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DIFFERENT EFFECTS OF THYMUS EXTRACTS ON HUMAN FIBROBLASTS AND CANCER CELLS IN CULTURE

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Summary

We studied the influence of soluble extracts of fetal and juvenile calf thymus on the proliferative capacity of human cells in culture. Low doses of thymus extracts increase nucleoside incorporation into DNA and RNA of fibroblasts and activate cell growth comparable to the effect of potent stimulators like fetal bovine serum or somatotropin . In contrast to these results thymus extracts cause a varying inhibition of DNA synthesis in cancer cells which is intensified by the addition of hydrocortisone. The opposite reaction in the fibroblast and cancer cell system may reflect a selective influence of the thymus extracts on the various cell types.

Introduction

The involvment of thymus in maturation of precursor T lymphocytes and development of cell-mediated immunity raised renewed interest when experiments discovered that immunocompetence of neonatally thymectomized animals was restored by grafting thymus tissue within a cell-impermeable chamber that allowed the passage of macromolecules (1). More direct studies showed that immunologically deficient mice recovered the capacity to

induce a graft-versus-host reaction after injection of crude and partially purified cell-free thymus extracts (2). Furthermore, metabolic deficiencies as a result of thymectomy were reversed by the administration of thymus extracts of different animal sources (3). On the basis of these findings several thymus-dependent factors were detected in the serum of animals and humans active in the regulation of various physiological processes. The essential role of these hormone-like substances encouraged the medical use of thymus preparations which now gain increasing importance in many fields of cure and therapy. Applications of thymus substances or purified hormones like thymosin affect growth and metabolism and were confirmed as successful agents for treatment of various diseases like immune infirmity, cancer and senility (4,5). In our experimental approach we intended to evaluate the molecular effects of thymus extracts on human fibroblasts and cancer cells in culture.

Results

The experimental conditions applied were close to the procedures reported by Gospodarowicz and Moran (6). Monolayer cultures of human fibroblasts and cancer cells were maintained in a resting phase during a 72 hours incubation in serum-deprived minimal medium containing 0.25% FBS. The bioassay utilizes the fact that the cells are still fully responsive to growth factors or mitogens and may initiate DNA synthesis following stimulation by appropriate substances; on the other hand, the response may be as well diminished by mitotic inhibitors or toxic components. Cell-free thymus extracts were prepared of either native tissue or dilutions of lyophilized organ powder after centrifugation and sterile filtration. Changes of metabolic events in the cell cultures were calculated according to the rate of ^{3}H-thymidine incorporation after addition of

the tissue extracts and compared with known proliferative stimulators like fetal bovine serum (FBS) and somatotropine (STH).

On the left side of Fig. 1 we see a striking increase of thymidine incorporation in MRC-5 cells (derived from human embryonic lung) after addition of native fetal thymus extracts (F.Th.). Although the final protein concentrations given in g/ml are very low the extract has a significantly higher stimulating activity than the comparatively submitted 1% of fetal bovine serum (final concentration 200 x 10^{-6} g/ml) or the 10^{-2} international units of somatotropin (a crude preparation with 50 x 10^{-6} g/ml). The initiation of DNA synthesis in these cells is not caused by unspecific reactions but exclusively depends on the addition of essential proteins or growth factors since solutions of bovine serum albumin (BSA) even in higher concentrations have no effect.

The right side of Fig. 1 demonstrates the influence on DNA synthesis of fibroblast cultures established from human skin biopsy (H.S.) which behave similar to the embryonic MRC-5 cells. We still find good results after incubation with lyophilized and redissolved organ powder of fetal thymus in the concentration of 10^{-6} g/ml but the influence of higher dilutions is less significant. Partial hydrolysis or denaturation of the organ substances reduces the cellular response which emphasizes the requirement of vital proteins for stimulation of DNA synthesis and metabolism in cell cultures of diploid state.

In contrast to these results are the effects of thymus extracts on cancer cells. In the case of Wish, a cell line derived from human amnion tissue, ^{3}H-thymidine incorporation after incubation with lyophilized and redissolved extracts of fetal (F.Th.) and of juvenile thymus (K.Th.) is significantly reduced (Fig. 2). Inhibition of Wish cell DNA synthesis is not expressed as a concentration-dependent reaction which may result of the nature of the organ solutions : Higher amounts lead to an increase of

