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BSC-1 growth inhibitor/type β transforming growth factor is a strong inhibitor of thymocyte proliferation

(keratinocytes/DNA synthesis/immunoregulator/interleukin 2)

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ABSTRACT **Growth inhibitor/type β transforming growth factor purified from BSC-1 cells and human platelets is shown to strongly inhibit the proliferation of Con A-stimulated mouse thymocytes. The inhibition can be achieved with growth inhibitor/type β transforming growth factor concentrations ≈1/10th those necessary to inhibit keratinocyte cultures. The inhibitory effect in thymocyte cultures can be reversed by the addition of interleukin 2. These findings suggest that growth inhibitor/type β transforming growth factor is a naturally occurring immunoregulator.**

In 1978, Holley *et al.* (1) reported that BSC-1 cells, an epithelial cell line from African green monkey kidney, release a factor into the medium that inhibits their own growth. By 1980, the inhibitor was considerably purified and found to act on epithelial but not on fibroblast cell lines (2). In 1984, Tucker *et al.* (3) showed that this growth inhibitor was closely related to type β transforming growth factor (GI/TGF-β) isolated from human platelets. Transforming growth factors were first described in 1978 as sarcoma growth factors by De Larco and Todaro and are released by tumor cells into the medium (4). They have gained widespread attention for their proposed ability to stimulate the growth of tumors *in vivo*, an effect that was termed autocrine stimulation (5, 6). Since most of these studies were performed with cell lines, we wanted to evaluate the effect of GI/TGF-β on primary cells in culture. We report here that GI/TGF-β acts as a strong inhibitor on thymocytes stimulated with Con A and that the sensitivity of these cells to the inhibitory effect of GI/TGF-β is 10 times higher than that of keratinocytes.

MATERIALS AND METHODS

Thymocytes were obtained from 5-week-old male C3H/HeJNCI mice. They were prepared and cultured as described by Ziegler and Unanue (7) and Mizel *et al.* (8); 1.5×10^6 cells in 0.5 ml of RPMI 1640 medium with 5% inactivated fetal calf serum were plated into each well of a 24- or 48-well tissue culture tray (Linbro Division, Flow Laboratories, Hamden, CT, or Costar, Cambridge, MA). Con A (2 μg/ml) with or without GI/TGF-β was added immediately and the cultures were incubated for 3 days with [^{3}H]thymidine (1 μCi/ml; 5 Ci/mM; 1 Ci = 37 GBq; Amersham) present for the last 18 hr of incubation. Cells were collected on glass fiber filters (Whatman) and the radioactivity was counted.

Primary mouse keratinocytes were prepared from newborn mice (various strains). They were cultured in 35-mm tissue culture dishes (Falcon) and their growth was arrested by serum starvation as described (9). GI/TGF-β was added to the cultures at the time of growth stimulation with 2% fetal calf serum. [^{3}H]Thymidine (2.5 μCi/ml; 5 Ci/mM) was added 14–17 hr later for 3 hr.

GI/TGF-β was prepared from either BSC-1 cell-conditioned medium using Bio-Gel P60 and HPLC (2) or from human platelets according to Assoian *et al.* (10). [One unit of inhibitor is equal to the amount of GI/TGF-β that gives a 50% inhibition of [^{3}H]thymidine incorporation in BSC-1 cells (2).] All GI/TGF-β fractions used in this study were a generous gift of R. W. Holley (The Salk Institute, San Diego, CA).

Rat interleukin 2 (Il-2) was obtained from Sigma. One unit is defined as the reciprocal of the dilution at which half-maximal [^{3}H]thymidine incorporation occurs using mouse cytolytic T lymphocytes.

