TRANSFORMING GROWTH FACTOR-β1 MODULATES THE EFFECT OF 1α,25-DIHYDROXYVITAMIN D₃ ON LEUKEMIC CELLS

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SUMMARY

The human leukemic cells HL-60, U937, KG-1 and THP-1 incubated with transforming growth factor-β1 (TGF-β1) were studied by examining cell surface antigens and macrophage-specific activities. The addition of 0.5 ng/ml (20 pM) of TGF-β1 with 1α,25-dihydroxyvitamin D₃ [1α,25(OH)₂D₃] induced more Leu-M3 (CD14)-positive cells (approximately 80%) than 5 × 10⁻⁵ M 1α,25(OH)₂D₃ alone did (30 to 50%), although original HL-60 cells did not express any Leu-M3 antigen at all. Tumor necrosis factor-α (TNF-α) with TGF-β1 and 1α,25(OH)₂D₃ was found to potentiate the expression of these surface antigens. Furthermore, the phagocytic activity was also induced strongly. The expression of CR3 (CD11b) antigen was also increased, and all Leu-M3-positive cells were found CR3-positive when HL-60, U937, and THP-1 cells were treated with these stimulants. In contrast, CR3 but not Leu-M3 was induced in KG-1 cells after the same treatment. This may indicate that the responsiveness of leukemic cells to TGF-β1 and 1α,25(OH)₂D₃ might vary depending on a differentiation stage of the target cells. Furthermore, K562 cells originated from a more undifferentiated precursor, were not able to respond to these two inducers. These results suggested that some of TGF-β superfamily proteins might represent potent modulators in hematopoiesis, especially in the development of monocytes-macrophages or their precursors.

Key words: HL-60 cell differentiation to macrophage; TGF-β1; 1α,25(OH)₂D₃; TNF-α phagocytosis; chemiluminescence.

INTRODUCTION

Human leukemic cell lines have proved to be useful for studying factors and conditions associated with a differentiation pathway to macrophages from their precursors (3). For example, 1α,25-dihydroxyvitamin D₃, [1α,25(OH)₂D₃] has been shown to differentiate HL-60 cells into macrophagelike cells (12).

Recently it has been reported that tumor necrosis factor-α (TNF-α) in the presence of 1α,25(OH)₂D₃, could regulate the differentiation of HL-60 cells into macrophagelike cells (30). In vivo differentiation of bone marrow precursor cells to macrophages, however, may be regulated by additional unknown cytokines. In this context, transforming growth factor-β1 (TGF-β1) is a molecule of considerable biological interest, belonging to a large gene family, most members of which have regulatory actions on cell growth and differentiation (1,6,29). Biologically inactive precursor substances of these gene products are known to be produced and secreted by virtually all types of cells. Antiproliferative effects of TGF-β1 have been reported on many types of cells including those associated with the immune system. Thus, TGF-β1 could suppress the activities of T cells, B cells, as well as NK cells (7,8,16,27). It has been shown too that monocyte functions could be modulated by TGF-β1 (25,28). These considerations have prompted us to perform a present series of experiments investigating a possible effect of TGF-β1 on the differentiation of macrophage lineage cells. In this paper, we will report results indicating that TGF-β1 is a potent co-factor to differentiate HL-60 and other related leukemic cells to mature macrophagelike cells in the presence of 1α,25(OH)₂D₃. The activity of TGF-β1 was synergistic with 1α,25(OH)₂D₃, and was further augmented by the addition of TNF-α.

MATERIALS AND METHODS

Cell culture conditions. Human leukemic cell lines, HL-60, U937 and KG-1, were kindly provided by Dr. Akagawa (National Institute of Health, Tokyo, Japan) and mycoplasma-free THP-1 (clone N) was obtained from Dr. H. Hemmi (Sagami Chemical Research Center, Kanagawa, Japan). K562 cells were supplied by Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). The HL-60 cells were used in most experiments and others were used in some experiments as specified in Results. The cells were maintained in RPMI 1640 supplemented with heat-inactivated 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C under 5% CO₂ in a humidified incubator. The initial cell density
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was 1 × 10^6 cells/ml in a 10-cm-diameter dish containing 10 ml of the culture medium and appropriate amounts of differentiation-inducers, as indicated in Results, were added at 0 time. After 3 d incubation, the cells were usually harvested by using a rubber policeman, washed, and used for experiments.

