EXPERIMENTAL BIOLOGY

Effects of Transforming Growth Factor-β_1 on Proliferation of Smooth Muscle Cells in Human Aortic Intima and Human Promonocytic Leukemia THP-1 Cells

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We studied the effects of transforming growth factor on proliferation of cultured smooth muscle cells from human aortic intima and proliferation and differentiation of human leukemia THP-1 promonocytes. Transforming growth factor inhibited proliferation of these cells, but stimulated differentiation of THP-1 cells. Therefore, transforming growth factor probably modulates proliferation and differentiation of smooth muscle cells and monocytes/macrophages involved in the pathogenesis of atherosclerotic damages.

Key Words: *transforming growth factor-β; proliferation; differentiation; atherogenesis*

Transforming growth factor-β (TGF-β) possesses various properties, regulates cell proliferation and differentiation, synthesis of the intercellular matrix, proteolytic activity, and migration of cells, and is involved in the pathogenesis of connective tissue diseases leading to fibrosis in humans and animals [6,12]. The role of TGF-β in atherogenesis is poorly understood. Our previous studies showed activation of the TGF-β system (TGF-β_1 and TGF-β_2), type I and II receptors, and Smad-2 and Smad-3 proteins transducing TGF-β signals during atherosclerotic damages to human aorta [1,2,4]. Smooth muscle cells (SMC) and monocytes/macrophages of the aortic intima are the main cells responding to TGF-β signals [4]. Experimental data allow us to elaborate new approaches to studying cell-cell interaction during atherosclerosis. This work was designed to study TGF-β-dependent cell effects, which probably play a key role in atherogenesis. Here we studied *in vitro* effects of TGF-β on proliferation and differentiation of SMC and monocytes/macrophages playing the major role in atherogenesis. Experiments were performed on primary culture of SMC from human aortic intima and culture of human leukemia THP-1 promonocytes, which is commonly used for studying proliferation and differentiation of promonocytes into monocytes/macrophages [5].

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MATERIALS AND METHODS

SMC were routinely isolated from human aortic intima after autopsy [9]. THP-1 cells of human promonocytic leukemia were obtained from the American Type Culture Collection (Rockville). SMC were grown on DMEM medium (Gibco) containing 15% fetal bovine serum. THP-1 cells were grown on RPMI-1640 medium (Gibco) containing 5% fetal bovine serum. The cells were cultured in an incubator at 5% CO_2.

To study the effect of TGF-β on SMC proliferation, these cells ($3\text{-}5\times10^5/cm^2$) were placed in 24-well plates. After attaining subconfluence, the cells were incubated a serum-free medium containing 4% Monomed A (CSL) for 24 h to inhibit proliferation. Test cultures were incubated in a serum-containing medi-

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um with increasing concentrations of TGF-β_1 (2, 4, and 6 ng/ml, R&D Systems Inc.) for 24 h. Control cells were cultured without TGF-β_1. ^{3}H-Thymidine was added into the culture medium, and radioactivity was measured on a Beckmann liquid scintillation counter after 14 h. Activity of control samples was taken as 100%.

THP-1 cells of human promonocytic leukemia were used to evaluate the effects of TGF-β on monocyte proliferation. These low differentiated cells intensively proliferate in the suspension culture. Differentiating agents induce their exit from the division cycle and promote terminal differentiation accompanied by cell adhesion to plastic. Promonocytes (500 cells/ml) were placed in 6-well plates. TGF-β_1 in a concentration of 4 ng/ml was added to test cultures. Adherent and nonadherent cells were collected and counted on days 3 and 5 of incubation.

The dynamics of THP-1 cell adhesion was studied to evaluate the effects of TGF-β_1 on promonocyte differentiation. Adherent cells were collected with trypsin, which was neutralized with the serum. The number of cells was estimated on a Cell Counter.

We performed 3 experiments in 3 repetitions. The results were analyzed by one-way ANOVA using SigmaStat 7.0 software. The data are presented as means ±standard errors.

RESULTS

TGF-β dose-dependently inhibited (especially, TGF-β_1 in a concentration of 6 ng/ml) ^{3}H-thymidine incorporation into SMC (Fig. 1), which attested to its antiproliferative effects on cultured cells.

