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RELATIONSHIP BETWEEN DEPRESSION OF HBsAg PRODUCTION AND DNA SYNTHESIS BY INTERFERONS IN HUMAN HEPATOMA CELL LINE

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Summary. — The influence of different interferons (IFNs) on HBsAg production and DNA synthesis was studied in PLC/PRF/5 cells using 30 I.U./ml of natural HuIFN-α, 25 I.U./ml of recombinant HuIFN-α_2, and 5 I.U./ml of natural murine IFN-α/β. All three IFN types inhibited significant inhibitory effect on HBsAg production during the second 24 hr-interval following their addition. After 96 hr HBsAg production had returned to normal levels. Natural HuIFN-α clearly depressed cellular DNA synthesis 24 hr after IFN addition which returned to normal within the next 24 hr. Recombinant HuIFN-α_2 influenced DNA synthesis only slightly and the mouse IFN-α/β showed no effect.

Key words: *interferons*; *hepatoma cells*; *HBsAg inhibition*; *DNA synthesis*

Introduction

IFNs with their large spectrum of activities have apparently an important role in the pathogenesis of viral infections in the organism as well as in regulations of cellular functions. Hepatitis B infection is a world-wide problem. It is still not clear what role IFN plays in its pathogenesis and to what extent and by which mechanism may IFN influence HBV (hepatitis B virus) replication. Increased levels of serum IFN were found in cases of acute hepatitis A and B (Levin *et al.*, 1982; Davis *et al.*, 1984). On the other hand, in patients with chronic course of hepatitis B infection, the level of IFN is decreased or below detection level (Poitrine *et al.*, 1985; Zachoval *et al.*, 1988). Chronic hepatitis B infection is directly related to the development of hepatocellular carcinoma (Bréchot, 1987).

In the present report we followed the effect of different IFNs on HBsAg production and DNA synthesis in one type of hepatocellular cells, i.e. in the PLC/PRF/5 cell line.

Materials and Methods

Cells. Human hepatoma cell line PLC/PRF/5 was cultured in plastic 96-well tissue culture microplates in Eagle's essential medium supplemented with 5% heat inactivated calf serum and antibiotics. The mouse LX cell line from the Imperial Cancer Research Fund Laboratories,

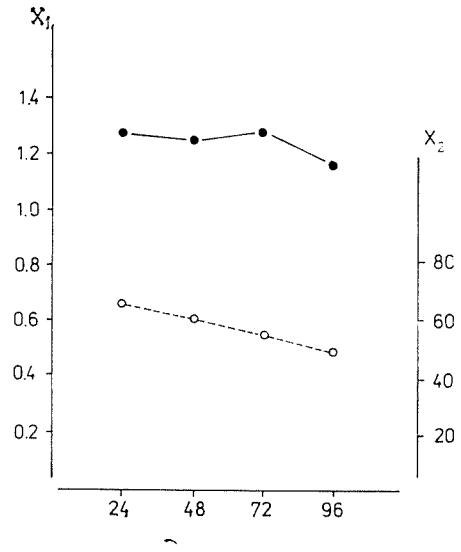

Fig. 1.
Extracellular production of HBsAg and DNA-synthesis in PLC/PRF/5 control cells
Abscissa: time in hours
X_1 — levels of HBsAg in optical density units ●——●
X_2 — ^{3}H-thymidine uptake × 10^3 cpm ○-----○

London, was used for IFN production. LX cells were maintained also in Eagle's essential medium supplemented with 5% heat inactivated calf serum and antibiotics.

Interferons. Semipurified natural HuIFN-α was prepared as described previously (Fuchsberger *et al.*, 1979). Recombinant HuIFN-α_2 (REAFERON) was obtained from the Ivanovsky Institute of Virology in Moscow, U.S.S.R. Mouse natural IFN was induced in LX cells by means of NDV and was partially purified by column SP-Sephadex G-25 chromatography as described previously (Fuchsberger *et al.*, 1975).

