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INTERLEUKIN-1 INDUCTION OF TUMOR NECROSIS FACTOR-α mRNA AND BIOACTIVE TUMOR NECROSIS FACTOR-α IN A PANCREATIC β-CELL LINE BY A MECHANISM REQUIRING NO DE NOVO PROTEIN SYNTHESIS

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Interleukin-1 (IL-1) has been implicated as an effector in insulitis of Type 1 (insulin-dependent) diabetes. Exposure of a β-cell line (βTC1) to IL-1β resulted in an increase of preproinsulin mRNA at 0.5 h followed by a gradual decrease. Tumor necrosis factor-α (TNF-α) mRNA expression by βTC1 cells was demonstrated 1-3 h after the addition of IL-1β. TNF bioactivity was detected in homogenates of βTC1 cells exposed to IL-1. The supplementation of cycloheximide (CHX) together with IL-1β resulted in the superinduction of TNF-α mRNA, suggesting that de novo protein synthesis is not required in IL-1-induced TNF-α mRNA expression. Endogenous TNF-α of β-cells may be involved in the islet lesion of type 1 diabetes.

There is much evidence to suggest that cytokines are involved in pancreatic β-cell destruction in Type 1 (insulin-dependent) diabetes. IL-1 at low concentrations enhances insulin release by increasing proinsulin biosynthesis (1-3), whereas at higher concentrations and during prolonged incubation it exerts a toxic effect on islet cells (1-9). Although the mechanism of unique bimodal action of IL-1 has not been clarified, it has been shown that the inhibition of insulin secretion by IL-1 requires gene transcription within the first hour of exposure to IL-1, followed by the synthesis of a peptide(s) (10).

Abbreviations: IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; CHX, cycloheximide; IFN, interferon; MHC, major histocompatibility complex.

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TNF-α is a candidate for the peptide expressed in early phase, since IL-1 and TNF-α share several common actions in islet cells, and IL-1 induces the production of TNF-α by macrophages. TNF-α has been shown to potentiate β-cell cytotoxicity of IL-1 (11). Furthermore, the combination of TNF-α and interferon-γ (IFN-γ) damages islet cells (12,13) and induces major histocompatibility complex (MHC)class II antigen expression (14,15). In this study, we showed using a β-cell line that TNF-α production is an early event in IL-1 actions preceding the suppression of preproinsulin gene transcription.

MATERIALS AND METHODS

Cell lines and cytokines

A β-tumor cell line (βTC1) (16,17) was kindly provided by Dr. K. Hamaguchi (Medical Collage of Oita, Oita, Japan), and maintained in RPMI 1640 medium (Gibco, NY) supplemented with 10% heat-inactivated fetal bovine serum and 20 mg/ml of gentamicin sulfate in 75 cm^2 or 25 cm^2 tissue culture flasks at 37 ℃. Recombinant human IL-1β (hIL-1β, 2×10^7 U/mg) and recombinant mouse IL-1α (mIL-1α, 8×10^8 U/mg) were provided by Otsuka (Tokushima, Japan) and Genzyme (MA), respectively.

DNA probes

Mouse TNF-α cDNA (18) was a gift from Genentech (CA). A 1,100 bp EcoRI fragment of mouse TNF-α in the plasmid was cloned in a pBR322-derived vector. Mouse preproinsulin cDNA was given by Dr. S. Efrat (Albert Einstein School of Medicine, NY), and mouse β-actin cDNA (19) was provided by Dr. S. Sakiyama (Chiba Cancer Center Research Institute, Chiba, Japan). The probes were labeled with [α-^{32}P] dCTP using a random primed DNA labeling kit (Boehringer Manheim, Germany).

Northern blot analysis

Total RNA was isolated from confluent βTC1 cells in 75 cm^2 flasks by the method of Chomczynski et al (20). Twenty micrograms of total RNA was denatured with 17.5% formaldehyde and 50% formamide, and electrophoresed through 1% agarose gels containing formaldehyde, and transferred onto nylon membranes (Schleicher & Schuell, NH). After prehybridization at 65 ℃ for 30 min, the membranes were hybridized with [^{32}P] dCTP-labelled cDNA probes using Rapid Hybridization Buffer (Amersham, UK) for 2 h at 65 ℃. After washing with standard saline citrate and sodium dodecyl sulfate at 68 ℃, the membranes were exposed to X-OMAT AR film (Kodak, NY) with an intensifying screen at -80 ℃ for 5 h to 14 days. The autoradiograms were quantitatively analyzed by densitometric scanning (Shimadzu, Kyoto, Japan). CHX was purchased from Wako, Osaka, Japan.

Bioassay of TNF

After culture in the absence or presence of IL-1, aliquots of the cell-free supernatants of confluent βTC1 cells in 25 cm^2 culture flasks were harvested. βTC1 cells were resuspended with trypsin-EDTA. After washing with RPMI-1640, the cells were destructed in 1 ml of phosphate buffered saline with a sonifier (Branson, CT), and centrifuged at 6,000 g. Supernatants of cell homogenates and medium samples were applied to the standard L929 cell cytotoxicity assay. The detection limit of the assay is approximately 0.1 U/ml.

