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Transcription of Class II MHC Gene by Interferon-γ in FRTL-5 cells[1]

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The intracellular mechanism by which interferon-γ induces the expression of class II major histocompatibility complex (MHC) antigen in nonlymphoid cells is not clear. The effect of recombinant rat interferon-γ (IFN-γ), and cycloheximide on the expression of class II MHC gene was studied using the techniques of immunocytochemical staining and northern blot analysis. IFN-γ induced de novo transcription of class II MHC gene and class II MHC antigen expression on the cell surface. Cycloheximide did not inhibit IFN-γ-induced class II MHC antigen expression in a dose-dependent manner indicating translational blockade. These results suggest that IFN-γ induces class II MHC antigen expression via de novo transcription of class II MHC gene leading to synthesis of new class II MHC molecule.

Key Words: *interferon-γ, class II MHC, transcription, cycloheximide.*

INTRODUCTION

Interferon-γ has been reported to induce aberrant expression of class II major histocompatibility complex (MHC) antigen in the cells usually not associated with immunological reactions such as thyrocytes (Hanafusa et al., 1983: Piccinini et al., 1987). Some investigators hypothesized that these cells expressing class II MHC antigen on their surface might present self-antigens to T lymphocytes causing autoimmunity (Bottazzo et al., 1983: Londei et al., 1985) but conflicting results have also been reported (Burkly et al., 1989: Schwartz, 1990). The detailed intracellular mechanism by which interferon-γ induces the aberrant expression of class II MHC antigen is also far from clear. Lee et al., (1991) reported that cycloheximide inhibited interferon-γ-induced class II MHC antigen expression in cultured rat thyroid cells. However, the control of transcription and translation of class II MHC gene by interferon-γ cycloheximide has not been widely studied in nonlymphoid cells (Neufeld and Davies, 1988).

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This study was performed to study the control of transcription and translation of class II MHC gene in a functioning rat thyroid cell line, i.e., FRTL-5 cells.

MATERIALS AND METHODS

Cell culture

FRTL-5 cells (kindly provided by Dr. Kohn, National Institute of Health, Bethesda, MD, USA) were cultured using a modification of the original method described by Ambesi-Impiombato et al. (Ambesi-Impiombato et al., 1980). In brief, the cells were cultured with Coon's modified Ham's F-12 medium containing 1 mU/ml bovine TSH (Armour, Kankakee, IL, USA), 10g/ml insulin (Nordisk, Gentofte, Denmark), 5 g/ml transferrin (Sigma, St. Louis, MO, USA). 10nM hydrocortisone (Sigma), 10ng/ml somatostatin (Sigma), 100 U/ml penicillin and 100 g/ml streptomycin (Sigma), 15 mM HEPES (Sigma), and 5% calf serum (Sigma) (6H medium) in a humidified atmosphere of 5% CO_2 at 37°C. Detached cells were plated in flat-bottomed 96-well culture plates (Flow, McLean, VA, USA) for immunocytochemical staining or in 100 mm culture dishes (Green Cross, Seoul, Korea) for RNA extraction.

Staining of class II MHC antigen with immunocytochemistry

The 6H medium in the 96-well plates of culture dishes

was changed to one containing recombinant rat interferon-γ (IFN-γ) (kindly provided by Dr. van der Meide, TNO Primate Center, Rijswijk, The Netherlands) and/or cycloheximide (Sigma) 3 days after plating. After 5 additional days of incubation, immunocytochemical staining of the viable cells was performed in the wells in situ using streptavidin-biotin-peroxidase complex kits (Biogenex, San Ramon, CA, USA) as described by Lee et al. (1991). In brief, OC-6 monoclonal antibody which is specific to one of the rat class II MHC antigen (RT1. B) (kindly provided by Dr. Mason, MRC Cellular Immunology Unit, Sir Williams Dunn School of Pathology, Oxford, UK) was applied to each well. The wells were washed with phosphate buffered saline (PBS) (Sigma) after incubation for 60 min at 37°C, and biotinylated anti-mouse immunoglobulin G was added. The wells were washed with PBS again after incubation for 30 min at 37°C, and peroxidase-labeled streptavidin solution was applied. The wells were washed with PBS and color reaction was developed with 3-amino-9-ethylcarbazole solution. Then the wells were observed under an inverted microscope (AO, Buffalo, NY, USA), and the percentage of the cells with intense purple color was calculated.