RESULTS

We first examined the effect of GI/TGF-β on primary cultures of mouse skin keratinocytes. A Bio-Gel P60-purified fraction of BSC-1 cell-conditioned medium was added together with 2% fetal calf serum to cultures of keratinocytes whose growth had been arrested by serum withdrawal. As shown in Fig. 1, a maximal inhibition of DNA synthesis in keratinocytes is obtained with ≈10 units/ml. Half-maximal inhibition occurs at ≈1.3 units/ml. Considering that 1 unit of highly purified GI/TGF-β equals ≈1 ng, the half-maximal inhibition at ≈1.3 units/ml is compatible with the finding of Moses *et al.* (11), who observed in a different system a half-maximal inhibition of human keratinocytes at 1 ng/ml.

To investigate the effect of GI/TGF-β on another type of primary cell, we used thymocytes—in part because of the better availability of these cells and their simple culture conditions compared to primary cells grown in monolayers. The same Bio-Gel P60-purified fraction of GI/TGF-β was added to mouse thymocyte cultures, together with Con A, at the time cells were plated. When [^{3}H]thymidine incorporation was measured 3 days later, a more pronounced inhibition than in keratinocyte cultures was observed, with a half-maximal inhibition at ≈1/10th the concentration of GI/TGF-β (Fig. 1).

We then obtained two highly purified preparations of GI/TGF-β derived, respectively, from BSC-1 cell-conditioned medium (2) and from human platelets (10). The dose responses with these two fractions in parallel cultures of thymocytes are presented in Fig. 2. The half-maximal responses to both preparations are at ≈0.1 ng/ml (4×10^{-12} M), confirming the earlier result with the less pure material that thymocytes are ≈10 times more sensitive than keratinocytes to GI/TGF-β. The GI/TGF-β derived from human platelets appears to give a maximal inhibition that is higher (95% versus 85% for the BSC-1 preparation), but the difference may not be significant.

The results indicate that GI/TGF-β inhibits the proliferation of thymocytes. At the present time, it is not clear how

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Abbreviations: GI/TGF-β, growth inhibitor/type β transforming growth factor; Il-2, interleukin 2.

