytokine production processes. We could make clear for the first time at a protein level that these cytokine productions by poly rI: poly rC are mediated by secreted IFN-β using a monoclonal antibody against human IFN-β. However, in our experiments, it seemed that amounts of secreted IFN-β necessary for cytokine productions are very little. Further, cytokine productions were not caused by addition of IFN-β alone, although there is a report that IFN-β by itself, used at high concentration, can directly induce IL-6 in human fibroblasts [Kohase et al., 1987]. So, we were interested in investigating how inducible IFN-β acts for cytokine productions and whether another signaling pathway of inducible IFN-β is existent in the cells in addition to that of secreted IFN-β. We have shown in this study that another cytokine synthesis pathway mediated by intracellular inducible IFN-β might be existent in the cells since we could find that intra-cellular syntheses of IFN-β and other cytokines are not inhibited by anti-human IFN-β antibody.

Meanwhile, we have also found that IL-1β could produce many kinds of cytokines such as IL-6, IL-8, G-CSF, GM-CSF, M-CSF, and TNF-α in human fibroblasts. Secretions of these cytokines were very late after stimulation similar to the production time courses by poly rI: poly rC stimulation. We were interested in cytokine production mechanisms by IL-1β, especially, in examining whether inducible IFN-β takes part in cytokine production processes. So far, it has been reported that cytokine productions by IL-1β are mediated by activation of transcription factors such as NF-κB and AP-1 via IL-1 receptor/TLR [Cao et al., 1996]; however, it is not clear whether IL-1β activates IRFs necessary for IFN-β gene activation. It has been already reported that IL-1β exerts an antiviral effect mediated by the expression of IFN-β in diploid fibroblasts [Van Damme et al., 1987b; Randolph-Habecker et al., 2002]; however, in these reports, increases of secreted protein and mRNA of IFN-β were observed only in combination with cycroheximide or in the cells infected with cytomegalovirus. Further, it has been shown that by IL-1β alone, secreted IFN-β has not been found in the culture supernatants and small amounts of IFN-β mRNA, which was only detectable with a high sensitive method, was produced [Van Damme et al., 1985]. We got similar results by the stimulation of IL-1β alone suggesting that cytokine productions by IL-1β might be also mediated by inducible IFN-β.

We have found for the first time that cytokine productions by poly rI: poly rC are extremely enhanced by treating the cells with a low dose of type 1 IFN prior to stimulation. This priming effects were blocked by cycloheximide similar to those in IFN-β production by poly rI: poly rC. It has been already reported that amounts of IFN and IFN mRNA produced in response to a variety of inducers were markedly increased by treating the cells with low doses of type 1 IFN before induction [Stewart et al., 1971; Fujita and Kohno, 1981; Raj and Pitha, 1981; Nir et al., 1985; Enoch et al., 1986; Xanthoudakis et al., 1989]. Thereafter, it was shown that IFN priming for IFN-β production is dependent on cellular protein synthesis [Fujita and Kohno,

1981]. Recently, it was elucidated that the priming effects of IFN are resulted from an action of IFN on the IFN-β promoter, that is, priming affected the activity of a specific region of the promoter of the human *IFN-β* gene [Dron et al., 1990]. However, there are not any reports about the priming effects of type 1 IFN on cytokine productions except IL-6 production [Kohase et al., 1987].

In this study, we have shown that inducible IFN-β plays a key role in cytokine production processes and that priming effects of type 1 IFN on cytokine productions might be indirect action that resulted from enhancement of IFN-β gene activation by poly rI: poly rC.

MATERIALS AND METHODS

Cell and Cell Culture

A human diploid fibroblast strain, designated FC1 [Sano et al., 1987], was isolated in our laboratory from a human neonatal foreskin which was kindly supplied by Dr. R. E. Gills (Providence Hospital, Anchorage, Alaska). Stock cultures of the fibroblasts were maintained as conventional monolayers in Eagle's MEM (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 5% FCS (Life Technologies, Grand Island, NY) using plastic tissue culture flasks with surface area of 75 cm^2 (Corning, NY). Experiments were usually performed using the cells of 20–45 population doubling level (PDL) with diploidy of over 95%. Experiments for cytokine productions were carried out using 25- and 75-cm^2 plastic culture flasks (Corning, NY) and 6-well plate for cell culture (Iwaki Glass, Tokyo, Japan). An efficient microcarrier culture system established in our laboratory as previously described [Sano et al., 1987, 1988; Ohashi et al., 2003a, 2003b] was also employed as a stable production system for cytokine productions. Briefly, Cytodex 1 (Pharmacia Fine Chemical AB, Uppsala, Sweden) which is made of cross-linked dextran and has a positive charge (N,N-diethylaminoethyl, DEAE) was used as microcarriers at 3 mg/ml. For the microcarrier cultures of 200-ml volume, 500-ml glass spinner flasks (Wilbur Scientific, Boston, MA) containing Eagle's MEM supplemented with 5% FCS were used. After the fibroblasts were propagated in plastic flasks, the cells were inoculated to microcarrier culture at the density of 2×10^5 cells/ml. The cells were cultured on microcarriers until $1–1.5 \times 10^6$ cells/ml by replacing to fresh medium every 2 or 3 days.

IFN-β and Other Cytokine Production

Confluent cells were primed for 24 h at 37°C with 100 international reference units (IU)/ml of IFN in Eagle's MEM containing 0.2% carboxymethyl cellulose (Daicel Chemical Industries, Osaka, Japan). IFN-α (Sumitomo Pharmaceuticals, Osaka, Japan), IFN-β (Toray Industries, Tokyo, Japan), and IFN-γ (Genentech, South San Francisco, CA) were used as priming IFN for IFN-β and other cytokine productions. After treating the cells with IFN, culture medium was replaced by fresh serum free medium containing 10 μg/ml of poly rI: poly rC (Yamasa Corporation, Chiba, Japan). Culture supernatants containing IFN-β and cytokines were harvested 5–6 days after poly rI: poly rC stimulation. The super-induction was carried out according to slightly modified procedure described by Vilcek et al. [1976]. Confluent cells treated with 100 IU/ml of IFN-β were stimulated with 10 μg/ml of poly rI: poly rC together with 10 μg/ml of cyclohexamide (Wako Pure Chemical, Osaka, Japan) for 4 h. Subsequently, 4 μg/ml of Actinomycin D (Makor Chemical, Jerusalem, Israel) was added to the cultures. After 1 h of incubation, the cells were washed with Eagle's MEM without serum and incubated at 37°C in fresh medium until culture fluids containing cytokines are harvested.

Antibody

A mouse anti-human IFN-β1 monoclonal antibody used to neutralize polyrI: poly rC or IL-1β-induced IFN-β was prepared in Toray Industries [Minagawa et al., 1989; Yamazaki et al., 1989]. A mouse anti-human MCAF monoclonal antibody having the same iso-type as that of IFN-β antibody was used as a control of anti-IFN-β antibody, which was also prepared by Toray Industries.

Quantifications of IFN-β and Cytokines

The quantities of IFN-β, IL-6, IL-8, and MCAF were determined by ELISA systems established in Toray Industries [Ida et al., 1989, 1990; Yamazaki et al., 1989]. Yields of GM-CSF, G-CSF, IL-1α, IL-1β, IL-11, RANTES, and TNF-α were assayed using ELISA kits purchased from R&D Systems (Minneapolis, MN). The amounts of GRO-α and GRO-β were

analyzed using ELISA kits developed by Immuno-Biological Laboratories (Gunma, Japan).