RESULTS

TNF-α mRNA expression induced by IL-1

To determine the time course of TNF-α gene transcription, βTC1 cells were incubated in the presence of hIL-1β at the doses of 5, 20, or 50 U/ml for up to 24 h (Fig. 1). TNF-α mRNA was not detected by the Northern blot analysis in cells cultured without the addition of hIL-1β. Exposure to the cytokine for 0.5 h resulted in slight expression of TNF-α mRNA by βTC1 cells; the maximal level of TNF-α mRNA was obtained at 1-3 h at any doses of hIL-1β used here. Longer exposure to hIL-1β showed the attenuation of TNF-α mRNA expression, although significant amounts of TNF-α mRNA were observed until 24 h.

To assess the optimum dose of hIL-1β to induce TNF-α, we next cultured βTC1 cells in the presence of hIL-1β at the concentrations of 0.1 to 200 U/ml, and mIL-1α at the concentrations of 0.1 to 20 U/ml (Fig. 2) for 3 h. Detectable TNF-α gene transcription was caused by 0.5 U/ml of hIL-1β, and the expression increased dose-dependently between 0.5 and 5 U/ml (Fig. 2a). In the presence of mIL-1α, Northern blot analysis showed detectable TNF-α mRNA at the dose of 1 U/ml, and the peak value was observed at the dose of 10 U/ml (Fig. 2b).

Effect of hIL-1β on preproinsulin mRNA levels

TNF-α mRNA of βTC1 cells incubated with 5 U/ml of hIL-1β was detected by Northern blot analysis. The membranes were then stripped off the TNF-α probe and reprobed for preproinsulin mRNA (Fig. 3). This experiment was repeated twice with essentially the same results. Densitometer scanning of the bands was carried out, and the means of preproinsulin/β-actin mRNA

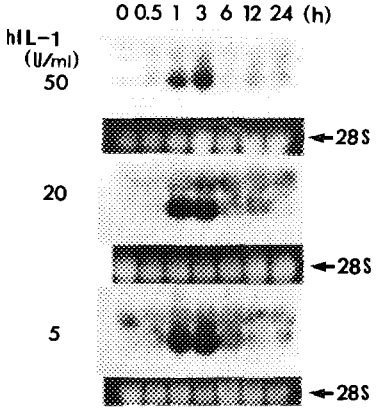

Fig. 1. Northern blot analysis of TNF-α mRNA expression by βTC1 cells induced by hIL-1β; 20 μg of total RNA was applied in all lanes. Cells were incubated in the presence of hIL-1β at the doses of 5, 20 and 50 U/ml for 0-24 h. Autoradiograph exposed for 7 days.

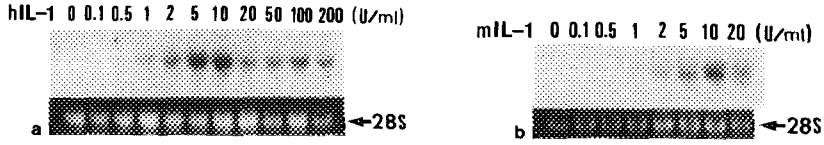

Fig. 2. Dose-responsiveness of TNF-α mRNA induction by hIL-1β (a) or mIL-1α (b). βTC1 cells were cultured for 3 h in the presence of IL-1. Northern blot analysis was performed to detect TNF-α mRNA; 20 μg of total RNA was applied in all lanes, and autoradiograph exposed for 7 days (a) or 14 days (b).

ratios in two experiments are presented in Fig. 4. We have observed maximal increase of preproinsulin mRNA for 0.5 h, and clear decrease of preproinsulin mRNA for 12 h of treatment with hIL-1β. The maximal value of preproinsulin mRNA was observed 0.5 h after the addition of hIL-1β, followed by a gradual decrease to the nadir at 12 h.

Detection of TNF-α bioactivity

TNF bioactivity was detected in cell homogenates 1 h after the addition of 5 U/ml of mIL-1α or hIL-1β (Fig. 5). At 3 h the bioactivity reached the maximal levels; 0.33 U/10^7 cells by hIL-1β, and 0.18 U/10^7 cells by mIL-1α. Intracellular TNF decreased to undetectable levels within 6 h. TNF activity was not detected in culture medium 1, 3, or 6 h after the supplementation of hIL-1β.

Effect of CHX on TNF-α gene transcription

CHX was added to the medium of βTC1 at the concentration of 10 mM 15 min before the supplementation of 5 U/ml hIL-1β. No TNF bioacitibity was detected in βTC1 homogenates after 3-h exposure to IL-1β. However, the level of TNF-α mRNA was rather elevated in CHX supplemented cultures (Fig. 6). CHX by itself induced slight expression of TNF-α mRNA.

Fig. 3. Effect of hIL-1β (5 U/ml) on the expression of preproinsulin mRNA (A). Same blots were rehybridized to β-actin probe (B). Essentially the same results were obtained in 2 separate experiments.

