EFFECT OF HOMOLOGOUS AND HETEROLOGOUS INTERFERONS ON PROTEOLYTIC ACTIVITY OF NORMAL AND TRANSFORMED CELLS

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Summary. — The authors investigated the effect of various types of interferons (INF) on the activity of intracellular neutral and cysteine proteinases (cathepsin B and H) in normal and transformed rat embryonic fibroblasts. A substantially stronger inhibitory effect was observed with all three types of IFN on the proteolytic enzymes in transformed cells if compared to that on normal cells. Heterologous IFN reduces the activity of cysteine proteinases by 33% and neutral proteinases by 79%. Homologous IFN strongly reduced the activity of cysteine proteinases int ransformed cells (59%) used in the experiments, but the inhibition of neutral proteinases remained nearly unchanged if compared with normal cells (18%).

Key words: *interferon*; *cell proteinases*; *inhibition*

Introduction

Interferons (IFN) are proteins capable to influence virus replication and cell growth and also to regulate the immune response. The efforts of clinical use of IFN are well known; in the last few years, many attempts have been made to use IFN as antitumor agents. Mechanisms of IFN action on transformed cells are not known and they have not been extensively investigated. It was shown that IFN exert several biochemical effects (Table 1) even though Baron *et al.* (1981) stated that these effects could not yet be linked to their causes. In addition to the effects listed by Baron *et al.* (1981) we demonstrated that IFN inhibits some intracellular proteolytic enzymes, i.e. the neutral and cystein proteinases (Schauer *et al.*, 1982).

The results of our previous experiments showed that all three types of IFN can have an inhibitory effect on the activity of the intracellular neutral proteinases but only β- and γ-IFN had a detactable effect on the proteolytic activity of B and H cathepsins (Schauer *et al.*, 1982). It seems that these enzymes could determine the invasiveness of transformed cells and could be of some importance in cancer metastasis formation (Quigley, 1979; Sträuli, 1980; Škrk *et al.*, 1982). It is also known that exogenous proteinases trypsin

and chymotrypsin can induce the transition of cells from G_1 to S phase and promote cell division (Brown and Stubblefield, 1974). By inhibiting proteinases, IFN could prevent this effect (Gutterman, 1981).

Materials and Methods

Preincubation of IFN samples with proteases. Purified intracelular neutral proteinases (Suhar *et al.*, 1982), cysteine proteinases B and H (Suhar *et al.*, 1981) and enzymes from the homogenates of rat embryonal fibroblasts were used. The latter cells have been used either as normal one after 140 passages (Filipič *et al.*, 1982) as spontaneously transformed cells.

The cells were considered transformed according to these characteristics: growth in the presence of 1% calf serum, agglutinability with ConA, doubling time in comparison to the primary cell culture, tumorogenicity in vitro, growth in medium with 0.01 mmol/l Ca^{2+}, invasiveness into the chicken embryonal heart, cloning efficiency (up to 40%), growth in polyacrylamide gel in the form of colonies, induction of tumours in newborn rats. As rat cells they were identified by the use of specific antisera.

After a preincubation of the enzyme samples with IFN preparations (30 000—150 000 unist of α-IFN, 1 200—6 000 units of β-IFN and 2 000—5 000 units of γ-IFN/mg of enzyme protein) at room temperature for 10 min, the activity of neutral and cysteine proteinases has been measured by means of thymus histones and benzoil-arginin-2-naphthyl amide (BANA) (Suhar *et al.*, 1981, 1982). Briefly, IFN preparations were added to 25 μl of cysteine proteinases and 100 μl of neutral proteinases or cell homogenates. After preincubation for cysteine proteinases BANA and for neutral proteinases, 1% calf thymus histones were added and incubated at 37 °C for 1 hr and 4 hr respectively. The inherent proteolytic activity of PBS pH 7.2, Eagle's medium, calf serum and IFN were negligible (for BANA: PBS — 0, Eagle's medium — 0.16, serum — 0, α-IFN — 0.2, β-IFN — 0, γ-IFN — 0 nmoles β-naphtylamine/min/mg, and for neutral proteinase activity: PBS — 0, Eagle's medium 0, serum — 0.01, α-IFN — 0.2, β-IFN — 0, γ-IFN — 0 nmoles Tyr/min/mg). Therefore as a control of proteolytic activity of the purified proteinases and cell homogenates the same volume of water was added instead of IFN. All 3 IFN types were tested separately.