The effect of TGF-β_1 on cell proliferation was studied by evaluating the dynamics of THP-1 cell count in the suspension at different terms of incubation with TGF-β_1. The count of cells incubated with TGF-β_1 on day 5 of culturing was 1.6-fold lower than in the control ($p<0.05$, Fig. 2), which indicated that TGF-β_1 inhibited cell proliferation.

We studied the effect of TGF-β_1 on cell adhesion to plastic to evaluate its influence on promonocyte differentiation. On day 5 of culturing with TGF-β_1, the count of adherent cells 7.2-fold surpassed the control (65.6±3.5 *vs*. 9.3±0.6%, $p<0.05$).

These results are consistent with published data on antiproliferative effects of TGF-β on various cells *in vitro*. Previous studies showed that TGF-β_1 inhibits proliferation of cultured SMC from rat aortic media, but the mechanisms underlying this effect remained unknown [7]. It was hypothesized that antiproliferative activity of TGF-β is associated with increased expression of cell cycle-inhibiting proteins [11]. Our previous experiments demonstrated that SMC and THP-1

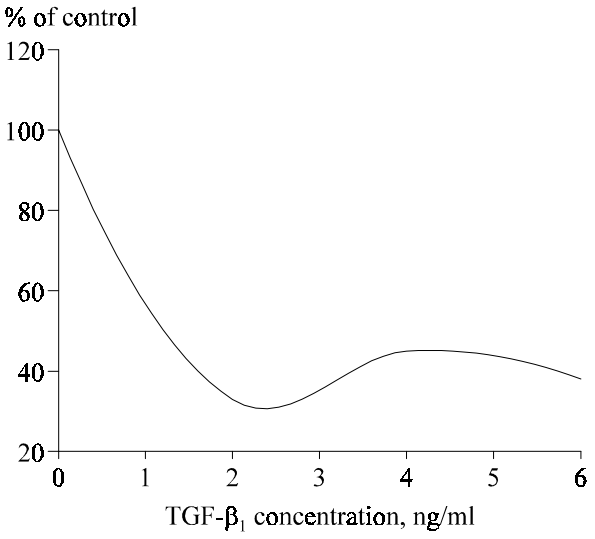

Fig. 1. Effects of TGF-β_1 on *in vitro* proliferation of smooth muscle cells (^{3}H-thymidine uptake) from human aortic intima.

cells express signal receptors for TGF-β [3] and Smad proteins transducing TGF-β signals from the membrane to cell nucleus. Smad-2 and Smad-3 proteins are the substrates for serine/threonine kinase of TGF-β receptors. Phosphorylated proteins migrate into the nucleus, bind to the specific sequence in gene promoters, and act as transcription promoters [8]. Promoters of genes encoding cell cycle inhibitors contain Smad protein-binding sequences [10]. Our previous studies showed that TGF-β in a concentration of 4 ng/ml stimulates expression of cell cycle inhibitors in THP-1 cells and cultured SMC from human aortic intima [2]. These data demonstrate a molecular mechanism underlying TGF-β-induced inhibition of proliferation of cul-

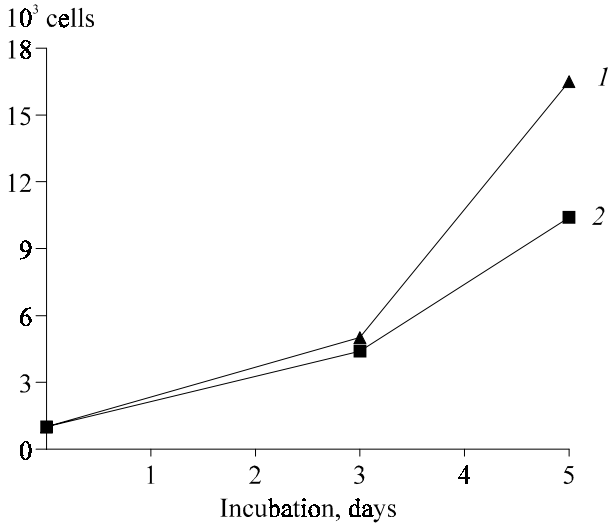

Fig. 2. Effects of TGF-β_1 on *in vitro* proliferation of promonocytes incubated in the culture medium with (*1*) and without 4 ng/ml TGF-β_1 (control, *2*).

tured SMC from human aortic intima and promonocytic leukemia THP-1 cells. Since these cells are similar to those involved in the pathogenesis of atherosclerosis in humans *in vivo*, our results probably reflect the development of atherosclerotic lesions in major vessels in humans.

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