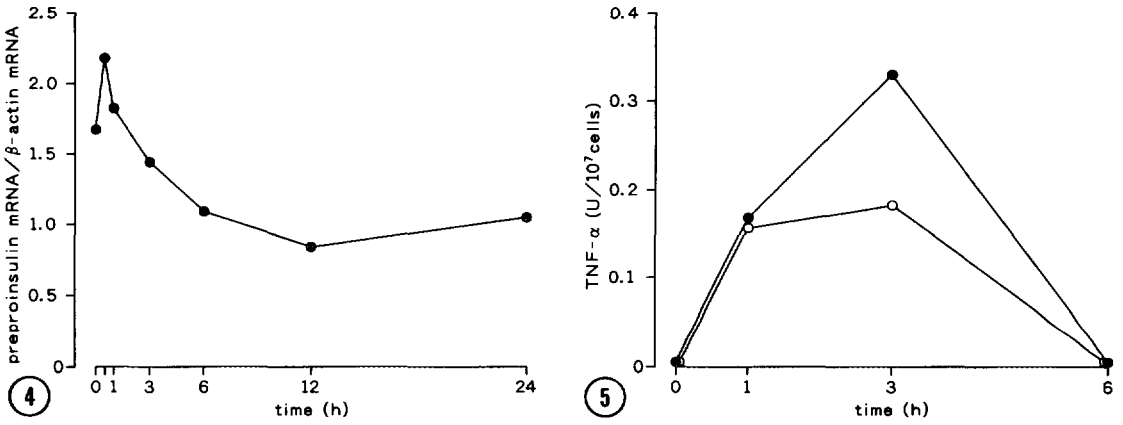

Fig. 4. Preproinsulin mRNA / β-actin mRNA ratios quantitated by scanning densitometry (ratio of OD units). Study conditions were described in the legend of Figure 2.

Fig. 5. TNF levels determined by bioassay in βTC1 cells induced by 5 U/ml of mIL-1α (o) or hIL-1β (●).

DISCUSSION

We demonstrated in this study that bioactive TNF-α was produced by βTC1 cells incubated with hIL-1β or mIL-1α. Although βTC1 is an insulinoma cell line derived from transgenic mice, it has been shown that the exposure of βTC1 to cytokines resulted in the responses similar to those of normal murine islet cells. IL-1 and IFN-γ inhibit DNA synthesis of βTC1 (17). IFN-γ augments the constitutive expression of MHC class I antigens, and induces the ectopic expression of class II molecules (17).

In this study βTC1 cells also showed bimodal responses in preproinsulin mRNA when exposed to IL-1; following the peak at 0.5 h, preproinsulin mRNA decreased below the initial level at 3 h. TNF-α bioactivity and mRNA expression were observed 1 h after the addition of IL-1β, and reached the maximal levels at 3 h. Although TNF bioactivity was not detected in

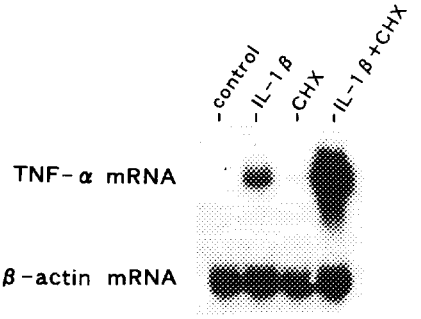

Fig. 6. Effect of CHX (10 mM) on TNF-α gene transcription in βTC1 cells induced by 5 U/ml of IL-1β.

culture medium of βTC1 cells stimulated by IL-1, there is a possibility that intracellular TNF-α works toxic to the very TNF-α producing cells, because it was reported that TNF-α-sensitive L929 fibroblasts were killed by the microinjection of TNF-α (21). TNF-α may act intracellularly without ligand-induced receptor activation at the cell surface, probably through the interaction with internalized IL-1 receptors.

Campbell et al. (22) reported that mouse islet cells and a rat insulinoma cell line RIN-m5F cells exposed to the combination of TNF-α and IFN-γ in vitro produced interleukin-6. Foulis et al. (23) showed immunoreactive IFN-α in β-cells in human type 1 diabetic pancreases, suggesting that chronic viral infection may underlie the pathogenesis of type 1 diabetes. However, IFN-α has little effect on β-cells in vitro. Since Higuchi et al. (24) recently reported that mice bearing a TNF-α transgene controlled by an insulin promotor developed severe insulitis, endogenous TNF-α expression may be involved in the progression of autoimmune insulitis.

Induction of TNF-α mRNA by IL-1 was independent of de novo protein synthesis, since CHX did not prevent expression of the gene. The superinduction of TNF-α mRNA by CHX may be attributable to the increased transcription of the gene and/or stabilization of TNF-α mRNA. Furthermore, CHX alone slightly induced the expression of TNF-α mRNA. CHX-induced TNF-α mRNA expression has been reported in various immune cells including macrophages (25, 26), T lymphocytes (27) and astrocytes (28). CHX may prevent the degradation of TNF-α mRNA by inhibiting the synthesis of a labile protein in βTC1 cells.

These observations showed that βTC1 cells have potential to produce TNF-α. The endogenous TNF-α of pancreatic β-cells may be involved in the progression of islet lesion in type 1 diabetes.

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