JOURNAL OF VIROLOGY, Feb. 1991, p. 968–971
0022-538X/91/020968-04$02.00/0

Vol. 65, No. 2

Interferon-Regulated *Mx* Genes Are Not Responsive to Interleukin-1, Tumor Necrosis Factor, and Other Cytokines

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Received 28 June 1990/Accepted 29 October 1990

Accumulation of *Mx* gene products in cells of patients and experimental animals has been recognized as a useful marker for detecting minute quantities of biologically active interferon (IFN). Goetschy et al. (J. Goetschy, H. Zeller, J. Content, and M. A. Horisberger, J. Virol. 63:2616–2622, 1989) reported that not only IFNs but also interleukin-1 (IL-1) and tumor necrosis factor (TNF) were potent inducers of the human *Mx* genes. However, we observed no *Mx* induction in cultured human fibroblasts or in human peripheral blood mononuclear cells treated with various concentrations of IL-1α or TNF-α. *Mx* induction was found in the spleens of mice treated with TNF-α or IL-1α, but this effect could be neutralized with antibodies to murine IFN-α/β. Of the other cytokines that we tested (IL-2, IL-6, and granulocyte-macrophage colony-stimulating factor), only IL-2 induced the *Mx* genes in peripheral blood mononuclear cells, but antibodies to human IFN-β efficiently neutralized this effect. Our results thus indicate that IFNs are the only cytokines with intrinsic *Mx*-inducing activity.

Mx genes of humans and other mammals are members of the large family of interferon (IFN)-regulated genes (for a review, see reference 10). The *Mx* genes are usually in a quiescent state but are expressed at a very high rate following exposure of cells to either IFN-α or IFN-β (1, 4, 11). On the basis of these observations, it was suggested that by measuring *Mx* gene products in leukocytes of patients, one might be able to monitor the effectiveness of IFN therapies (3, 9). In an animal model system, Mx has already proved to be an extremely sensitive marker for IFN action (5). High levels of *Mx* gene products were found in the peripheral blood mononuclear cells (PBMC) of patients suffering from viral infections (3, 17) or other disease (18) which had little or no detectable IFN activity in the serum, again indicating a superior sensitivity of this type of indirect IFN assay.

To critically evaluate Mx as a marker for IFN action, it was important to define the specificity of the Mx response. In particular, it was crucial to determine whether other cytokines could induce the *Mx* genes. Goetschy et al. (4) reported that in addition to IFN, tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) were excellent *Mx* inducers in human fibroblasts. However, we were unable to verify these results. We now show that both IL-1α and TNF-α failed to activate the *Mx* genes of PBMC and cultured fibroblasts. Of the other cytokines that we tested, only high concentrations of IL-2 weakly induced *Mx* in PBMC, but this effect could be neutralized with antibodies to IFN-β. Treatment of mice with IL-1α or TNF-α led to the accumulation of *Mx* gene products in the spleen cells, but treatment of mice with antibodies to IFN-α/β prior to cytokine injection abolished *Mx* induction, suggesting that IFNs are the only cytokines with intrinsic *Mx*-inducing activity.

***Mx* induction in PBMC by different cytokines.** PBMC were isolated from fresh blood by standard Ficoll-Paque gradient centrifugation, the cells were incubated for 6 h in the presence of various cytokines, and total RNA was prepared and analyzed for *Mx* mRNA content by the RNA slot blotting technique (3). Briefly, RNA samples (usually 20 μg) were serially twofold diluted in a solution containing yeast tRNA; the first six dilutions were then blotted to nitrocellulose, and the membrane was hybridized to radiolabeled human *MxA* cDNA (1). Under the high-stringency conditions used, this probe detected mainly *MxA* mRNA and showed very little cross-hybridization to *MxB* mRNA, the product of the second human *Mx* gene (1). Unstimulated PBMC from healthy volunteers contained barely detectable concentrations of *Mx* mRNA (Fig. 1). Incubation of PBMC with recombinant human (rHu) IFN-α2 (10^8 U/mg; a gift from Biogen SA, Geneva, Switzerland) at 1,000 U/ml resulted in approximately 100-fold elevated *Mx* mRNA pools. Treatment of PBMC for 6 h with rHu IL-1α (10^7 U/mg; Boehringer Mannheim) at either 10 or 100 U/ml did not result in significantly elevated *Mx* mRNA pools (Fig. 1). Treatment of PBMC with rHu IL-6 (2×10^8 U/mg; Boehringer Mannheim) at 20 or 200 U/ml or with rHu TNF-α (7×10^6 U/mg; a gift from G. Brandner, Freiburg, Federal Republic of Germany) at 100 or 1,000 U/ml also failed to induce the *Mx* genes to significant levels (Fig. 1). Similarly, treatment of PBMC with rHu granulocyte-macrophage colony-stimulating factor (5×10^7 CFU/mg; a gift from C. Huber, Innsbruck, Austria) at 10 to 10,000 U/ml for 3 or 6 h did not lead to elevated *Mx* mRNA pools (data not shown).

