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EFFECT OF INTERLEUKIN-6 ON CELL PROLIFERATION OF FRTL-5 CELLS

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SUMMARY: We studied the effects of interleukin-6(IL-6) on DNA synthesis and cyclic AMP production in rat thyroid FRTL-5 cells. When cells were incubated with IL-6 in the presence or absence of IGF-I, cell proliferation was not observed. By contrast, IL-6 stimulated DNA synthesis in a dose dependent manner when TSH was added concomitantly. On the other hand, IL-6 did not modulate the cAMP accumulation in the presence or absence of TSH. These data demonstrate that, like IGF-I, IL-6 may be able to act as a growth factor through activation of a mitogenic signal transduction pathway different from A-kinase in FRTL-5 cells.

Interleukin-6 (IL-6) is a pleiotropic cytokine that is produced by many kinds of cells and acts on various tissues (1). IL-6 induces B-cell differentiation; production of acute phase proteins in liver cells; proliferation and differentiation in T cells; growth in mesangial cells. IL-6 regulates immune responses and may play an important role in autoimmune disease.

The thyroid gland is one of the main target organs involved in autoimmune disorders. It is reported that thyroid epithelial cells from autoimmune thyroiditis produce IL-6 in vivo and in vitro (2-4). However, the influence of IL-6 on autoimmune thyroid disease has been reported

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Abbreviations used are: IL-6; interleukin-6, TSH; thyroid-stimulating hormone, IGF-I; insulin-like growth factor-I.

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only in few studies. In particular, it remains unclear whether IL-6 stimulates the cell cycle progression of thyroid cells. Taken together, we examined the effects of IL-6 on cell proliferation and cAMP level of FRTL-5 cells.

Materials and Methods

Materials: Recombinant human IL-6 (specific activity; $1X10^7$U/mg) was purchased from Genzyme; [methyl-^{3}H]Thymidine from DuPont; Yamasa cyclic AMP assay kit from Yamasa Shoyu Co.(Tokyo, Japan); TSH from Sigma; IGF-I from Calbiochem. Other materials and chemicals were obtained from commercial sources.

Cell culture and assay for DNA synthesis: The FRTL-5 cells were seeded into 24-well Costar tray in Ham's F-12 medium containing 5% newborn calf serum, TSH (100μU/ml), transferrin (5μg/ml), insulin (10μg/ml), somatostatin (10ng/ml), cortisone (10nM), and glysyl-L-histidyl-L-lysine acetate (10ng/ml) with some modification from the previous description, and were incubated to be subconfluent, then the cells were incubated in serum - and hormones - free F-12 medium for 2 days to be growth-arrested. For measurement of DNA synthesis, cells were incubated in Ham's F-12 medium containing 1% BSA, [^{3}H]thymidine (37kBq/ml), recombinant IL-6 (0-10^5U/l) for 48 hours with or without TSH (10μU/ml) or IGF-I (20ng/ml). The reaction was stopped by addition of 10% trichloroacetic acid and the radioactivity in acid-insoluble materials was counted in a liquid scintillation spectrometer.

Measurement of cyclic AMP production: Cells were incubated in Hanks' solution without NaCl containing 1% BSA, 20mM Hepes, 0.5mM 3-isobutyl-1-methylxanthine and IL-6 (0-10^5U/l) with or without TSH (10μU/ml). Supernatants were removed after 2 hours and the cAMP released into the supernatants was measured by RIA [Yamasa cyclic AMP assay kit, Yamasa]. Each assay was performed in triplicate.

Results

Effect of IL-6 on DNA synthesis

As shown in Fig.1, IL-6 alone had no effect on [^{3}H]thymidine incorporation. By contrast, IL-6 stimulated DNA synthesis in a dose-dependent manner in the presence of 10μU/ml TSH. Moreover, IL-6 did not enhance cell proliferation induced by 20ng/ml IGF-I (Fig.2).

Effect of IL-6 on cAMP production

We next examined the effect of IL-6 on the cAMP accumulation. Figure 3 shows that IL-6 did not change basal nor TSH-induced cAMP concentrations.

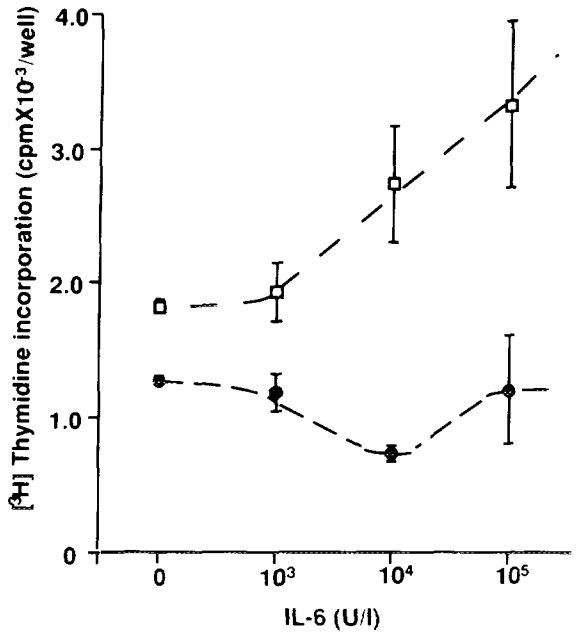

Fig.1. Stimulatory action of IL-6 on DNA synthesis of FRTL-5 cells. The quiescent cells were incubated with various concentrations of IL-6 in the presence (▫) or absence (•) of 10μU/ml TSH. The results are shown with mean±SE for triplicate determinations.