HBsAg determination. Culture supernatants were assayed for HBsAg presence using commercially available ELISA kits (Sevatest HBsAg ELISA micro, SEVAC Praha) as previously described (Stanček *et al.*, 1987).

Cell growth inhibition assay. Confluent monolayers of PLC/PRF/5 cells were treated with IFNs for 24 hr. Culture medium was replaced every day with a fresh one. At the chosen intervals ^{3}H-thymidine was added to control and IFN-treated cells in an amount of 37 kBq/well. After 4 hr incubation the plates were frozen and thawed, the materials were transferred on a strip of fiberglass filter paper (Whatman GF/B) using a domestic harvester, washed with 10-fold volume of PBS and dried. The strip of paper was cut into pieces containing one material each. The pieces were transferred to scintilation solution and the radioactivity was measured in a Packard counter.

Results

Influence of different IFNs on HBsAg production and DNA synthesis in PLC/PRF/5 cells

Four plastic microplates and 15×10^3 PLC/PRF/5 cells per well were used in each experiment. Following 3 days incubation at 37 °C the culture media were changed for fresh ones and different IFNs were added. Each microplate contained control (untreated) cells, cells treated with either 30 I.U./ml of natural HuIFN-α or 35 I.U./ml of recombinant HuIFN-α_2, or with 5 I.U./ml of natural HuIFN-α/β. IFN activities were estimated by titration

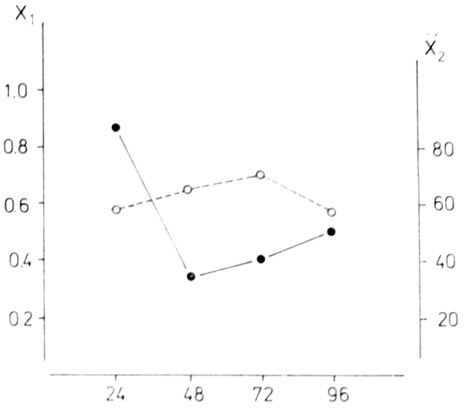

Fig. 2.
Effect of natural HuIFN-α on HBsAg production and DNA-synthesis in PLC/PRF/5 cells
Abscissa: time in hours
X_1 — levels of HBsAg in optical density units ●——●
X_2 — ^{3}H-thymidine uptake × 10^3 cpm ○-----○

in PLC/PRF/5 cells according to international standard procedures. The activity of mouse IFN in homologous mouse cells was 5×10^4 I.U./ml. After 24 hr incubation, medium samples were taken for HBsAg evaluation. ^{3}H-labelled thymidine was added then to the wells of the first microplate for tracing DNA synthesis. To the remaining 3 plates fresh IFN-free medium was added. This procedure was repeated each day until the last plate was used, i.e. 96 hr after IFN addition.

The results are shown in Figs. 1—4. Almost identical data were obtained in the three successive experiments. Each plotted point on the curves represents an average value from 5 parallels. Fig. 1 represents production of HBsAg and DNA synthesis in control untreated cells. The amount of HBsAg was almost unchanged, the DNA synthesis was slightly decreased. No significant difference was seen between the HBsAg levels before and 24 hr after IFN addition. Fig. 2 demonstrates significant HBsAg inhibition during the second 24 hr-interval after natural IFN-α treatment. The level of *s* antigen has returned to normal after 96 hr. On the other hand, inhibition of

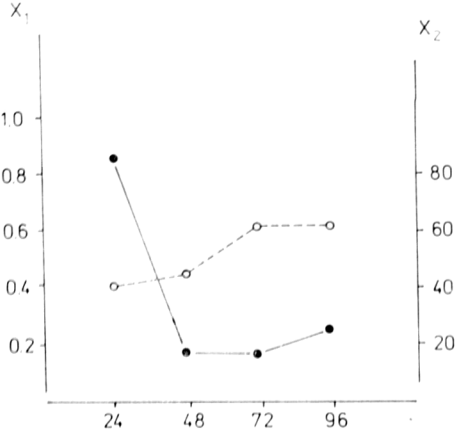

Fig. 3.
Effect of recombinant HuIFN-α_2 on HBsAg production and DNA-synthesis in PLC-PRF/5 cells
Abscissa: time in hours
X_1 — levels of HBsAg in optical density units ●——●
X_2 — ^{3}H-thymidine uptake × 10^3 cpm ○-----○