Isolation of RNA and Semi-Quantitative RT-PCR Analysis

Total RNA was prepared from fibroblasts primed with 100 IU/ml of IFN-β and stimulated with 10 μg/ml of poly rI: poly rC using RNA isolation reagent (ISOGEN, Nippon Gene, Tokyo, Japan or TRIZOL, Invitrogen, San Diego, CA) according to the manufacturer's instructions. The concentration of the RNA was determined by measuring the absorbency at A_{260}/A_{280}, and the purity was confirmed by formaldehyde gel electrophoresis. To analyze altered gene expression, mRNA was transcribed into cDNA using SuperScript RT-PCR system purchased from Invitrogen (San Diego, CA). cDNA was produced from 100 ng to 1 μg of total RNA by reverse transcription (RT) reaction. PCR reactions were carried out using GeneAmp 2400 PCR System (Applied Biosystems, Foster City, CA) or PCR thermal cycler MP (TAKARA, Tokyo, Japan). Reversibly transcribed products of 5 μl were used for each PCR reaction and all of PCR reactions were carried out in pertinent cycle number determined by ensuring amplification in the linear range using 20, 25, 30, and 35 cycles experimentally. The resulting amplified products were electrophoresed on 2% agarose gel containing ethidium bromide or 3% NuSieve 3:1 agarose gel (TAKARA, Tokyo, Japan). Photographs of the gel visualized by UV illumination were then digitally scanned and the band intensities were quantified using ATTO Densitograph software Ver4.0 (ATTO, Tokyo, Japan). Expression ratios were determined by dividing the band intensity of the product of interest by that of the corresponding band of G3PDH or β-actin. The primer pairs used for semi-quantitative RT-PCR were as follows. Primer pairs of IFN-β, ENA-78, G-CSF, MGSA/GRO-β, MCAF/MCP-1, PF-4, IP-10, and IL-11 were created from the sequences in cording region not containing intron based on the data base of GenBank. Other primer pairs were purchased from companies. IFN-β, 5′-AATTGCTCTCCTGTTGTGCTTCTCC-3′ (F) and 5′-TGACTGTAGTCCTTGGCCTTCAG-3′ (R) (GenBank no. NM002176, nt 99–557), 459-bp product; IL-6, 5′-TCTCAGCCCTGAGAAAG-GAGAC-3′ (F) and 5′-GAAGAGCCCTCAGGCT GGACTG-3′ (R) (Toyobo, Cat. no. RPP-108), 438-bp product; IL-8, 5′-CGATGTCAGTGCC ATAAAGACA-3′ (F) and 5′-TATGA ATTCT-CAGCCCTCTT-3′ (R) (Toyobo, Cat. no. Rpp-109), 203-bp product; IL-1β, 5′-ATGGCAGAAG-TACCTAAGCTCGC-3′ (F) and 5′-ACACAAAT TGCATGGTGAAGTCAGTT-3′ (R) (Clontech, Cat. no. 5422-3), 802-bp product; GM-CSF, 5′-CTGCTGCTGAGAATGAAACAG-3′ (F) and 5′-TGGACTGGCTCCCAGTCAAAG-3′ (R) (CLP, Cat. no. 5231H), 286-bp product; TNF-α, 5′-GAGTGACAAGCCTGTAGCCCATGTTGTAG CA-3′ (F) and 5′-GCAATGATCCCA AAGTA-GACCTGCCCAGACT-3′ (R) (Toyobo, Cat. no. Rpp-110), 444-bp product; ENA-78, 5′-TACA-GACCACGCAGGGAGTT-3′ (F) and 5′-ACAA ATTTCCTTCCCGTTCT-3′ (R) (GenBank no. X78686, nt 261–379), 119-bp product; G-CSF, 5′-TGCTGCTCGGACACTCTCTG-3′ (F) and 5′-CTGGGCAAGGTGGCGTAGAA-3′ (R) (GenBank no. M17706, nt 275–651), 377-bp product; MGSA/GRO-β, 5′-TGCTGCTCCTGCTCCTG GTA-3′ (F) and 5′-CATTCCGCCCATTCTTGA GT-3′ (R) (GenBank no. NM0015 11, nt 138–329), 192-bp product; PF-4, 5′-TTGCTGCTCCT GCCACTTG-3′ (F) and 5′-CCTTC CATTCTT CAGCGTGG-3′ (R) (GenBank no. NM002619, nt 62–247), 186-bp product; MC AF/MCP-1, 5′-CTCGCTCAGCCAGATGCAA-3′ (F) and 5′-GG TGGTCCATGGAATCCTG-3′ (R) (GenBank no.S71513, nt 117–321), 205-bp product; IP-10, 5′-ATGAATCAAACTGCGAT TCTG-3′ (F) and 5′-TCTTGATGGCCTTCGAT TCT-3′ (R) (GenBank no. NM001565, nt 67–316), 250-bp product; IL-11, 5′-TGCACAGCT GAGGGA CAAAT-3′ (F) and 5′-ACGGCCCAGTCAAGT GTCAG-3′ (R) (GenBank no. NM000 641, nt 307–702), 396-bp product; G3PDH, 5′-ACCA CAGTCCATGCCATCAC-3′ (F) and 5′-GTGAT GGCATGGACTGTGGT-3′ (R) (Invitrogen, Cat. no. 10336-022), 452-bp product; β-actin (Takara, Cat. no. 6623), 275-bp product.

Northern Blot Analysis

Poly(A)$^+$RNA was isolated from disruption of fibroblast cells (FC1) stimulated with poly rI: poly rC after priming the cells with IFN-β by incubating with oligo-(dT) cellulose, and washing and eluting retained Poly(A)$^+$RNA using Fast Track mRNA isolation kit (Invitrogen, San Diego, CA). Poly(A)$^+$RNA (2 μg on each lane) was separated by gel electrophoresis in a 1% agarose/2.2 M formaldehide gels and transferred overnight to nylon filters (Hybond N, Amersham Pharmacia Biotech, Piscataway, NJ) in 20× SSC. Northern blot hybridization

was carried out using nucleotide fragments labeled with [α-^{32}P] dCTP as probes, and hybridized filters were autoradiographed. Probes used were full-length IL-6, GM-CSF, and IFN-β cDNA. After washing, the filter was exposed to the imaging plate for 2 h at room temperature and analyzed using Bioimage Analyzer, BAS 2000 (Fuji Photo Film Co., Tokyo, Japan). Changes in the levels of IL-6, GM-CSF, and IFN-β Poly(A)$^+$RNAs were then normalized relative to β-actin Poly(A)$^+$RNA levels by scanning densitometry.

RESULTS

Priming Effects of Type 1 IFN on Poly rI: Poly rC-Induced Cytokine Productions

Human diploid FC1 treated with low doses of human IFN-β secreted a variety of cytokines as well as IFN-β in large quantities by stimulation of poly rI: poly rC. Treating the cells with 100 IU/ml of natural IFN-β for 18–24 h resulted in a 5- to 40-fold increase of cytokine yields (Fig. 1A). Priming effects were caused only when the cells were treated with IFN-α or -β at the concentration of over 100 IU/ml similar to IFN-β production (Fig. 1B). Priming effects of IFN-β on cytokine productions were inhibited by the addition of cycloheximide (Fig. 1C). The cells treated with cycloheximide of 5–10 μg/ml together with priming IFN-β were cultured for 24 h, thereafter, the culture fluids were replaced with fresh medium containing poly rI: poly rC without cycloheximide. This result indicated that some novel protein synthesis is necessary for the expression of priming effects for cytokine productions same as the case of IFN-β production. Meanwhile, effects of pretreatment with IFN-β on mRNA expressions of IL-6, GM-CSF, and IFN-β were also examined by Northern blot analyses (Fig. 4B, lanes 1–6). Fibroblasts grown in plastic culture flasks (75 cm^2) were treated or not treated with 100 IU/ml of IFN-β for 24 h and stimulated with 10 μg/ml of poly rI: poly rC. The cells were harvested at 4, 10, 30 h after stimulation for mRNA isolation using Fast Track mRNA isolation kit (Invitrogen, San Diego, CA). Poly(A)$^+$ RNA was separated by gel electrophoresis and transferred overnight to nylon filters. After Northern blot hybridization using nucleotide fragments labeled with [α-^{32}P] dCTP as probes, hybridized filters were autradiographed. Probes used were full-length IL-6, GM-CSF, and IFN-β cDNA. The filter was exposed to the imaging plate and analyzed using Bioimage Analyzer, BAS2000 (Fuji Photo Film Co.). Figure 4B has also demonstrated that priming the cells with IFN-β resulted in increases of mRNA expressions of IL-6 and GM-CSF as well as IFN-β.

Late Syntheses of Poly rI: Poly rC-Induced Cytokines

In super-induction method which is well-known as an efficient production system for early induced IFN-β by poly rI: poly rC stimulation, only a very little cytokines were produced in human fibroblasts primed with IFN-β (Fig. 2A). However, when the cells were treated with poly rI: poly rC alone, cytokine yields were increased 10- to 100-fold compared with those obtained in super-induction method, although IFN-β production was extremely decreased (Fig. 2B). The maximum production of IFN-β was observed at 24–48 h after poly rI: poly rC stimulation, however, production peaks of other cytokines were observed 2 or 3 days later than IFN-β production. In these experiments, culture fluids were replenished with fresh Eagle's MEM without poly rI: poly rC and serum every 2 days and cytokine contents in each supernatant were measured using ELISA systems for each cytokine.