RNA Extraction

Total RNA was extracted from cultured cells using a modification of the original method described by Gilsin et al., (1974). In brief, 2 ml of 4 M guanidium thiocyanate (Boehringer Mannheim Biochemicals, Indianapolis, IN, USA) homogenization buffer was applied to each confluent culture dish. The lysate was layered onto a cushion of 5.7 M CsCI (Sigma), 0.01 M EDTA (Sigma) (pH 7.5). Then ultracentrifugation was done at 32,000 rpm (rotor type SW 41), 20°C for 24 hrs. The pellet was dissolved in appropriate volume of TE solution containing 0.1% SDS (pH 7.6) Bethesda Research Laboratories, Gaithersburg, MD, USA). RNA was precipitated with addition of 0.1 volume of 3M sodium acetate and 3 volume of ice-cold ethanol. The precipitated RNA was washed with 70% ethanol after centrifugation at 12,000 G, 4°C for 10 min and was stored in ethanol at −70°C until use.

DNA Probe

Transformation of E. Coli was done using a pUC 19 plasmid containing RTI. D (one of the rat class II MHC genes) insert kindly provided Dr. Davies, Mount Sinai Medical Center, New York, NY, USA) on BL medium/agar/ampicillin plates according to a method previously described (Davis et al., 1986). Transformed E. coli was cultured in 60 ml Circlegrow Medium (BIO 101, Inc. La Jolla, CA, USA) and plasmid DNA was extracted employing a kit (Circleprep, BIO 101, Inc., La Jolla, CA, USA.) Extracted plasmid DNA was linearized with HindIII (Bethesda Research Laboratories) and radiolabelled using a multiprime random labelling kit (Amersham, UK) and 32p-dCTP (Du Pont, Wilmington, DE, USA). Finally it was purified with Sephadex G-50 columns (Quick Spin Column, Boehringer Mannheim Biomedicals, Mannheim, Germany).

Hybridization

Stored RNA was reprecipitated and dissolved in dihydroethyl pyrocarbonate (Sigma)-treated distilled water. Then hybridization of RNA with the radiolabelled RTI. D probe was done according to a method previously described (Ausubel et al., 1987). In brief, electrophoresis of the RNA was done in 1.2% agarose/formamide denaturing gel at 100V for 3 hrs. Then denatured RNA in the gel was transferred to a nylon membrane (Schleicher & Schuell, Keene, NH, USA). The nylon membrane was baked for 2 hrs in a vacuum oven at 80°C before prehybridization and hybridization overnight at 42°C. Included in the hybridization solution were radiolabelled probe of 500,000 cpm/ml, 25 mM KPO4 (pH 7.4), 5×SSC, 5×Denhardt's solution, 100 g/ml salmon sperm DNA (Sigma), 50% formamide (Bethesda Research Laboratories) and 2% dextran sulfate (Pharmacia, Uppsala, Sweden). The nylon membrane was washed in 0.1×SSC with 0.1% SDS 8 times for 15 min each at 65°C and exposed to X-ray film in the presence of intensifying screens at −70°C. Densitometry was done on autoradiograms using a scanning densitometer (Helena Lab., Beaumont, TX, USA). A β-actin cDNA probe, which was used as a control, was labelled also using a multiprime random labelling kit (Amersham, UK).

RESULTS

Effect of cycloheximide on IFN-γ-induced class II MHC antigen expression

RT1. B antigen, which was not detected in the basal state (6H medium alone), was induced in 88.0±3.3% (mean ±SD: n=3) of FRTL-5 cells cultured for 5 days in the 6H medium containing 100 Uml IFN-γ. When cycloheximide of 0.01, 0.1, 1, and 10 g/ml was added to the 6H medium containing 100 U/ml IFN-γ, 77.5±8.9, 10.9±9.0, 3.0±3.6, and 0% of the cells were RT1.B antigen-positive, respectively, showing a dose-dependent inhibition of IFN-γ-induced RTI. B expression by cycloheximide (Lee et al., 1991).

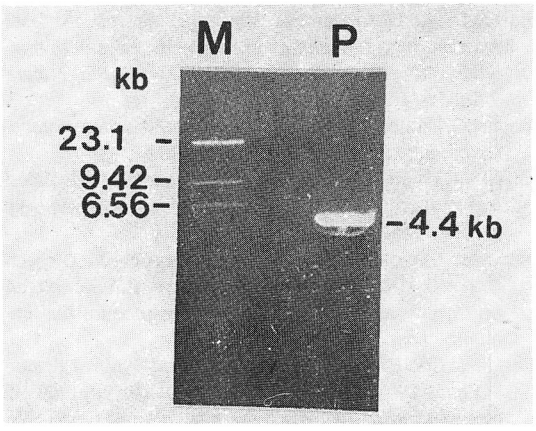

Fig. 1. Ethidium bromide staining of minigel for the extracted plasmid (Lane P) with the HindIII digestion of lamda DNA (M). The size of 4.4 kb was equal to the sum of the sizes of the RTI.D insert and pUC 19 plasmid.