Discussion

Our present study show IL-6 stimulates cell proliferation of FRTL-5 cells only in the presence of TSH. Additionally, IL-6 does not increase the basal

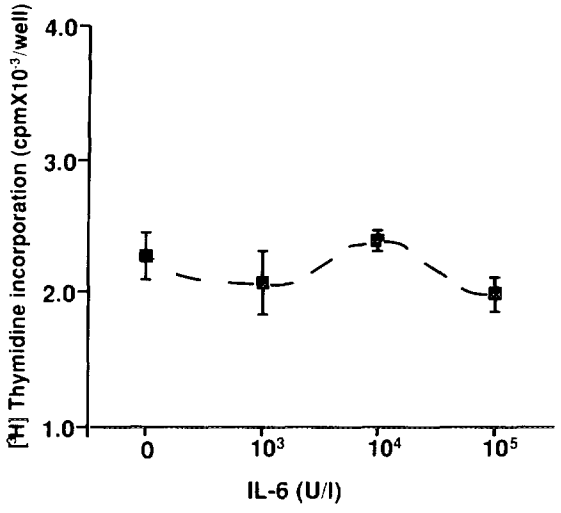

Fig.2. Effect of IL-6 on IGF-I - stimulated DNA synthesis. Cells were incubated with various concentrations of IL-6 in the presence of 20ng/ml IGF-I. The results are shown with mean±SE for triplicate determinations.

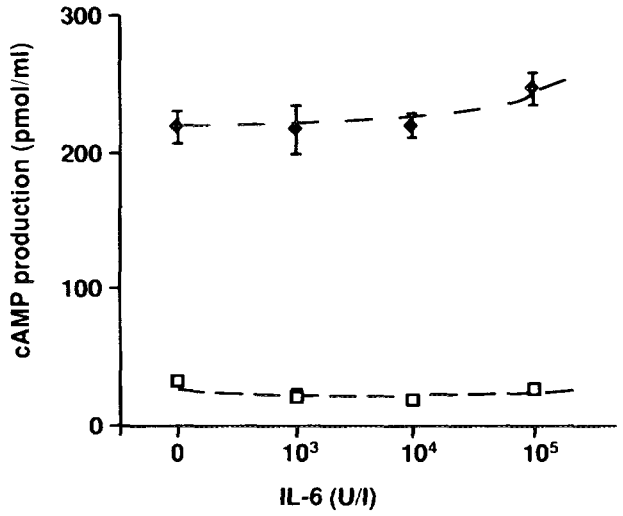

Fig.3. Effect of IL-6 on cAMP production. The quiescent cells were incubated with various concentrations of IL-6 in the presence(◆) or absence(□) of 10μU/ml TSH. The results are shown with mean±SE for triplicate determinations.

cAMP level and does not enhance the cAMP production by TSH. Proliferation of cells can be induced by combined addition of the growth factors. In FRTL-5 cells, TSH can make cells to respond to IGF-I. Our previous studies showed IGF-I can cause sustained Ca^{2+} influx through activation of Gi protein only in TSH-pretreated FRTL-5 cells and this long lasting Ca^{2+} entry is necessary for cell cycle progression (5). Taken these data together, IL-6 may possibly activate a mitogenic signal transduction pathway independent from cAMP-A kinase signaling system in TSH-treated FRTL-5 cells.

In previous works, IL-6 showed little effect on cell proliferation of FRTL-5 cells (6,7). These studies, however, used assay medium (5H or 6H) containing high concentrations of insulin, probably masking the mitogenic action of IL-6. Therefore, it seems difficult to evaluate possible progression effects of IL-6 in these experiments. There is another example where IL-6 acts as a second factor in cell proliferation. IL-6 induces cell growth of quiescent mesangial cells in the presence of serum (8). Thus, IL-6 can activate a mitogenic signal pathway through synergism with many growth factors of serum.

In autoimmune thyroid disease, the thyroid gland is infiltrated with many monocytes and these cells generate various cytokines, including IL-6, which act directly on the adjacent follicular epithelium. Additionally, monocyte-derived IL-1 enhances IL-6 production by thyroid cells themselves (9,10). The present findings raise the possibility that autocrine or paracrine IL-6 stimulates thyroid cell growth and enhances the development of goiter that is characteristic of autoimmune thyroiditis.

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