The synthesis patterns of cytokine mRNAs were investigated using semi-quantitative RT-PCR analysis (Fig. 3). FC1 cells grown in 75-cm^2 culture flasks were primed with 100 IU/ml of IFN-β and stimulated with 10 μg/ml of poly rI: poly rC. The cells were harvested at 0, 3, 6, 9, 24, 33 h after stimulation with cell scraper and total RNA was isolated using RNA isolation reagent (ISOGEN). cDNA was produced using 100 ng of total RNA by SuperScript RT-PCR system (Invitrogen, Tokyo, Japan). PCR reactions were performed using 5 μl of reversibly transcribed products in pertinent cycle number determined by ensuring amplification in the linear range using 20, 25, 30, and 35 cycles experimentally. Sequences of used primer sets were shown in Materials and Methods. Amplified PCR products were electrophoresed using 2% agarose gel containing ethidium bromide and were visualized by UV illumination. Photographs were then digitally scanned and the band intensities were quantified using ATTO Densitograph software Ver4.0 (ATTO). Expression ratio shown in Figure 3 was determined from the values obtained by dividing the band

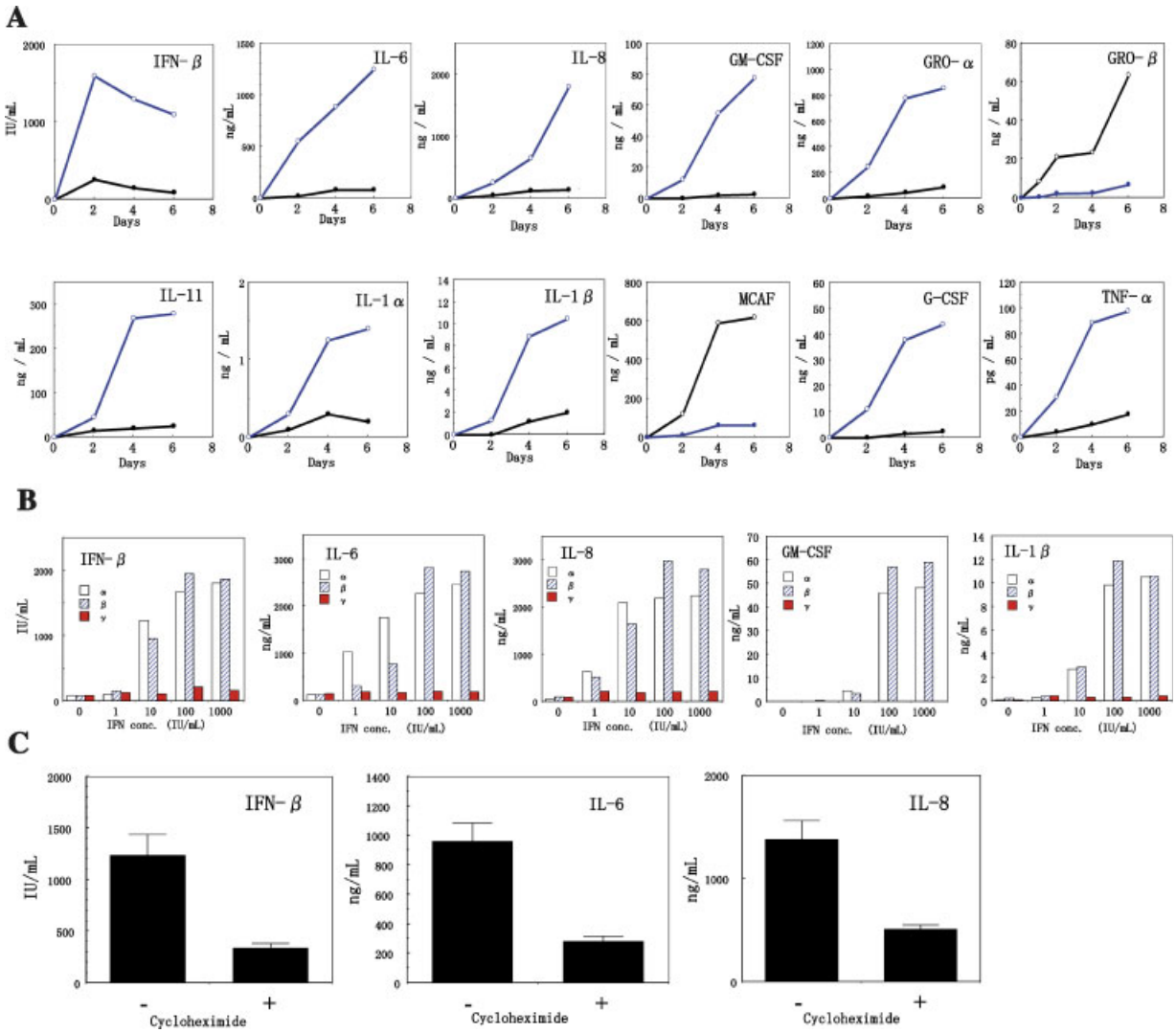

Fig. 1. Priming effects of type 1 IFN on cytokine productions by poly rI: poly rC. **A**: Priming effects of IFN-β on IFN-β and other cytokine productions. Fibroblasts (FC1 cells) grown in a microcarrier culture (1×10^6 cells/ml) were primed (+) with human IFN-β (100 IU/ml) for 24 h or not primed (−) prior to poly rI: poly rC stimulation (10 μg/ml). Culture supernatants were drawn out every 2 days to determine the yields of cytokines. Quantification of IFN-β and other cytokines were assayed using ELISA system for each cytokine. **B**: Quantities of type 1 IFN necessary for priming. The cells were treated with 1–1,000 IU/ml of IFN-α, -β, and -γ for 24 h before poly rI: poly rC stimulations (10 μg/ml). Culture fluids were harvested on the 5th day after induction for the analyses of cytokine contents. **C**: Inhibition of priming effects by cycloheximide. The cells were treated with cycloheximide (10 μg/ml) together with priming IFN-β (100 IU/ml) for 24 h. The culture fluids were replenished with fresh medium containing 10 μg/ml of poly rI: poly rC without cycloheximide and harvested on the 5th day after induction for analyzing the contents of IFN-β, IL-6, and IL-8. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

intensity of the product by that of the corresponding band of G3PDH. All of these cytokine mRNAs were synthesized later than IFN-β mRNA similar to the production patterns of cytokines demonstrated in Figure 2B. The maximum expressions of cytokine mRNAs were observed 6–9 h alike after poly rI: poly rC stimulation, while peak of IFN-β was 2–4 h.

Cytokine mRNA synthesis patterns were also examined by Northern blot analyses. The cells primed with 100 IU/ml of IFN-β were induced with 1 or 10 μg/ml of poly rI: poly rC and harvested with cell scraper at 4, 10, 30 h after stimulation. To investigate the expression levels of IL-6, GM-CSF, and IFN-β mRNA, poly(A)$^+$RNA was isolated from disruption of