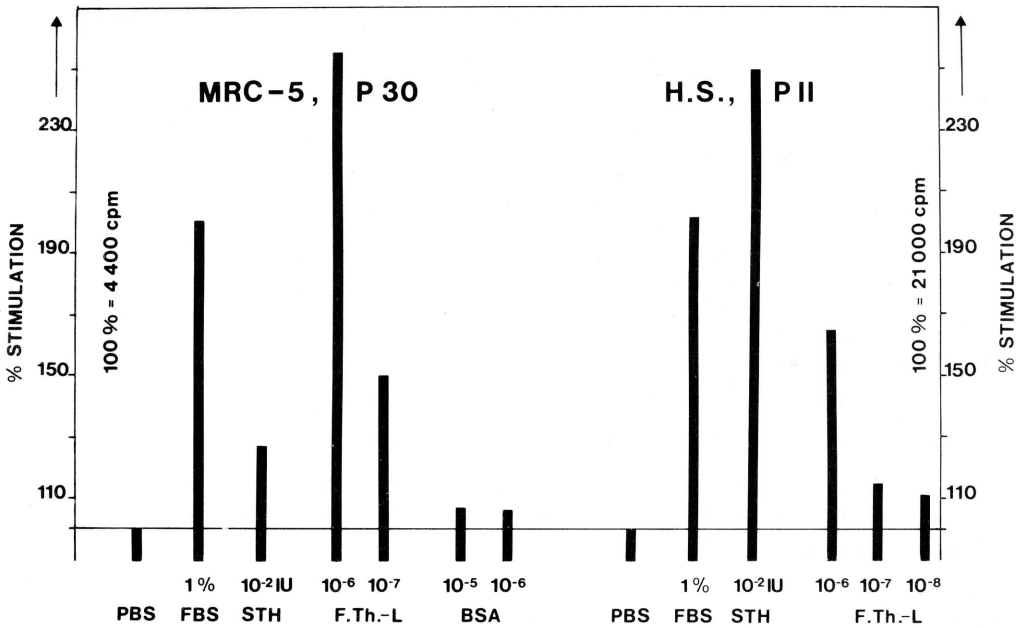

Fig. 1.
Stimulation of DNA synthesis in MRC-5 cells, passage 30, after incubation with phosphate-buffered saline (PBS = control), fetal bovine serum (FBS), somatotropin (STH), native fetal calf thymus extract (F.Th.) and bovine serum albumin (BSA). Stimulation of DNA synthesis in skin fibroblast cultures (H.S.), passage 11, after incubation with lyophilized and redissolved extract of fetal calf thymus (F.Th.). Final protein concentration given in g/ml.

nucleoside incorporation and to a stimulation of the cancer cells possibly due to the supply of nutritional substances. Additional dilutions (10^{-9} g/ml) of the tissue-specific metabolic inhibitors have little or no influence. Compared to the situation in diploid cell cultures the activation of the cancer

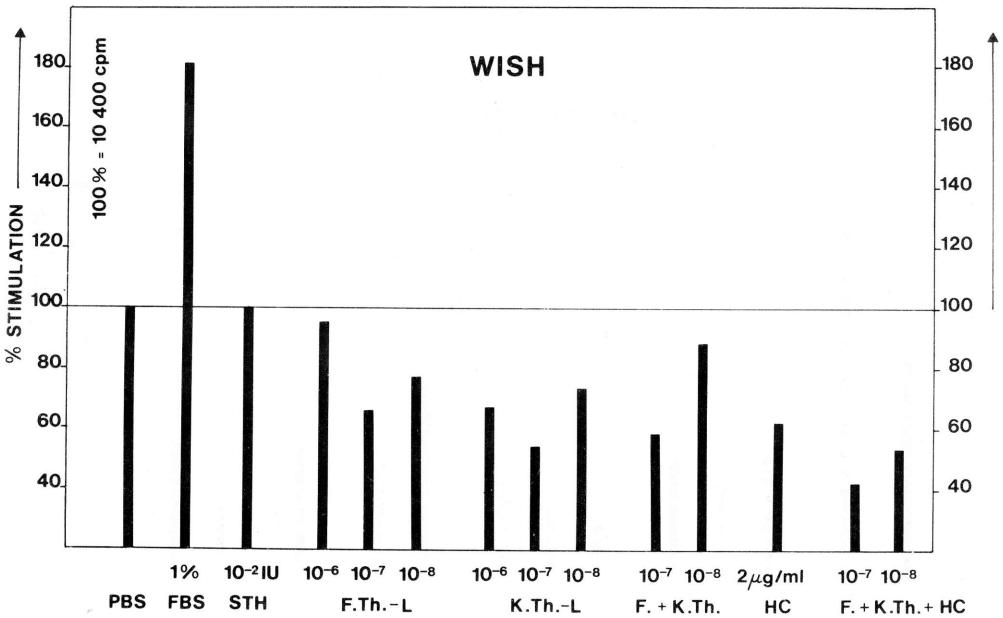

Fig. 2.