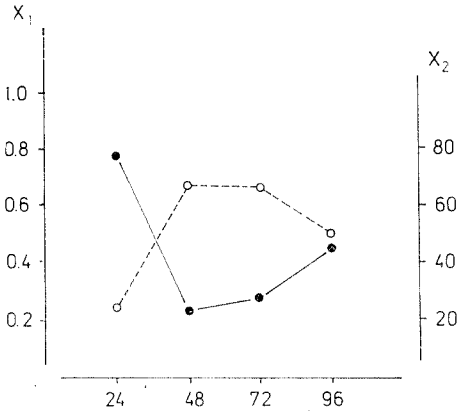

Fig. 4.
Effect of natural HuIFN-α/β on HBsAg production and DNA-synthesis in PLC/PRF/5 cells
Abscissa: time in hours
X_1 — levels of HBsAg in optical density units ●——●
X_2 — ^{3}H-thymidine uptake × 10^3 cpm ○·····○

DNA synthesis is the most expressed 24 hr after IFN addition. Recombinant IFN-α_2 showed similar influence on HBsAg production as the natural HuIFN-α (Fig. 3), however, its influence on DNA synthesis was negligible. Mouse IFN-α/β had the same effect on PLC/PRF/5 cells as human IFNs (Fig. 4) except on DNA synthesis which was not apparently influenced.

Discussion

In our previous report (Hajnická *et al.*, 1987) we described the significant influence of human leukocyte IFN-α on HBsAg production by PLC-PRF/5 cells in both fast growing and confluent stationary monolayer cultures. These results are in a certain disagreement with other reports (Desmyter *et al.*, 1981; Manzin *et al.*, 1986; Ren *et al.*, 1988). A possible explanation for the controversy is our finding of the maximal HBsAg inhibition at 24—48 hr after IFN treatment. Other authors checked HBsAg production only 3—5 days after IFN treatment. At that time the amount of the antigen produced during the first and the last days apparently overlaps the period of maximal HBsAg inhibition since its accumulation over certain level is limited (Desmyter *et al.*, 1981).

In the present report we attempted to find out whether the inhibitory effect of IFN on HBsAg production is specific or it is a mere consequence of its antiproliferative activity. HBsAg production and DNA synthesis in PLC/PRF/5 cells were therefore simultaneously checked after cell treatment with either HuIFN-α or recombinant HuIFN-α_2 or natural MuIFN-α/β. Our decision to check mouse IFN activity comes from Shouval's report (Shouval *et al.*, 1983) on the role of IFN and NK cells in tumourigenicity of human hepatoma cells in nude mice. Though mouse IFN showed no anti-VSV activity in human embryonic cells, it was slightly active in PLC/PRF/5 cells. This effect was several times higher, however, in homologous mouse system (1 : 10 000).

All IFN types used in this work showed an inhibitory effect on HBsAg production but differed significantly in their influence on DNA synthesis.

The most expressed inhibition was seen after natural HuIFN-α treatment. Though it may not be excluded that excess proteins or other molecules present in the semipurified natural IFNs may be responsible for this effect. By 24—48 hr after the IFN treatment the DNA synthesis was returning to the normal levels while HBsAg production reached it much later. Only slight DNA inhibition could be seen after recombinant HuIFN-α_2 treatment and none with HuIFN-α/β.

Based on our data it may be concluded that the inhibition of HBsAg synthesis is not due to cell growth inhibition. Though it is still possible to anticipate a minute role of antiproliferative activity of IFN, the ability of IFN-treated PLC/PRF/5 cells to recognize the changes taking place after viral and cellular DNA integration is highly probable.

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