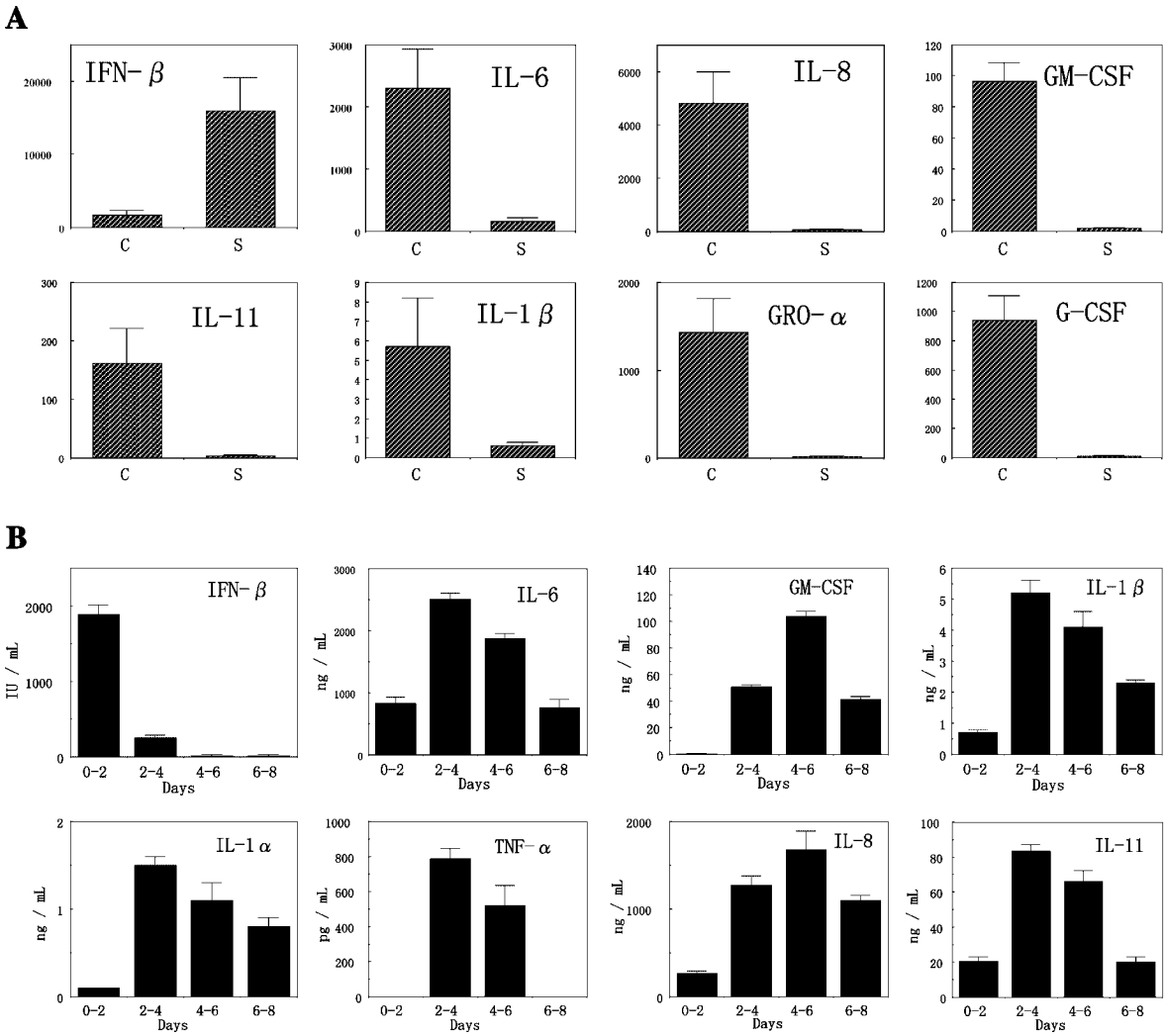

Fig. 2. Production patterns of poly rI: poly rC-induced IFN-β and other cytokines. **A**: Comparison of IFN-β and other cytokine yields in super-induction method (S) and stimulation by poly rI: poly rC alone (**C**). FC1 cells (1×10^6/ml) primed for 24 h with IFN-β (100 IU/ml) were stimulated with 10 μg/ml of poly rI: poly rC in Eagle's MEM without serum. Maximum yields of IFN-β (Day 2) and other cutokines (Day 5) were compared (IFN-β yield: IU/mL, cytokine yield: ng/mL). **B**: Production time courses of IFN-β and other cytokines in simple stimulation by poly rI: poly rC. After priming, the cells were stimulated with 10 μg/ml of poly rI: poly rC and the culture fluids were replaced with fresh medium without serum every 2 days for quantification.

the cells using Fast Track mRNA isolation kit (Invitrogen, San Diego, CA). Poly(A)$^+$RNA was separated by agarose gel electrophoresis and transferred to nylon filters. Northern blot hybridization was carried out using nucleotide fragments labeled with [α-^{32}P] dCTP prepared from full-length IL-6, GM-CSF, and IFN-β cDNA as probes. The filter was analyzed using Bioimage Analyzer, BAS 2000 (Fuji Photo Film Co.). As shown in Figure 4B (lane 4, 5, 6, 8, 9, 10), maximum expressions of GM-CSF and IL-6 mRNA were observed 10 h after stimulation and the peak of IFN-β mRNA was 4 h corresponding to the results from RT-PCR analyses demonstrated in Figure 3.

Cytokine Productions Responded to Repeated Stimulation With Poly rI: Poly rC

As shown in Figure 4A, IL-6, IL-8, and GM-CSF productions were enhanced in response to repeated stimulations of poly rI: poly rC which caused tolerance of IFN-β production. Culture media were replaced every day with fresh medium containing 10 μg/ml of poly rI: poly rC without serum. Cytokine productions were augmented by twice stimulations, and

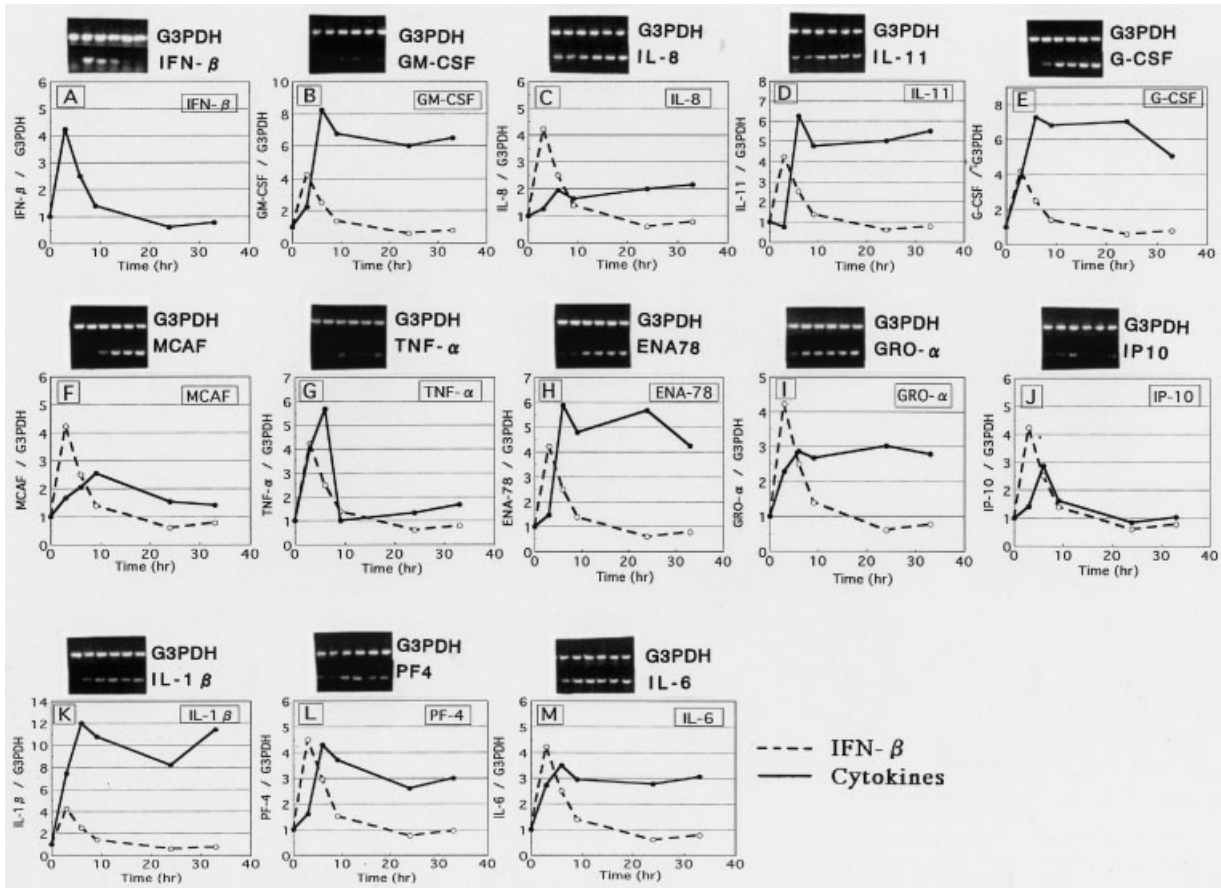

Fig. 3. Synthesis patterns of IFN-β and cytokine mRNA using semi-quantitative RT-PCR. FC1 cells grown in 75-cm^2 culture flasks (1×10^7 cells/flask) were primed with IFN-β (100 IU/ml) for 24 h and were stimulated with poly rI: poly rC (10 μg/ml). The cells were harvested at 0, 3, 6, 9, 24, 33 h after stimulation using cell scraper. Total RNA was isolated from cell pellet using RNA isolation reagent (ISOGEN, Nippon Gene) and concentration of the RNA was determined from the absorbency at A_{260}/A_{280}. cDNA was produced using 100 ng of total RNA under the conditions of 50°C, 30 min and 94°C, 2 min. employing SuperScript RT-PCR system (Invitrogen). PCR reactions were performed using 5 μl of reversibly transcribed products in pertinent cycle number determined by ensuring amplification in the linear range using 20, 25, 30, 35 cycles experimentally. Sequences of a set of every cytokine primers were shown in Materials and Methods. Amplified PCR products were electrophoresed using 2% agarose gel containing ethidium bromide and photographs of the gel visualized by UV illumination were digitally scanned. Band intensities were quantified using ATTO Densitograph software. Expression ratios were determined by dividing the band intensity of the product by that of the corresponding band of G3PDH.

enhancement of cytokine mRNA syntheses were certified in Northern blot analyses (Fig. 4B). Second stimulation was done on the following day after initial stimulation which is the most efficient timing in twice stimulations. FC1 cells grown in culture flasks (75 cm^2) were treated with 100 IU/ml of IFN-β for 24 h and stimulated once or twice with 1 or 10 μg/ml of poly rI: poly rC for Northern blot analyses. The cells were harvested at 4, 10, 30 h after initial stimulation and poly(A)$^+$RNA was isolated from disruption of the cells using Fast Track mRNA isolation kit (Invitrogen, San Diego, CA). Poly(A)$^+$RNA was separated by gel electrophoresis in a 1% agarose/2.2 M formaldehyde gels and transferred to nylon filters. Northern blot hybridization was carried out using nucleotide fragments labeled with [α-^{32}P] dCTP prepared from full-length IL-6, GM-CSF, and IFN-β cDNA as probes. The filter was analyzed using Bioimage Analyzer, BAS 2000 (Fuji Photo Film Co.). As shown in Figure 4B (lanes 6, 7, 11, 12), syntheses of GM-CSF and IL-6 mRNA were increased by twice stimulations with 1 or 10 μg/ml of poly rI: poly rC supporting the results shown in Figure 4A.

