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SYNTHESIS OF 23 K ACUTE-PHASE PROTEIN BY HBV GENOME CARRYING PLC/PRF/5 HUMAN HEPATOMA CELLS

V. HÁJNICKÁ[1], N. FUCHSBERGER[2], D. STANČEK[1], A. VOLNÁ[1], L. ČERVEŇÁKOVÁ[1]

[1]Institute of Preventive and Clinical Medicine, 833 01 Bratislava; and [2]Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Czechoslovakia

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Summary. – Elevated synthesis of 23 K protein by human hepatoma PLC/PRF/5 cells was observed after their treatment with conditioned medium from concanavalin A stimulated peripheral-blood monocytes. Increased amount of this protein was first determined 4 hr after the treatment and its maximal level was reached 48 hr later. The role of the 23 K protein remains so far unknown.

Key words: cytokines; acute-phase proteins; hepatoma cells; PLC/PRF/5 cells

Introduction

Cytokines are involved in the immune inflamatory and other mechanisms (Trotta, 1991). Tissue injury or infection usually lead to increased production of certain plasma proteins known as acute-phase proteins. The major source of these proteins is liver. Cytokines that mqdulate the synthesis of acute-phase proteins are mainly IL-1, IL-6, tumour necrosis factor (TNF), transforming growth factor (TGF) and interferon-gamma (Andus *et al.*, 1988; Mackiewicz *et al.*, 1990; Mackiewicz *et al.*, 1991; Magielska-Zero *et al.*, 1988). Mazuski with coworkers described synthesis of 23 K murine hepatocyte secretory protein by *in vivo* induction with endotoxin or after *in vitro* stimulation by lipopolysaccharides, glucocorticoides, IL-1 and IL-6 (Mazuski *et al.*, 1988; 1989). However, they were not able to identify this proteins in human model (Bankey *et al.*, 1990).

We studies the effect of cytokines secreted after concanavalin A (ConA) stimulation by normal peripheral blood mononuclear (PBM) cells in the human hepatoma cell line PLC/PRF/5.

Materials and Methods

Cell cultures. The human hepatoma cell line PLC/PRF/5 (Alexander *et al.*, 1976) was obtained from the Ivanovsky Institute of Virology, AMS, Moscow. The cells were cultured in Legroux flasks in Eagle's essential medium supplemented with 5 % heat inactivated calf serum and antibiotics.

PBM cells were obtained by modification of Böyum's method (Böyum, 1976) with Ficoll-Hypaque

density gradient centrifugation of heparinized blood samples of normal volunteers. For ConA stimulation 10^6 PBM cells/ml were incubated for 48 hr in RPMI 1640 medium supplemented with 10 % of heat-inactivated human serum, antibiotics and 20 μg/ml of ConA.

PLC/PRF/5 induction. PLC/PRF/5 cells were treated with the supernatants of ConA stimulated PBM cells for the required interval. The cells were then washed three times with phosphate buffered saline and lysed in buffer containing 1 % Triton X-100, 0.5 % sodium deoxycholate and 0.1 % sodium dodecyl sulphate and centrifuged at 20 000 x g for 30 min.

Soluble proteins were analysed by a modified SDS-polyacrylamide gel electrophoresis method (Laemli, 1970). It includes polyacrylamide slab gel gradient (7.5–15 % acrylamide). After electrophoresis the gels were silver stained.

Results and Discussion

Fig. 1 shows the protein patterns of control cells and cells treated for 24 hr with supernatants obtained from ConA stimulated PBM cells. Clear stimulation of the production of a 23 K protein was observed after the treatment. The time course of the 23 K protein induction in PLC/PRF/5 cells is documented in Fig. 2. Electrophoresis profiles were compared 6, 24, 48, 72 and 96 hr after the cytokine mixture addition. An elevated level of the 23 K protein was first detected 4 hr after the treatment (data not shown) and its maximal level was reached after 48 hr.