Preparation of IFN. Alpha IFN. Human leukocyte IFN was prepared by the technique described by Cantell and Hirvonen (1978). Leukocytes were obtained through the lysis with 0.83% ammonium chloride. They were resuspended in the Eagle's medium containing 4% human agamma serum. Induction of IFN was performed by the superinduction method (Cantell and Hirvonen, 1978). The leukocyte suspension (10^7 cells/ml) was treated with 100—300 units/ml of homologous IFN overnight. On the next day, Sendai virus (Cantell strain) 100 HU units/ml was added. After incubation, the supernatant was obtained by centrifugation at 1 800 g. The supernatant was further purified through the KSCN precipitation, and than with pH changes that cause selective precipitation. The nett product had approximately 6×10^4 units/ml, with specific activity of 10^4 units/mg protein.

Table 1. Biochemical effects of interferons*

Inductions of new cellular proteins including:
ds RNA-activable protein kinase that phosphorylates ribosome-associated proteins and eIF-2
Protein phosphatase
2′-phosphodiesterase
Alteration of initiation factor eIF-2
Induction of 2′, 5′-oligoadenylic acid synthetase
Activation of endonuclease by 2′,5′-oligoadenylic acid
Alteration of tRNA concentrations
Changes in glycosyl transferase
Membrane transport and binding alterations
Inhibitition of some intracellular proteolytic enzymes (neutral and cystein proteinases)**

* Baron *et al.* (1981);
** Schauer *et al.* 1982.

Beta IFN. Rat fibroblast IFN was prepared from rat embryonal fibroblasts (REF) growing in monolayer. A spontaneously transformed cell line was obtained at the Institute of Microbiology, Medical Faculty, Ljubljana (Filipič *et al.*, 1982). IFN was induced by the technique described by Coupin *et al.* (1978) and Illinger *et al.* (1976). Rat β-IFN was then partially purified by trichloroacetic acid precipitation followed by ammonium sulphate (40—60%) precipitation.

Induction of IFN was performed by the method described above. The nett product had approximately 10^3—10^4 units of IFN per ml, as a product of about 1 000-fold purification.

Gamma IFN. Human lymphocyte IFN was prepared by the technique described by Ankel *et al.* (1980). Lymphocytes were obtained from the whole blood with Ficoll-Paque technique. They were suspended in the Eagle's medium with 4% foetal calf serum (Flow) (approximately 10 cells/ml). IFN was then induced with phytohaemagglutinin (PHA) (Bacto) in a concentration of 200 μg/ml. The suspension was incubated for three days at 37 °C. Subsequently, the cells were removed by centrifugation. The supernatant was used without further purification as γ-IFN. This IFN preparation had 1 500—3 000 units/ml.

Results and Discussion

All three types of IFN preparations showed an inhibitory effect on the activity of purified neutral intracellular proteinases, but cathepsins B and H were inhibited only by beta and gamma IFN (Fig. 1). The greatest activity on neutral proteinases was shown with α-IFN, which reduced their activity by 41%. The proteolytic activity of cathepsin B was with β-IFN reduced by 64%, and the activity of cathepsin H by 76%. Both types of cysteine proteinases were inhibited in their activity by γ-IFN (29 and 44% respectively).

All types of IFN preparations reduced the activity of intracellular proteinases in homogenates of normal rat embryonic fibroblasts. The suppression was similar, but in the majority of cases less pronounced than observed with purified proteinases (Fig. 2). The effect of IFN on the activity of these enzymes is much greater in transformed than in normal cells. Fig. 2 shows that γ-IFN reduces the activity of cysteine proteinases by 33% and the activity of neutral proteinases by as much as 79%. This reducing effect was observed despite of the fact that a heterologous system was used for assay.

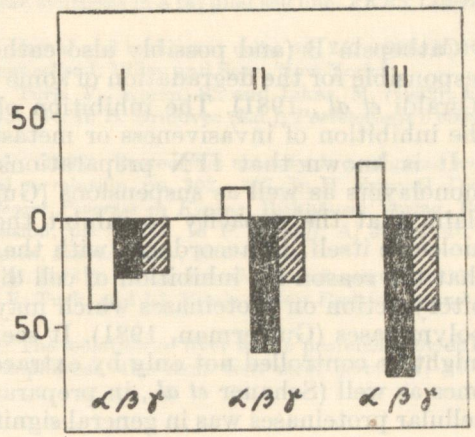

Fig. 1.