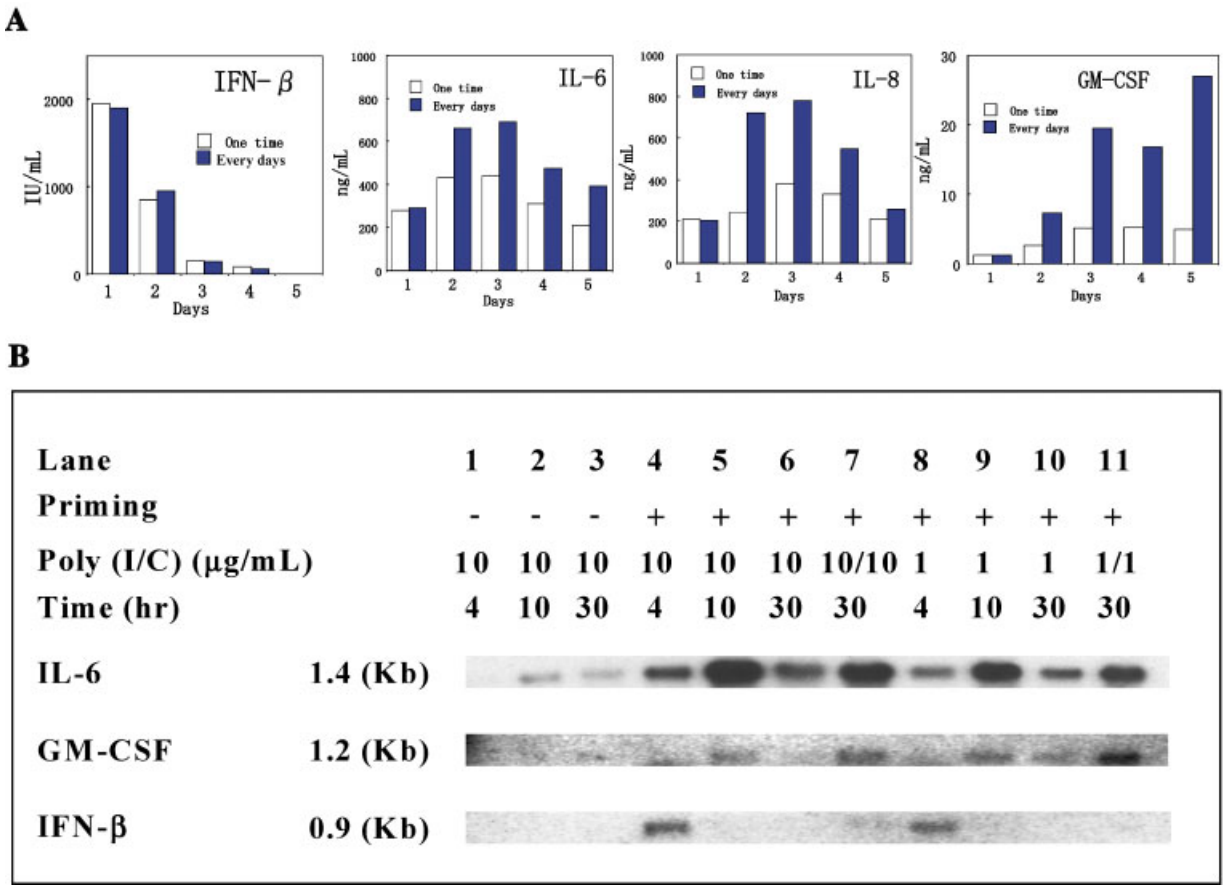

Fig. 4. Cytokine productions by repeated stimulations of poly rI: poly rC. **A**: Cytokine productions by repeated stimulations were compared with those in only initial stimulation. After initial addition of poly rI: poly rC (10 μg/ml) to primed FC1 cells (1×10^6/ml, microcarrier culture), culture fluids were replenished every day with fresh media containing (■) or not containing (□) poly rI: poly rC (10 μg/ml). The cytokine yields were quantified by ELISA systems for each cytokine. **B**: Northern blot analyses for expression levels of IFN-β, IL-6 and GM-CSF mRNA. FC1 cells primed or not primed with IFN-β (100 IU/ml) were stimulated with 1 or 10 μg/ml of poly rI: poly rC. Expressions by twice stimulations with 1 or 10 μg/ml of poly rI: poly rC were also investigated. Poly(A)$^+$RNA was isolated from the cells at 0, 4, 10, and 30 h after stimulation and mRNAs were analyzed by the method described in Materials and Methods. Expressions of IL-6 and GM-CSF mRNAs were promoted by twice stimulations with 1 or 10 μg/ml of poly rI: poly rC (**Lanes 7 and 11**) and maximum expressions of these cytokines were observed at 10 h after stimulation (**Lanes 5 and 9**). These cytokine mRNA expressions were also enhanced by priming the cells with 100 IU/ml of IFN-β.

Inhibition of Poly rI: Poly rC-Induced Cytokine Productions by Anti-Human IFN-β Antibody and Enhancement by Addition of IFN-β

Results shown in Figures 2, 3, and 4B have suggested that the autogenous IFN-β might be involved in the production processes of the other cytokines. To certify this concept, effects of specific monoclonal antibody against human IFN-β on cytokine productions were examined. FC1 cells primed with 100 IU/ml of IFN-β were stimulated with 10 μg/ml of poly rI: poly rC in the presence of various concentrations of anti-human IFN-β antibody. To avoid influence of non-specific inhibition by murine immunoglobulin, an anti-human MCAF monoclonal antibody having the same iso-type of murine IgG as that of IFN-β antibody was employed as a control. Productions of IL-6, GM-CSF, IL-8, GRO-β, and RANTES as well as IFN-β were suppressed by addition of the monoclonal antibody against human IFN-β dependent on the concentrations (Fig. 5A). As MCAF monoclonal antibody has not exerted non-specific inhibition on cytokine productions at the concentration of 1–5 μg/ml, effect of IFN-β antibody on the inhibition patterns of cytokine productions were investigated using 2 μg/ml of the antibody.

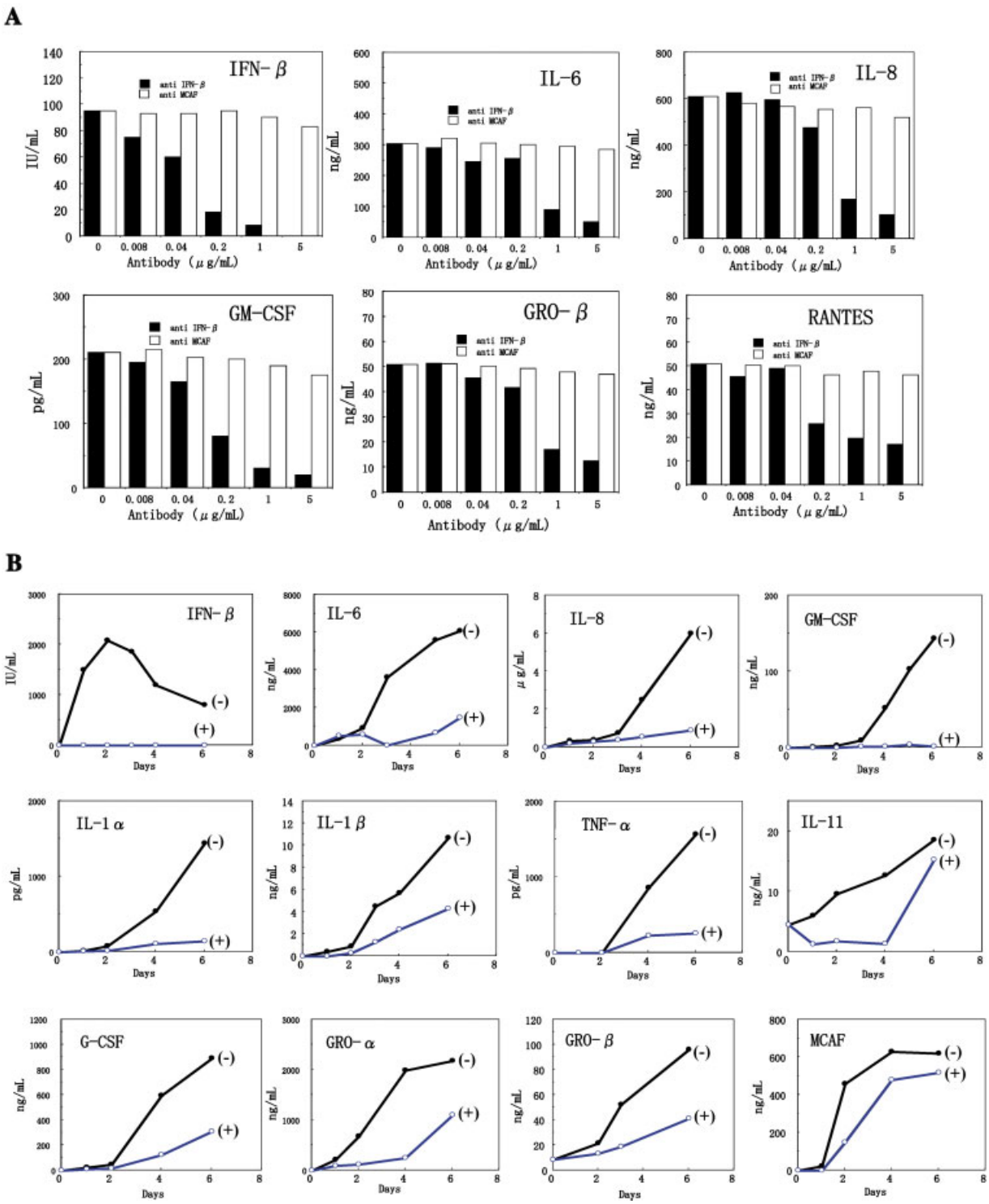

Fig. 5. Inhibition of cytokine productions by anti-human IFN-β monoclonal antibody. **A**: IFN-β and other cytokine productions were suppressed by a monoclonal antibody against human IFN-β in a dose dependent manner. FC1 cells grown in 6-well plates (0.8×10^6 cells/well) were primed with IFN-β (100 IU/ml) for 24 h and were stimulated with 10 μg/ml of poly rI: poly rC in the presence of various concentrations of anti-human IFN-β antibody (■). Monoclonal antibody against human MCAF was also used as a control of murine IgG of IFN-β antibody (□). culture fluids were harvested on the 5th day after induction for quantification of IFN-β and cytokines. **B**: Inhibition patterns of cytokine productions by human IFN-β antibody. The cells primed with IFN-β (100 IU/ml) were incubated for 6 days in the absence (−) or presence (+) of 2 μg/ml of anti-human IFN-β antibody together with 10 μg/ml of poly rI: poly rC. Culture fluids were harvested every 1 or 2 days for quantification by ELISA systems. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