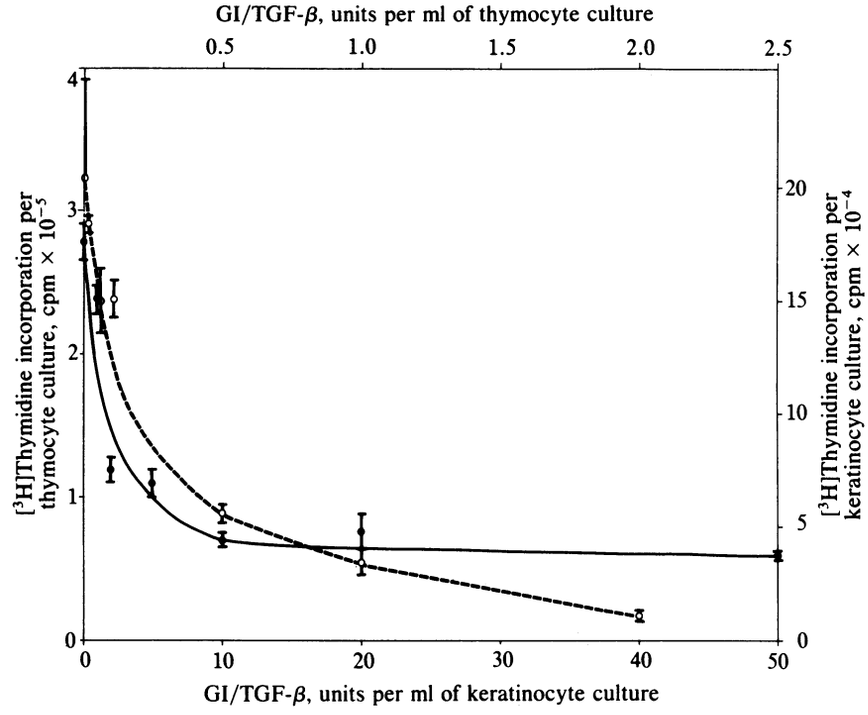

FIG. 1. Inhibition of [³H]thymidine incorporation into primary mouse skin keratinocytes (solid line) and Con A-stimulated mouse thymocytes (broken line). Bio-Gel P60-purified GI/TGF-β from BSC-1 cell-conditioned medium (2) was added to keratinocyte cultures at the time of stimulation with 2% fetal calf serum and to thymocytes together with Con A at the initiation of the cultures. The incorporation in unstimulated (serum-free and GI/TGF-β-free) keratinocyte cultures was 27,700 ± 1850 cpm. The incorporation in unstimulated thymocyte cultures was 264 ± 70 cpm. As observed in a separate experiment with thymocytes, there is no further significant decrease of thymidine incorporation with GI/TGF-β concentrations between 2 and 50 units/ml. Each point represents the average of triplicate cultures ± SEM.

GI/TGF-β acts. Increasing the concentration of Con A has no effect on the inhibition by GI/TGF-β (data not shown). It is generally assumed that subsequent to lectin addition to lymphocytes, Il-2 is produced, which in turn causes the proliferation of cells in the culture. We therefore added Il-2 in increasing concentrations to thymocyte cultures that simultaneously received Con A with and without GI/TGF-β. Data presented in Fig. 3 demonstrate that the inhibitory effect of GI/TGF-β on Con A-stimulated thymocytes can be reversed by the addition of Il-2. This effect of Il-2 on the response of thymocytes to GI/TGF-β is similar to the effect of epidermal growth factor in counteracting inhibition of growth of BSC-1 cell cultures by GI/TGF-β (2).

DISCUSSION

GI/TGF-β is reported to inhibit the growth of cell lines of epithelial origin and to stimulate fibroblast cell lines, which

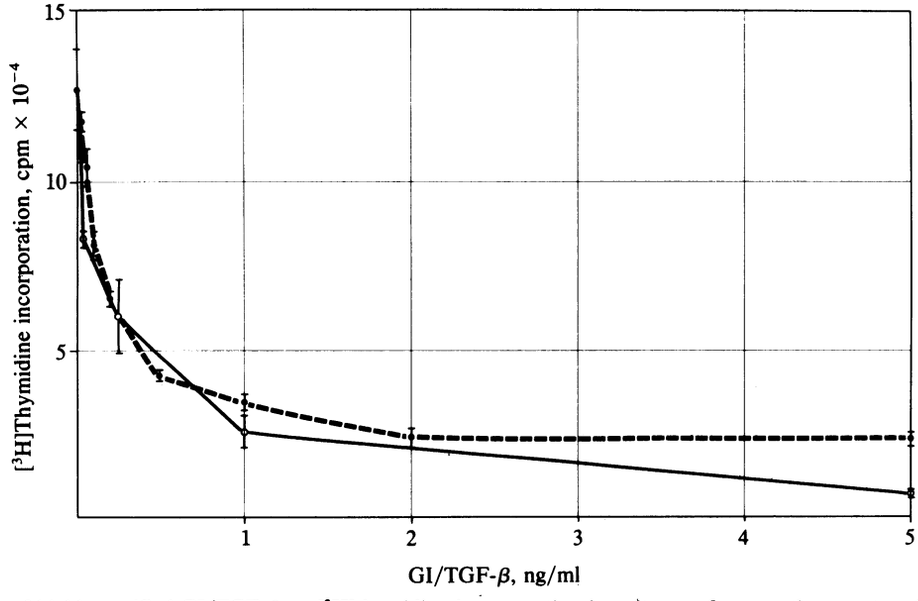

FIG. 2. The effect of highly purified GI/TGF-β on [³H]thymidine incorporation in cultures of mouse thymocytes. GI/TGF-β purified by HPLC was added together with Con A at the time of plating. Each point represents the average of triplicate cultures ± SEM. Solid line represents data with GI/TGF-β from BSC-1 cells. Broken line represents data with GI/TGF-β from human platelets. Unstimulated cultures showed an incorporation of 244 ± 52 cpm.