PBMC treated for 6 h with rHu IL-2 (10^7 U/mg; a gift from E. Liehl, Sandoz, Vienna, Austria) at 10,000 or 50,000 U/ml contained *Mx* mRNA pools that were significantly higher than those in the untreated control cells but much lower than those in cells treated with IFN-α (Fig. 2). To determine whether the observed *Mx* induction by IL-2 was caused by cytokine-induced IFN, we performed the IL-2 induction in medium containing antibodies that neutralize the different IFNs. Addition of 1,000 neutralizing units of horse antibodies to human IFN-β (Boehringer Mannheim) per ml completely blocked *Mx* induction by IL-2, whereas similar concentrations of neutralizing horse antibodies to human IFN-α (Boehringer Mannheim) or rabbit antibodies to human IFN-γ (a gift from M. Aguet, Zürich, Switzerland) failed to block *Mx* induction by IL-2 (Fig. 2). This result suggested

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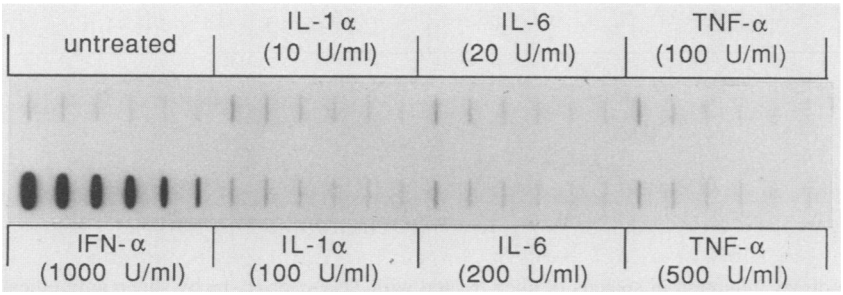

FIG. 1. No *Mx* induction after treatment of human PBMC with rHu IL-1α, rHu IL-6, or rHu TNF-α. PMBC were prepared from fresh blood by Ficoll-Paque gradient centrifugation, and samples of 5×10^6 cells were incubated for 6 h at 37°C in 1.5 ml of Dulbecco modified Eagle medium containing 10% fetal calf serum and various cytokines at the indicated concentrations. Total RNA was isolated (1) and serially twofold diluted, and the first six RNA dilutions were blotted to a nitrocellulose membrane. The membrane was hybridized under standard high-stringency conditions to the radiolabeled 2.2-kb *Eco*RI-*Sma*I fragment of human *MxA* cDNA (1).

that the high doses of IL-2 used in our experiments induced PBMC to produce IFN-β, which in turn activated the *Mx* genes.

No *Mx* induction by TNF-α and IL-1α in human fibroblasts. The data presented above did not agree with the conclusions of Goetschy et al. (4) that TNF-α and IL-1 were good inducers of the *Mx* genes. Since the latter experiments were performed with a fibroblast cell line, we investigated the cytokine-induced *Mx* response of low-passage human foreskin fibroblasts. These cells responded to IFN-α by accumulating high concentrations of *Mx* mRNA, but the *Mx* mRNA pools remained unchanged at a very low level in the fibroblasts that were treated with 250 or 1,000 U of TNF-α per ml or with 10 or 20 U of IL-1α per ml (Fig. 3A). Since IL-1 and TNF were described as inducers of IL-6 (2), we stripped the nitrocellulose membrane and rehybridized it to radiolabeled IL-6 cDNA (7). Fibroblasts treated with IL-1α and TNF-α contained eight- and twofold-elevated pools of IL-6 mRNAs, respectively (Fig. 3B), demonstrating that both cytokine preparations contained biologically active protein.

In a second series of experiments, we investigated whether varying the growth conditions would make the *Mx* genes responsive to TNF-α or IL-1. Variations included the fetal calf serum concentration and the density of the cell monolayers. In addition, we used a second cell line, HFL cells (1). We failed to observe *Mx* induction by TNF-α or IL-1 under all conditions tested (data not shown).