As shown in Figure 5B, productions of IL-6, GM-CSF, IL-1α, IL-1β, TNF-α, IL-11, G-CSF, IL-8, GRO-α, GRO-β, and MCAF as well as IFN-β were suppressed alike with elapse by addition of monoclonal IFN-β antibody, although suppressed production profiles were slightly different respectively. Meanwhile, effects of additive IFN-β to the cultures primed with IFN-β and induced with poly rI: poly rC on cytokine productions were examined in a microcarrier culture. Culture fluids were replaced at 2 h after poly rI: poly rC stimulation with fresh medium without serum containing 0.1–1,000 IU/ml of IFN-β. Culture supernatants were harvested on the 5th day after stimulation and amounts of IL-6 and IL-8 were determined using each ELISA kit. As shown in Figure 6, each cytokine yield was increased about twofold by addition of IFN-β indicating that secreted IFN-β mediates subsequent cytokine productions. We could not detect any cytokines in culture supernatants, although various concentrations of IFN-β were added to the cultures which were not stimulated with poly rI: poly rC (data not shown).

These results have suggested that inducible IFN-β in human fibroblasts might be essential in the production processes of the other cytokines.

Intra-Cellular IFN-β and Other Cytokine Productions in the Presence of Anti-Human IFN-β Antibody

To confirm whether IFN-β induced by poly rI: poly rC must be secreted from the cells to mediate cytokine productions, synthesis patterns of intra-cellular IL-6, GM-CSF as well as IFN-β in the presence of anti-human IFN-β antibody were investigated. The cells primed with 100 IU/ml of IFN-β and stimulated with 10 μg/ml of poly rI: poly rC were cultured in the presence or absence of 5 μg/ml of a monoclonal antibody against human IFN-β which is sufficient dose to neutralize secreted IFN-β. The cells were harvested every several hours until 7 days and sonicated to recover intra-cellular cytokines. Supernatants obtained from disrupted cells by centrifugation were analyzed for cytokine contents by ELISA systems. As shown in Figure 7, intra-cellular IL-6 and GM-CSF as well as IFN-β were synthesized showing similar production patterns to secreted cytokines regardless of excess existence of antibody against human IFN-β. This results have suggested that alternative intra-cellular signaling pathway of inducible IFN-β might be in existence in addition to the pathway by secreted IFN-β to mediate cytokine productions.

Augmentation of IFN-β mRNA Expression by IL-1β

IL-1β has also stimulated productions of numerous kinds of cytokines in human fibroblasts (FC1 cells). These cytokines were secreted late after stimulation similar to the production patterns by poly rI: poly rC stimulation demonstrated in Figure 2B. The maximum productions of IL-6 and IL-8 were observed 2 or 3 days after IL-1β stimulation and secretion of

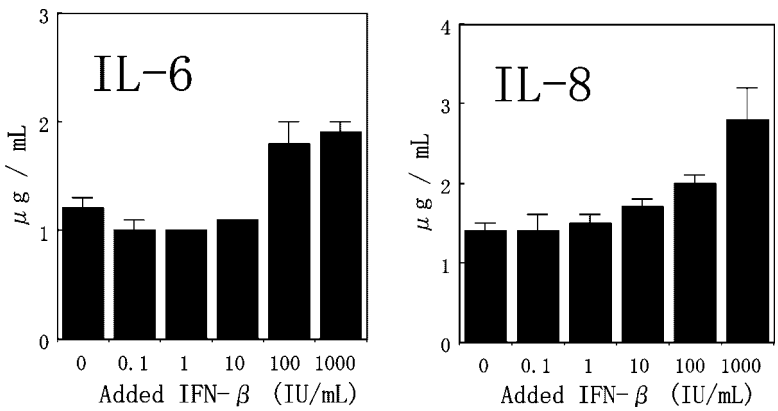

Fig. 6. Effect of addition of IFN-β on cytokine productions. The cells grown in microcarrier culture were treated with IFN-β (100 IU/ml) for 24 h at 37°C and culture fluids were replaced with fresh medium (Eagle's MEM) without serum containing 10 μg/ml of poly rI: poly rC. After 2 h cultivation, culture fluids were replenished again with fresh medium without poly rI: poly rC containing 0.1–1,000 IU/ml of IFN-β and were harvested on the 5th day after poly rI: poly rC stimulation. Quantifications of IL-6 and IL-8 were carried out using each ELISA system.

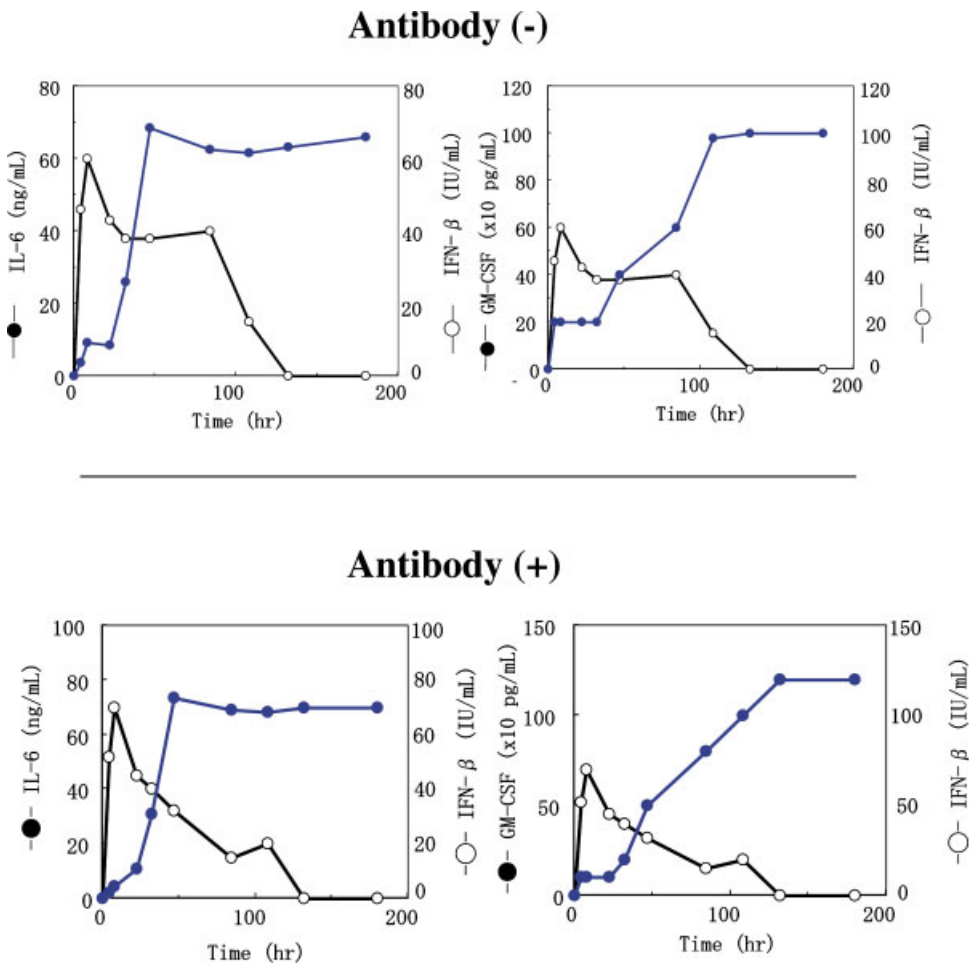

Fig. 7. Intra-cellular IFN-β and cytokine productions in the presence of anti-human IFN-β antibody. FC1 cells of 1×10^6 grown in 25-cm^2 flasks were primed with 100 IU/ml of IFN-β for 24 h and stimulated with 10 μg/ml of poly rI: poly rC in the absence or presence of 5 μg/ml of anti-human IFN-β antibody. The cells were harvested with elapse using cell scraper (Corning, NY) into 15 ml centrifuge tubes (Corning, NY) containing 5 ml of Eagle's MEM without serum. The cells were centrifuged at 1,000 rpm for 5 min and washed with the same volume of PBS (−) three times. The cell pellets were suspended in 100 μl of Eagle's MEM without serum and sonicated for 30 s using Handy Sonic model UR-20P (TOMY SEIKO, Saitama, Japan). The cell lysates were centrifuged at 3,000 rpm for 10 min to get the supernatant for quantification of cytokine contents. Production patterns of intra-cellular cytokines (●) were compared with that of IFN-β (○). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