DNA synthesis in Wish cells after incubation with PBS, FBS, STH, lyophilized and redissolved extract of fetal calf thymus (F.Th.), juvenile calf thymus (K.Th.), mixtures of both extracts (F.+K.Th.) and hydrocortisone (HC). Final protein concentration given in g/ml.

cells by fetal bovine serum and somatotropin is less pronounced. The combination of the extract mixtures with hydrocortisone (HC) intensifies the inhibitory effect and decreases the ^{3}H-thymidine incorporation to values as low as 40 - 50% of the untreated control.

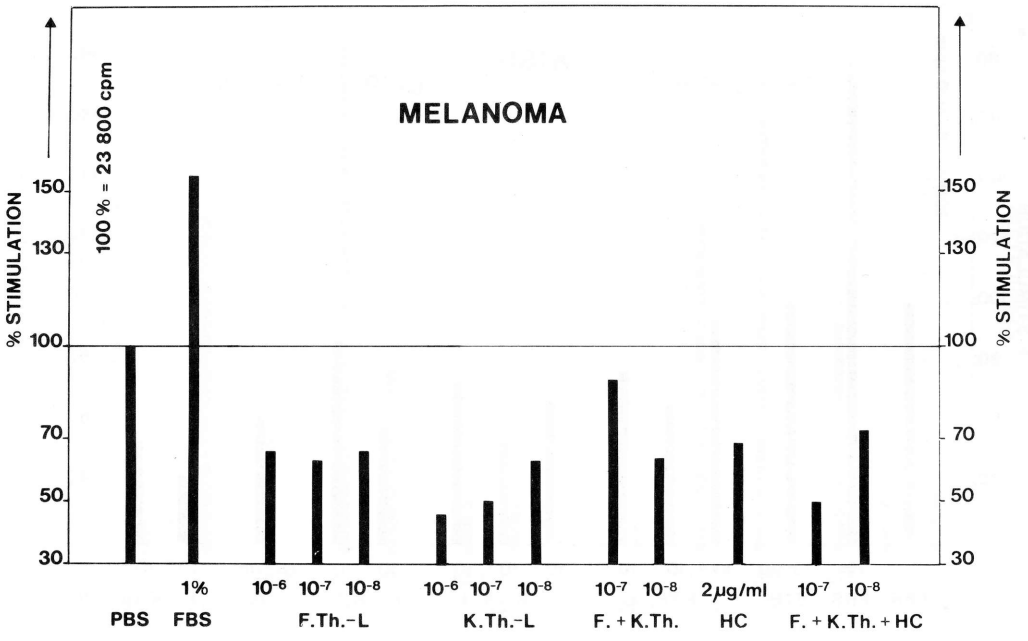

Fig. 3.

DNA synthesis in a human melanoma cell line after incubation with PBS, FBS, STH, lyophilized and redissolved extract of fetal calf thymus (F.Th.), juvenile calf thymus (K.Th.), mixtures of both extracts (F.+K.Th.) and hydrocortisone (HC). Final protein concentration given in g/ml.

Similar results were seen after incubation of a human melanoma cell line with lyophilized and redissolved thymus extracts (Fig. 3). An average of 40% reduction of DNA synthesis was obtained with fetal and juvenile thymus. The inhibition is effective at a broad concentration range but less amplified in the combination of the extract mixtures with hydrocortisone.

Discussion

Although it is well-known that thymectomy or age-associated deficiencies of thymus function increase the development of neoplasia and even stimulate tumor growth, only few reports exist concerning the influence of thymus factors on human cells in culture. Data published mostly deal with maturation studies of precursor T cells into immunologically competent T lymphocytes or the expression of T cell surface antigens.

Our results illustrate the action of calf thymus extracts on different cell types and demonstrate that the thymus gland contains and/or secretes several biologically active factors which may play an important role in the physiological regulation of cells and organs not involved in immunological processes.

Already at low extract concentrations we detected a stimulation of DNA synthesis and metabolism in diploid human cells of different origin. On the other hand, thymus substances in a concentration range of 10^{-6} - 10^{-8} g/ml exerted a significant decline on DNA synthesis in 2 human cancer cell lines which may provide evidence for the existence of tissue-specific factors with opposite activities. In contrast to the stimulation of diploid cell growth which resulted in a better multiplication and higher cumulative cell number we were not able to influence cancer cell proliferation permanently. Reports on *in vivo* studies with thymus substances revealed inhibitory effects to chemically induced tumors with incidence of volume reduction and of malignancy (3). Based on successful approaches of clinical applications and therapy with thymus substances we may conclude that thymus factors provoke immune reactivity, influence metabolism and neoplastic growth and restore functions in patients with thymus-dependent immunological deficiencies.

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