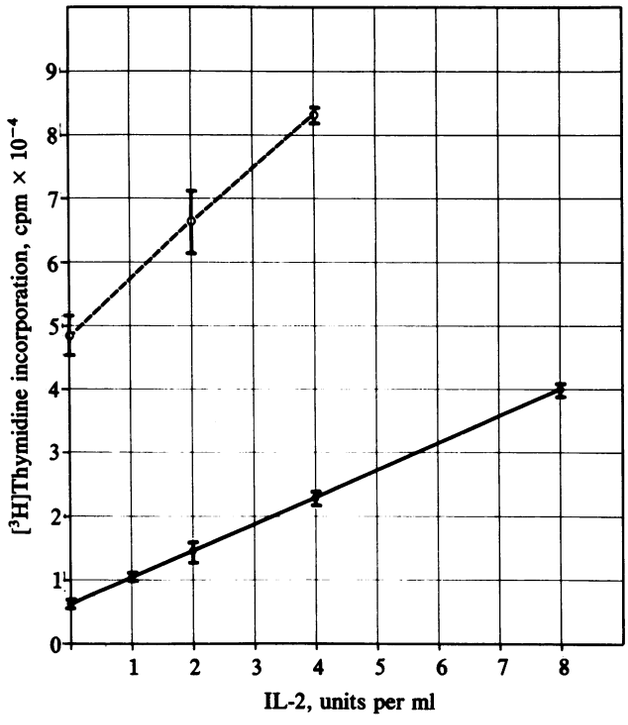

FIG. 3. The effect of Il-2 on the inhibition of [³H]thymidine incorporation in mouse thymocyte cultures by GI/TGF-β. Solid line: Con A (2 μg/ml) and GI/TGF-β (2 ng/ml) were added with rat Il-2 at the concentrations indicated. The GI/TGF-β used here is the same BSC-1-derived fraction used in Fig. 2. The same result was obtained with GI/TGF-β from human platelets (data not shown). Broken line: Con A (2 μg/ml) without GI/TGF-β was added with Il-2 at the concentrations indicated. Unstimulated cultures showed an incorporation of 141 ± 21 cpm.

are of mesenchymal origin (11). Our results with primary mouse keratinocytes presented here, as well as the observation that GI/TGF-β alone without additional epidermal growth factor stimulates DNA synthesis in cultures of primary mouse fibroblasts arrested by serum starvation (unpublished results), are in agreement with these previous reports. From this viewpoint, the results obtained with GI/TGF-β in mouse thymocyte cultures are unanticipated, since the overwhelming majority of these cells are of mesenchymal origin, and the small number of epithelial cells that may be cultured with them are not expected to respond to Con A.

The results with thymocytes shown in Fig. 3 indicate that GI/TGF-β and Il-2 have opposing actions in the immune response. At the present time, one cannot say whether this is due to an inhibition by GI/TGF-β of the production and release of Il-2 from lymphocytes, whether GI/TGF-β binds directly to Il-2 or to its receptor, or whether the opposing actions occur through unrelated mechanisms. If these observations made with thymocytes are representative for circulating T lymphocytes, it is tempting to speculate that under *in vivo* conditions tumors may not only release substances that stimulate their own growth, but that these substances may also inhibit the body's immune response, thus providing the tumor cells with an additional advantage.

In addition, GI/TGF-β may help to elucidate the molecular and cellular basis of T-lymphocyte activation. Considering the widespread occurrence of GI/TGF-β (10, 12, 13) and the recent finding of TGF-β mRNA in peripheral blood lymphocytes (14), it will be interesting to investigate whether GI/TGF-β bears any relationship to lymphokines or to other possible inhibitors of the immune response or whether this inhibition is specific for precursor T cells.

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