***Mx* induction by cytokines in the spleens of mice.** We next

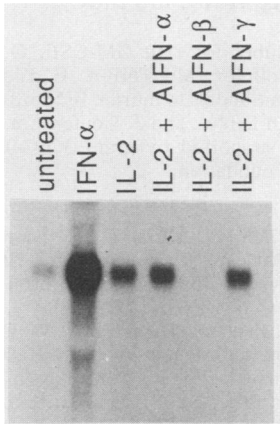

FIG. 2. Induction of *Mx* by high doses of IL-2 in PBMC and blockage of induction by IFN-β-neutralizing antibodies. PBMC (5×10^6 cells in 1.5 ml of medium) were treated for 6 h with 50,000 U of rHu IL-2 per ml in the presence or absence of horse antibodies to human IFN-α (AIFN-α), horse antibodies to human IFN-β (AIFN-β), or rabbit antiserum to human IFN-γ (AIFN-γ) at 1,000 neutralizing units per ml. RNA was isolated from the various cell cultures (1), and samples (20 μg) were used to prepare a Northern (RNA) blot (11). The membrane was then hybridized under standard high-stringency conditions to the radiolabeled 2.2-kb *Eco*RI-*Sma*I fragment of human *MxA* cDNA (1).

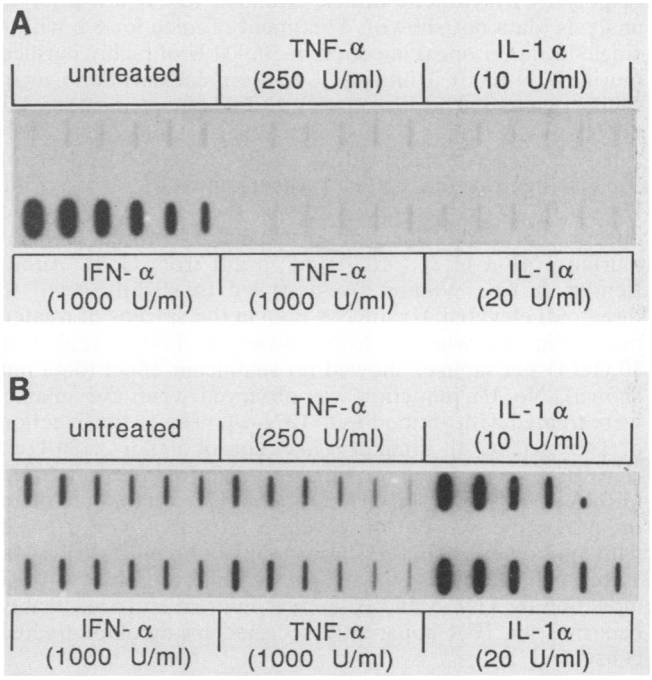

FIG. 3. No *Mx* induction observed after treatment of human fibroblasts with IL-1α or TNF-α. Confluent monolayer cultures of human foreskin fibroblasts were treated for 6 h with the indicated concentrations of either rHu IFN-α2, rHu IL-1α, or rHu TNF-α. A slot blot was prepared from serially twofold-diluted RNAs, and the membrane was hybridized under standard high-stringency conditions to the radiolabeled 2.2-kb *Eco*RI-*Sma*I fragment of human *MxA* cDNA (1) (A) or a radiolabeled 500-bp fragment of human IL-6 cDNA (7) (B).

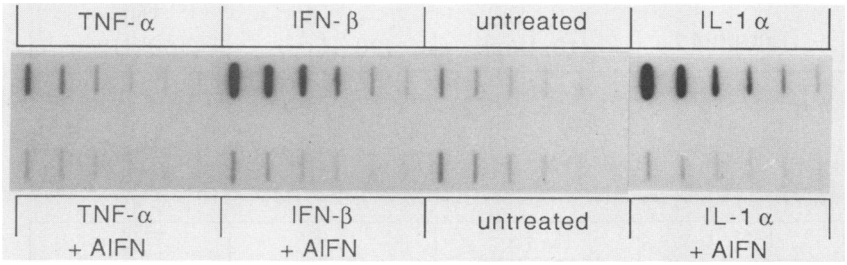

FIG. 4. Neutralization of *Mx* induction in spleens of mice treated with TNF-α or IL-1α by sheep antibodies to murine IFN-α/β. Thirty minutes prior to intraperitoneal injection of either 50,000 U of recombinant murine TNF-α, 2,000 U of rHu IL-1α, or 50,000 U of murine IFN-β, BALB.A2G-*Mx* mice received an intravenous injection (25 μl) of 250,000 neutralizing units of sheep antibodies to murine IFN-α/β (AIFN) or an equal volume of buffered saline. The animals were killed 6 h after the onset of the cytokine treatment, RNA was prepared from their spleens (1), and samples (30 μg) were serially twofold diluted, blotted to nitrocellulose membranes, and hybridized under standard high-stringency conditions to the radiolabeled 2.3-kb *Bam*HI-*Bam*HI fragment of murine *Mx1* cDNA (13).