IFN-β was not detected by ELISA system as shown in Figure 8A. To investigate whether IFN-β production is involved in cytokine production processes by IL-1β, effects of IL-1β on the expression of IFN-β mRNA were examined using semi-quantitative RT-PCR analysis. Fibroblasts grown in 75-cm^2 culture flasks were stimulated with 20 ng/ml of IL-1β in Eagle's MEM without serum. The cells were harvested at 4, 8, 24, 30 h after IL-1β stimulation with cell scraper and total RNA was isolated using RNA isolation reagent (TRIZOL, Invitrogen). RT-PCR analysis was performed using 1 μg of total RNA and the amplified products were electrophoresed by 3% NuSieve 3:1 agarose gel (TAKARA). Photographs of the gel visualized by UV illumination were digitally scanned and the band intensities of IFN-β were quantified by comparing with those of β-actin using densitograph software (ATTO). Sequences of a set of IFN-β primers were shown in Materials and Methods. Expression of IFN-β mRNA was augmented at 4 h after IL-1β stimulation as shown in Figure 8B suggesting that activation of IFN-β mRNA by IL-1β might affect cytokine productions. To certify whether a small amount of secreted IFN-β mediates cytokine productions by IL-1β, productions of IL-6 and IL-8 were examined in the presence of various concentrations of anti-human IFN-β antibody. As shown

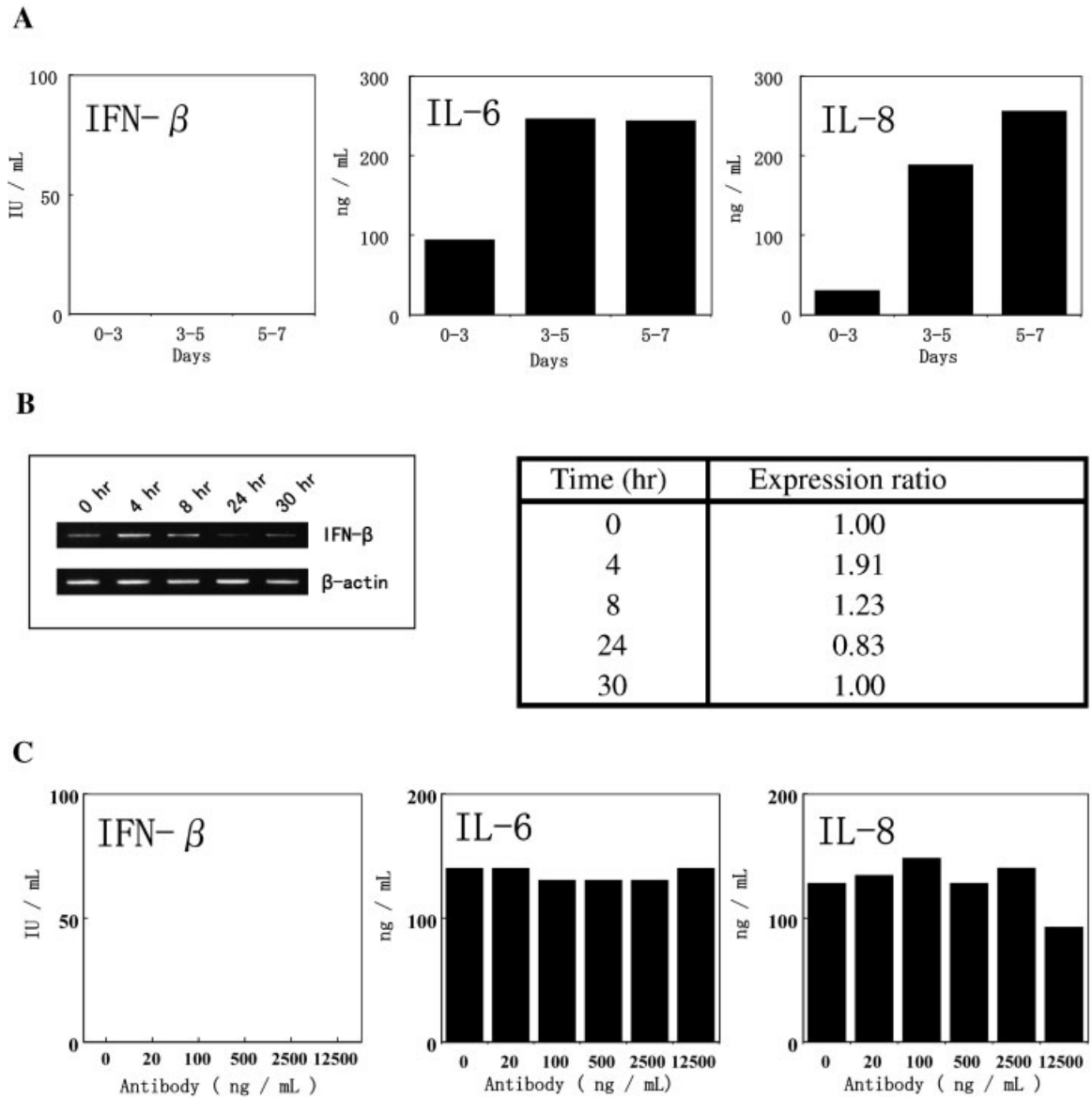

Fig. 8. Cytokine productions by IL-1β. **A**: Production time courses of IL-1β-induced cytokines. The cells cultured in 6-well plates (0.9×10^6 cells/well) were stimulated with 20 ng/ml of IL-1β (Pepro Tech, London) in serum free Eagle's MEM and culture fluids were replenished with fresh medium every 2 or 3 days. The contents of IL-6, IL-8, and IFN-β were quantified by each ELISA system. IFN-β was not detected. **B**: Enhancement of IFN-β mRNA expression by IL-1β. Fibroblasts proliferated in 75-cm^2 plastic flasks (1.0×10^7 cells/flask) were stimulated with 20 ng/ml of IL-1β in serum free Eagle's MEM and the cells were harvested at 4, 8, 24, 30 h after addition of IL-1β with cell scraper. Total RNA was isolated using RNA isolation reagent (TRIZOL, Invitrogen) and concentration of the RNA was determined from the absorbency at A_{260}/A_{280}. RT-PCR analyses for IFN-β were performed using 1 μg of total RNA. RT reactions were carried out at 30°C for 10 min, 45°C for 30 min, 99°C for 5 min, and 5°C for 5 min. PCR reactions for IFN-β were performed in 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. RT-PCR analyses for β-actin were performed using 0.1 μg of total RNA and PCR reactions were done in 30 cycles under the same conditions of the case of IFN-β using PCR thermal cycler MP (TAKARA). The amplified products of 5 μl were electrophoresed using 3% NuSieve 3:1 agarose gel (TAKARA) and visualized by UV illumination. Photographs were digitally scanned and the band intensities were quantified using ATTO densitograph software (ATTO). Expression ratios of mRNA were determined by dividing the band intensity of each product by that of the corresponding band of β-actin and were shown as ratios against the value at 0 h (1.00). Sequences of a set of IFN-β primers were shown in Materials and Methods. Primer sets for β-actin were purchased from TAKARA. **C**: Productions of IL-6 and IL-8 were not suppressed by IFN-β antibody. The cells grown in 6-well plates were stimulated with 20 ng/ml of IL-1β in the presence of 20–12,500 ng/ml of the antibody against human IFN-β. Culture supernatants were harvested on the 5th day after IL-1β stimulation.

in Figure 8C, IL-6 and IL-8 productions were not suppressed by addition of antibody suggesting that IL-1β-induced cytokine productions might be not mediated by secreted IFN-β, although IFN-β mRNA is augmented by IL-1β.