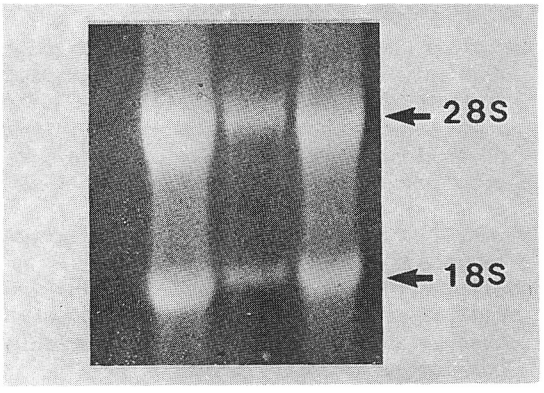

Fig. 2. Ethidium bromide staining of RNA after denaturing gel electrophoresis. Distinct 28S and 18S bands indicated the good quality of the extracted RNAs.

Transcription of class II MHC gene by IFN-γ

The size of the HindIII-linearized plasmid extracted from transformed E. coli was 4.4 kb in a minigel running, which was equal to the sum of the sizes of the RTI.D insert and pUC 19 plasmid (Fig. 1). Ethidium bromide (Boehringer Mannheim, Mannheim, Germay) staining of RNA after denaturing gel electrophoresis showed distance 28S and 18S ribosomal RNA bands, which represented the good quality of the extracted RNA (Fig. 2).

Northern blot analysis of RNA showed RTI. D transcript of 1.4 kb in the RNA from IFN-γ treated cells, while no such transcript was observed in the RNA from the cells cultured in 6H medium only. The RTI. D transcript was also observed in the RNA from the cells cultured with 100 U/ml IFN-γ and 10 g/ml cycloheximide. The intensity of the RTI. D transcript from the cells cultured with both IFN-γ and cycloheximide seemed to be weaker than that from the cells cultured with IFN-γ only; however, the ratio between the 2 bands (about 4:1) was similar to that between the intensities of the β-actin transcript of the same 2 lanes judging from the scanning densitometry (Fig. 3).

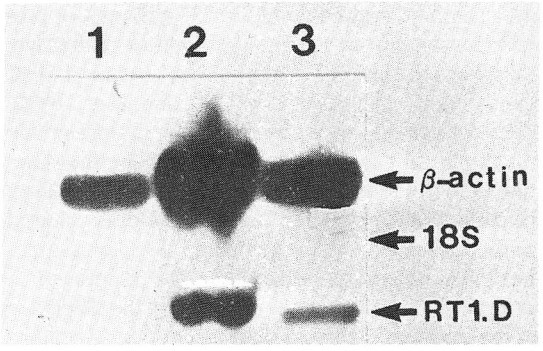

Fig. 3. The intensity of the RTI. D transcript after northern hybridization. RTI. D transcript of 1.4 kb was not visible in the control state (Lane 1): however, it was visible in the IFN-γ-treated state. The ratio between the intensity of RTI. D transcript from IFN-γ-treated cells (Lane 2) and that from cells treated with IFN-γ and cycloheximide (Lane 3) was about 4:1, which was similar to the ratio between the intensities of the β-actin transcript of the same 2 lanes judging from the scanning densitometry.

DISCUSSION

De novo protein synthesis seems to be necessary for IFN-γ-induced RTI. B expression in FRTL-5 cells because cycloheximide inhibited it in a dose-dependent manner. Moreover, IFN-γ-induced de novo transcription of class II MHC gene which could leads to synthesis of new class II MHC molecules. These data on the transcription of class II MHC gene by IFN-γ are similar to those reported by Neufeld et al. (1988) although stringency of hybridization was different between their study and ours. It is not yet known how IFN-γ induces de novo transcription of class II MHC genes. Blanar et al. (1989) reported that interferon-γ-induced a DNA-binding factor, IBP-1, which binded to the interferon response sequence (IRS) located upstream of the class I MHC structural gene. No such data has been reported in the induction of class II MHC gene by interferon-γ, however, intracellular machinery might work.

Although cycloheximide is primarily an inhibitor of

translation, it might also block the synthesis of proteins having a positive or negative effect on the synthesis and/or turnover of the mRNA of class II MHC genes (Woodward et al., 1989; Makino et al., 1984). Therefore, there was a possibility that cycloheximide might alter the level of class II MHC gene transcription by IFN-γ. However, the difference in the intensity of class II MHC transcript between the cells cultured in the presence of IFN-γ alone and IFN-γ plus cycloheximide seems to reflect only the difference in amount of the loaded RNA considering the difference in the intensity of the β-actin transcript. So cycloheximide seems to inhibit IFN-γ-induced class II MHC antigen expression not by transcriptional but by translational control. The present data suggest that IFN-γ induces class II MHC antigen expression via de novo transcription of class II MHC gene leading to synthesis of new class II MHC molecule.

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