wished to examine the *Mx* gene activation by cytokines in vivo and therefore decided to work with Mx^+ mice, which possess a functional *Mx1* gene (12). We administered the cytokines via the intraperitoneal route in a total volume of 0.5 ml of isotonic buffer, sacrificed the animals 6 h after the onset of the cytokine treatment, prepared total RNA from the spleen, and measured the *Mx* content in the different RNA preparations by RNA slot blotting. A radiolabeled murine *Mx1* cDNA fragment (13) was used as a hybridization probe. The spleens of untreated control mice contained low but readily detectable pools of *Mx* mRNA. The *Mx* mRNA pools were more than 100-fold increased in the spleens of mice that received a single intraperitoneal injection of 100 μg of poly(I-C) (synthetic double-stranded RNA) 6 h prior to analysis (data not shown). Treatment of mice for 6 h with a single intraperitoneal injection of 50,000 U of highly purified murine IFN-β (10^8 Units/mg; Lee Biomolecular, San Diego, Calif.) resulted in approximately 10-fold-elevated spleen *Mx* mRNA pools (Fig. 4). This induction was completely abolished in mice treated with a single intravenous dose of 250,000 neutralizing units of sheep antibodies to murine IFN-α/β (6) (a gift from I. Gresser, Paris, France) prior to injection of IFN. Injection of 50,000 U of recombinant murine TNF-α (1.2×10^7 U/mg; a gift from G. R. Adolf, Bender & Co., Vienna, Austria) led to slightly (two- to threefold) elevated *Mx* mRNA pool in the spleens of treated mice (Fig. 4), whereas lower doses of TNF-α (2,000 or 10,000 U per mouse) showed no significant effect (data not shown). No *Mx* induction was observed when the animals were treated with antibodies to IFN-α/β prior to the injection of TNF-α (Fig. 4). Similarly, injection of 500 or 2,500 U of rHu IL-1α led to approximately eightfold elevated *Mx* mRNA pools in the spleens of treated mice, but induction of the *Mx1* gene was not observed in mice that were treated with antibodies to IFN-α/β prior to injection of IL-1 (Fig. 4). Taken together, these data suggested that the observed *Mx* induction by TNF-α and IL-1α was indirect and presumably occurred via IFN apparently secreted by cytokine-treated cells.

Conclusions. Our experiments with fresh human PBMC, with cultured human fibroblasts, and with laboratory mice clearly showed that neither IL-1α nor TNF-α possessed intrinsic *Mx*-inducing activity. Of the other cytokines, only IFN-α and IFN-β were potent inducers of the *Mx* genes. We showed elsewhere that IFN-γ has very low intrinsic *Mx*-inducing activity in human fibroblasts (1). It thus appears that Mx would qualify as a sensitive and specific biochemical marker for the action of IFN-α and IFN-β.

Our results are in conflict with an earlier report by Goetschy et al. (4) which showed that IL-1 and TNF-α were excellent *Mx* inducers in a human fibroblast cell line. We have no satisfactory explanation for this discrepancy. We believe that cytokine-induced IFN was most likely the true *Mx* inducer in the latter experiments. This would explain why, in contrast to IFNs, these cytokines needed ongoing protein synthesis for *Mx* gene activation as reported by Goetschy et al. (4). Indeed, IL-1 and TNF-α were shown to induce minute quantities of IFN-β in some cell lines (8, 14, 15). It is conceivable, therefore, that the human embryonic lung cell line used by Goetschy et al. (4) was exceptional in that it readily produced IFN-β in response to treatment with TNF-α or IL-1. Our conclusions are supported by some recent Western immunoblotting data of others (16) which show that MxA protein is not produced by PBMC treated for 24 h with TNF-α or IL-1.

The weak *Mx* induction by IL-2 that we observed was most likely also indirect and resulted from the action of induced IFN-β, as neutralizing antibodies to IFN-β blocked this effect. This result would thus indicate that IL-2 has a potential to induce IFN-β in PBMC.

We thank C. Huber for rHu GM-CSF, G. Brandner for rHu TNF-α, G. R. Adolf for rMu TNF-α, E. Liehl for rHu IL-2, I. Gresser for sheep antibodies to murine IFN-α/β, M. Aguet for rabbit antiserum to human IFN-γ, and J. Content for IL-6 cDNA.

This work was supported by grant 3.597-0.87 from the Swiss National Science Foundation.

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