DISCUSSION

We have investigated cytokine production profiles and mechanisms in human fibroblasts stimulated with poly rI: poly rC or IL-1β to understand the meaning of cytokine productions or cytokine network in living body. In our production system, numerous kinds of cytokines and chemokines were produced in human fibroblasts stimulated with these inducers and cytokine productions by poly rI: poly rC were extremely enhanced by treating the cells with type 1 IFN similar to priming effect for IFN-β production. It has been already shown that some cytokines such as IL-6, IL-8, and CSFs are produced in human fibroblasts stimulated with poly rI: poly rC or IL-1β [Kohase et al., 1987; Van Damme et al., 1987a,b, 1989a,b; Fibbe et al., 1988] and that some inflammatory cytokine gene activations are caused following IFN-β gene activation by poly rI: poly rC [Yamamoto et al., 2003]. Although human fibroblasts have been known as potent cells for IFN-β production, it has almost not been examined about production profiles or mechanisms of a variety of cytokines produced in human fibroblasts to aim at understanding physiological significance of cytokine productions in the cells. Hitherto, cytokine productions have been examined in a short time for 24–48 h, but we were able to find a lot of cytokines by examining production time courses for a long time. Some differences in the production profile between IFN-β and other cytokines were observed. The most distinctive feature among them was different production time courses (Fig. 2B). Contrary to a rapid production of IFN-β, most of other cytokines appeared more slowly. It has been reported that IFN-β mRNA is detected as early as 1.5 h after addition of poly rI: poly rC and induction does not require protein synthesis [Cavalieri et al., 1977b]. Our results about IFN-β production corresponded to this as shown in Figures 2–4. However, maximum synthesis of cytokine mRNA was observed 6–10 h after stimulation suggesting that newly protein syntheses are necessary for cytokine productions. In super-induction method which has been contrived to produce IFN-β efficiently in consideration of early expression of IFN-β mRNA [Vilcek et al., 1976], cytokines were produced only a very little (Fig. 2A) reflecting late expressions of cytokine mRNA. In our experiments using poly rI: poly rC alone, most of cytokines were produced comparatively in large quantities, although IFN-β yields were very little (about one-tenths of those obtained in super induction method) (Fig. 2). These results have suggested that IFN-β induction might be involved in cytokine production processes. As these cytokine productions were decreased by neutralizing secreted IFN-β with anti-human IFN-β antibody, we could make clear for the first time at a protein level that cytokine productions are mediated by secreted IFN-β. However, cytokine yields did not seem to depend on the quantities of secreted IFN-β since quantities of secreted IFN-β were very little (about 0.5–5 ng/ml) in comparison with cytokine yields, although slight increase of cytokine yields was shown by addition of IFN-β (Fig. 6). Recently, some molecules relating to IFN-β gene activation by double-stranded RNA have been identified and now, molecular mechanisms of subsequent IFN-β-inducible gene activation containing cytokine productions are being studied actively. It has been shown that TLR3 is a receptor for double-stranded RNA produced by viruses [Alexopoulou et al., 2001; Matsumoto et al., 2002] and is necessary for IFN-β gene activation. In consequence of IFN-β gene activation, mRNA of some cytokines such as RANTES, MCAF/MCP-1, and IP-10 were also augmented [Yamamoto et al., 2002, 2003]. Later, an adaptor molecule designated TICAM-1 (Toll-IL-1 receptor domain (TIR) containing adaptor molecule)/TRIF (TIR domain containing adaptor inducing IFN-β) which can physically bind TIR domain of TLR3 and activate IFN-β promoter in response to poly rI: poly rC was identified [Oshiumi et al., 2003; Yamamoto et al., 2003]. TICAM-1/TRIF is known to activate IFN-β promoter by means of three transcription factors of NF-κB, AP-1, and IRF-3. In addition to TLR3 ligand, TLR4 ligand LPS has been also shown to induce IFN-β [Kawai et al., 2001; Yamamoto et al., 2003]. Meanwhile, it has been known that cytokine productions by IL-1β are mediated by activation of transcription factors such as NF-κB and AP-1 via IL-1 receptor/TLR [Cao et al., 1996], however, it is not clear whether IL-1β-induced cytokine productions are mediated by IFN-β

gene activation. By means of above-mentioned signaling pathways, various kinds of cytokines found in our experiments might be produced. However, there is little information about molecular studies related to cytokine gene expressions in human fibroblasts. It was interested in examining how inducible IFN-β acts for cytokine productions. Until now, it has not been clear whether inducible IFN-β is secreted or not secreted to mediate cytokine productions because most of the studies about cytokine productions have been carried out only at mRNA expression levels. If inducible IFN-β must be secreted for cytokine productions, cytokines should be produced even by addition of IFN-β. However, in our experiments, we could not detect any cytokines in the culture fluid of fibroblasts treated with high concentrations ($> 10^4$ IU/ml) of human IFN-β, although there is a report that IFN-β by itself, used at high concentration, can directly induce IL-6 in human fibroblasts [Kohase et al., 1987]. Therefore, it was considered that secreted IFN-β might act for cytokine productions together with some another signals by poly rI: poly rC. There are some reports about indirect actions of poly rI: poly rC mediated by secreted IFN-β. It has been shown that cell growth regulation or anti-virus effects in human fibroblasts stimulated with poly rI: poly rC were owing to secreted IFN-β [Vilcek et al., 1987; Van Damme et al., 1987b].

To confirm that intra-cellular IFN-β might be also taken part in cytokine productions, intra-cellular syntheses of IFN-β and other cytokines were examined. Production patterns of intra-cellular IFN-β and other cytokines were similar to those of secreted proteins (Fig. 7), and intra-cellular cytokine productions were not inhibited by anti-human IFN-β antibody. As it has been already examined that the antibody is not internalized into the cells, it was considered that intra-cellular cytokines might be synthesized via intra-cellular IFN-β by means of different way from that of secreted IFN-β since secreted IFN-β should be neutralized by the antibody, although a possibility that secreted IFN-β might be remained slightly over the cells cannot be denied. Meanwhile, inhibition patterns of cytokine productions caused by anti-human IFN-β antibody have shown a tendency to recover along with elapse, although the inhibition curves were respectively different as shown in Figure 5. It has also suggested that another pathway of inducible IFN-β might be existent for cytokine productions. Hereafter, it should be clarified how IFN-β induces various kinds of cytokines at a molecular level.

Meanwhile, we have shown that cytokine productions by poly rI: poly rC are extremely enhanced by treating the cells with type 1 IFN similar to IFN-β production. Various studies about priming effects of type 1 IFN on IFN-β production by poly rI: poly rC have been carried out; however, there are not any reports about priming effects for cytokine productions until now. In our experiments, it was shown that treating the cells with more than 100 IU/ml of IFN-a or -β resulted in a 5- to 40-fold increase of cytokines similar to IFN-β production (Fig. 1A,B). It has been shown that IFN priming for IFN-β production is dependent on cellular protein synthesis [Fujita and Kohno, 1981]. In the same way, priming effects on the productions of IL-6 and IL-8 were also blocked by the addition of cycloheximide which is a reversible inhibitor for protein synthesis (Fig. 1C). These data suggested that there might be existent some common mechanism in priming effects on IFN-β and other cytokine productions. However, it was considered that priming effects for cytokine productions might be indirect action caused by enhancement of signaling pathway for IFN-β gene activation by poly rI: poly rC since cytokines were produced a few days after IFN-β production.

Further, repeated stimulation with poly rI: poly rC caused the tolerance of IFN-β production, but not in the case of the cytokines (Fig. 4A). As a matter of course, cytokine mRNAs were augmented different from IFN-β mRNA when fibroblasts were stimulated twice with poly rI: poly rC (Fig. 4B). In twice stimulations, the second stimulation of poly rI: poly rC was most effective when poly rI: poly rC was added on the next day after initial stimulation (Data not shown). As cytokine productions were continued by twice stimulations even in the cells not primed with IFN-β (data not shown), it was considered that a small amount of IFN-β induced by initial inoculation of poly rI: poly rC resulted in enhancement of ctokine productions by second stimulation with poly rI: poly rC as a priming IFN-β.

On the other hand, cytokine productions by IL-1β were very late similar to those by poly rI: poly rC. However, we could not detect secreted IFN-β using a high sensitive ELISA kit (Toray).

Recently, it has been shown that signaling pathway of IL-1β mediated by Toll-IL-1 receptor is different from those mediated by TLR3 or TLR4 for cytokine productions and cytokine productions by IL-1β are not mediated by IFN-β gene activation [Kaisho and Akira, 2001; Kaisho et al., 2001; Yamamoto et al., 2003]. However, it has been already reported that IL-1β exerts an anti-viral effect on human fibroblasts by inducing expression of IFN-β [Van Damme et al., 1987b; Randolph-Habecker et al., 2002]. In these reports, secreted IFN-β has not been found in the culture supernatants and a small amount of IFN-β mRNA, which was only detectable with a high sensitive method, was produced by IL-1β alone. Increases of secreted IFN-β and its mRNA were observed only in combination with cycloheximide or in the cells infected with cytomegalovirus. In the same way, we could not at all detect secreted IFN-β by IL-1β stimulation and as a matter of course, cytokine productions were not inhibited by the addition of anti-human IFN-β antibody as shown in Figure 8C. However, we could find that IFN-β mRNA was augmented in fibroblasts stimulated with IL-1β alone (Fig. 8B). These data suggested that cytokine productions by IL-1β might be also mediated by IFN-β gene activation.

In this study, we have shown that inducible IFN-β takes part in cytokine production processes suggesting autogenous IFN-β might play a key role in cytokine productions caused as one of the self-defence mechanisms to bacterial or viral infection or inflammation in living body and that priming effects of type 1 IFN on cytokine productions might be indirect action resulting from enhancement of IFN-β gene activation by poly rI: poly rC.

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