Effect of interferons alpha (empty columns), beta (black columns) and gamma (dashed columns) on the activity of purified intracellular proteinases
I — neutral proteinase; II — cathepsin B; III — cathepsin H.

In transformed cells, cystein proteinases have been inhibited by the use of homologous β-IFN by 59% but to a much lesser extent this inhibition was noted in normal cells. The exception was a similar inhibition of the activity of neutral proteinases by β-IFN in normal and transformed cells (18%). The effect of α-IFN effect was similar as with purified enzymes.

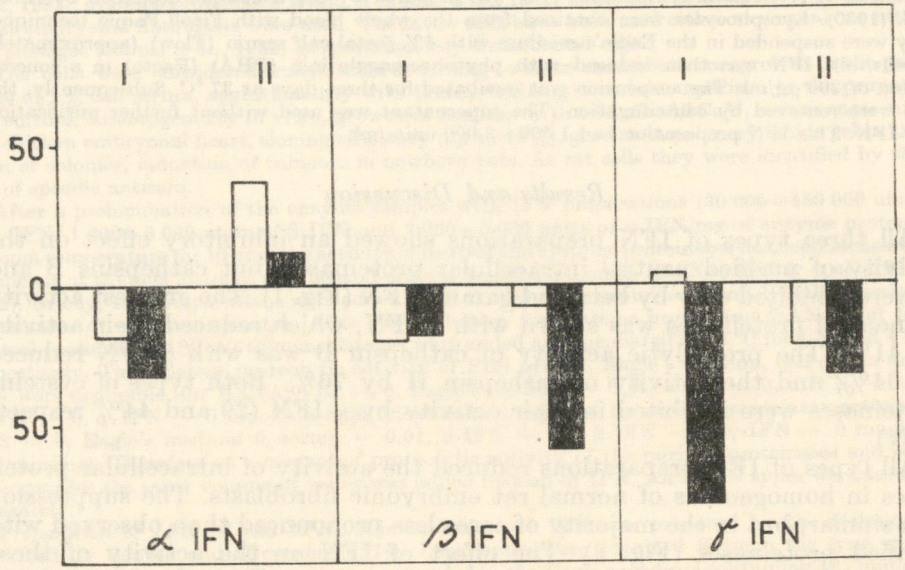

Fig. 2.

The effect of interferons alpha, beta and gamma on the activity of intracellular proteinases in normal and transformed rat embryonic fibroblasts
I — neutral proteinase; II — cathepsins B+H.
Empty columns: normal cells; black columns: transformed cells.

Cathepsin B (and possibly also cathepsin H) could belong to proteinases responsible for the degradation of some components of intercellular substance (Giraldi *et al.*, 1981). The inhibition observed could also be responsible for the inhibition of invasiveness or metastasis formation in cancer.

It is known that IFN preparations inhibit division of cells growing in monolayers as well as suspensions (Gutterman, 1981). A number of authors claim that the capacity to inhibit the growth of cells is inherent to IFN molecule itself. In accordance with the results of our experiments we believe that the reason for inhibition of cell division by IFN could be also the inhibitory action on proteinases which may act as regulatory proteins for DNA polymerases (Gutterman, 1981). It seems conceivable that the cell division might be controlled not only by extracellular proteinases but by intracellular ones as well (Schauer *et al.*, in preparation). The reduction of the activity of cellular proteinases was in general significantly greater in transformed than in

normal cells. These observations support on a molecular level the view that the inhibition of multiplication of transformed cells by IFN is greater than the inhibition of the growth of normal cells (Evans, 1982).

Preliminary measurements of the effect of IFN on intracellular proteinases in cells obtained from the peritoneal fluid (ascites) of patients with ovary cancer showed — even if the number of patients observed was small — that IFN should be applied to humans with caution, as to the time of its application IFN could reduce the intracellular proteinases at a time, when their level had already decreased, in response to the tumour (Schauer *et al.*, in preparation).

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