We consider the 23 K protein as cytoplasmic since it could be detected in cell homogenates after removing cell membranes. A protein of this molecular

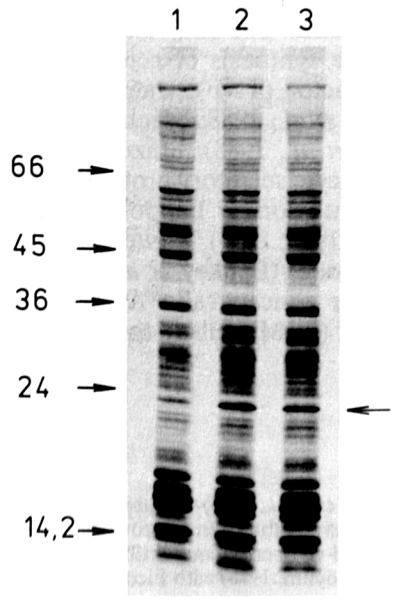

Fig. 1
Stimulation of 23 K protein synthesis in PLC/PRF/5 cells with cytokines by ConA stimulated PBM cells
Protein profiles of control cells (lane 1) and cells treated for 24 hr with two different cytokine preparations (lanes 2, 3). The left arrows indicate the position of the size markers (M_r values). The right arrow indicates the 23 K protein.

weight was first described by Mazuski and coworkers as secretory protein produced by mouse hepatocytes (Mazuski *et al.*, 1988; 1989). Our results confirm the presence of similar protein in human hepatoma cells by using supernatants of ConA stimulated PBM cells containing among other products also cytokines. The role of 23 K protein in the immune response of both animals and humans is so far unknown.

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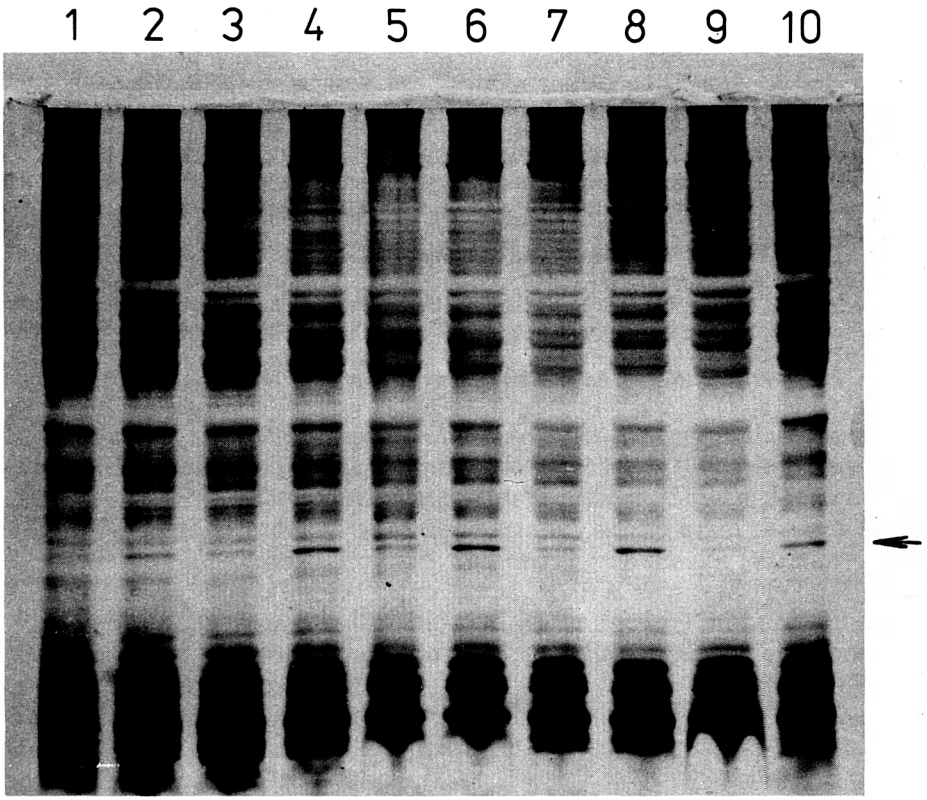

Fig. 2
The time course of synthesis of the 23 K protein in PLC/PRF/5 cells
Protein profiles of control cells (lanes 1, 3, 5, 7, 9) and cells exposed to conditioned medium from ConA stimulated PBM cells (lanes 2, 4, 6, 8, 10) for 6, 24, 48, 72 and 96 hr. The arrow indicates the position of the 23 K protein.

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