Biologicals and chemicals. Mouse monoclonal anti-trinitrophenol (TNP) antibodies (lgG2a and IgG2b) were kindly supplied by Drs. Majima and Ishida (Tohoku University, Sendai, Japan). Monoclonal antibodies, anti-Leu-M3 (fluorescein-conjugated and phycoerythrin-conjugated) and anti-CR3 (phycoerythrin-conjugated), were purchased from Becton Dickinson Co. (Mountain View, CA), interferon-γ (IFN-γ) from Genzyme Corp. (Boston, MA), porcine TGF-β1 and human TGF-β1 from R & D Systems, Inc. (Minneapolis, MN), and Polybead fluorescent microspheres (0.75-μm diameter) from Polysciences, Inc. (Warrington, PA). FBS was obtained from Bocknek Laboratories Inc. (Canada). 1α,25(OH)2D3 was supplied by Chugai Pharmaceutical Co., Ltd. Retinoic acid (RA) was obtained from Sigma Chemical Co. (St. Louis, MO). The 1α,25(OH)2D3 and RA were dissolved in ethyl alcohol. Porcine and human TGF-β1s were dissolved in 4 mM HCl containing 1 mg/ml crystalline bovine serum albumin (BSA). Recombinant human TNF-α was dissolved in RPMI 1640 containing 10% FBS.

Detection of cell surface antigens. The cells were removed from dishes using a rubber policeman, washed twice with cold phosphate buffered saline (PBS) containing 0.3% BSA and 0.05% sodium azide (designated, BSA-PBS), and resuspended in 50 μl of BSA-PBS. The cells were treated with 5 μl of appropriate monoclonal antibodies. The cell suspension was incubated on ice for 30 min. After the incubation the cells were washed twice with BSA-PBS. Immediately after labeling the cells with fluorescent antibodies, they were fixed with 2.5% formaldehyde. If necessary the cells were treated with the second antibodies in the same manner. Among the monoclonal antibodies used in a series of experiments, Leu-M3 is a typical surface antigen of monocyte-macrophage (24) and CR3 is linked with the macrophage functions (10). Fluorescence was determined with a flow cytometer, FACStar (Becton Dickinson Co.).

Measurement of phagocytic activity. The cells treated with the indicated inducer(s) were suspended in RPMI 1640 containing 10% FBS and 0.02% Polybead fluorescent microspheres, transferred to a culture tube, and incubated for several hours to equilibrate the medium with 5% CO2:95% air. Then the tubes were sealed and incubated at 37°C overnight while they were gently shaken. After incubation, the cells were washed 3 times with cold BSA-PBS to remove the excess amounts of latex beads. The number of the cells incorporating the fluorescent beads was determined using a flow cytometer FACStar, and the percentage of cells ingesting the beads was obtained.

Detection of release of reactive oxygen intermediates mediated by human receptor for Fc portion of IgG (FcγRI). FcγRI-mediated reactive oxygen intermediate release was assayed with the method of Majima et al. (11). Briefly, mouse monoclonal anti-TNP-IgG2a and anti-TNP-IgG2b bind with human FcγRI and FcγRII, respectively. When the differentiated HL-60 cells were mixed with the correspondent monoclonal antibody and TNP-conjugated sheep red blood cell (SRBC), the antibody-SRBC complex was incorporated into the cells through FcγRI or FcγRII. The

![Fig. 1. Photomicrograph of a phase contrast preparation of HL-60 cells treated with TGF-β1 or 1α,25(OH)2D3, or both. Cells (1 × 10^6 cells) were cultured in 10 ml of RPMI 1640 containing (a) 10% FBS alone; supplemented with (b) 5 × 10^-8 M 1α,25(OH)2D3; (c) 1.0 μg/ml TGF-β1 and 5 × 10^-8 M 1α,25(OH)2D3; at 37°C for 3 d under 5% CO2:95% air, respectively. Differentiated cells were stained with anti-Leu-M3 antibody; their positive populations were determined by flow cytometry as shown in Fig. 2.](image-url)