T Cells

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The Winged-Helix Transcription Factor Trident is Expressed in Actively Dividing Lymphocytes

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Abstract

We recently identified the winged-helix transcription factor Trident and described its expression pattern in synchronized fibroblasts. We have now studied *Trident* expression in cell lines, differentiating thymocytes and in lymphocytes derived from peripheral blood. During T cell differentiation, expression peaked in the actively dividing immature single positive cells. In peripheral blood lymphocytes, expression of *Trident* mRNA was absent, but could be induced upon stimulation with mitogens *in vitro*. These observations imply a function for Trident in dividing lymphocytes.

Introduction

Transcriptional regulation is dependent on the action of sequence-specific DNA binding proteins. Eukaryotic transcription factors are usually classified according to their characteristic structural domains involved in DNA binding. Examples are the homeobox, helix-loop-helix, leucine zipper and POU domains (reviewed in 1). The fork head or winged-helix motif has originally been identified in the product of the *Drosophila* homeotic gene *fork head* (2) and the liver-specific protein HNF-3a in rat (3). Over eighty members of this family have since then been cloned from many species ranging from yeast to man. Members of this class of transcription factors have been shown to play important roles in development, tumorigenesis and in cell-type-specific gene expression (for a recent review, see 4).

Recently, we have described the isolation and characterization of Trident, a member of the winged-helix family in mouse (5). In adult mice, *Trident* expres-

Abbreviations: PBL = peripheral blood lymphocytes; FCS = fetal calf serum; PHA = phytohaem agglutinin; PMA = phorbol myristate acetate

sion is restricted to the thymus, whereas in the mouse embryo expression is ubiquitous. When fibroblasts are synchronized and subsequently stimulated to enter the cell cycle by serum addition, the *Trident* gene is transcriptionally activated upon entry into the S phase. In M phase the Trident protein is phosphorylated, presumably by p34^{cdc2} (5, 6).

In this study, we report expression of *Trident* in a large number of cell lines and the expression pattern during the differentiation of T lymphocytes. Furthermore, we show that expression in peripheral blood lymphocytes is induced upon activation with mitogens *in vitro*.

Materials and Methods

Northern blotting

Total RNA was isolated with RNazol (Cinna-Biotex) and 15 µg per lane was analyzed on a 1% agarose gel containing 2% formaldehyde. RNA was transferred to nitrocellulose, hybridized and washed under standard conditions (7). Poly A⁺ RNA from sorted thymocytes was isolated and analyzed as described (8).

Cell lines and stimulation assays

Cells were grown in RPMI 1640 (Gibco) supplemented with 5% heat-inactivated FCS and antibiotics. Peripheral blood lymphocytes were obtained by Ficoll-Isopaque density centrifugation of blood from normal donors or from a chronic lymphatic leukemia patient. In the stimulation assay, peripheral blood lymphocytes were stimulated with 5 µg/ml phytohaemagglutinin (PHA) or 5 ng/ml phorbol myristate acetate (PMA).

Results and Discussion

Ubiquitous expression of *TRIDENT* in cell lines

To test whether the tissue-restricted expression was reflected in a differential expression in cell lines, total RNA from twenty human cell lines was analyzed for *TRIDENT* expression by Northern hybridization (Fig. 1). A single band of approximately 3.5 kb could be detected in all lanes, representing cell lines ranging from B and T lymphoid, myeloid and erythroid to several carcinoma cell lines. This result suggested a role for Trident in cycling cells.

Expression of Trident in developing thymocytes

Trident expression was subsequently followed during the differentiation of T lymphocytes. Subpopulations of thymocytes were separated by fluorescence-activated cell sorting (FACS), based on the expression of surface markers CD25, CD4, CD44, CD8 and CD3, which define an ordered progression in thymocyte differentiation (8). *Trident* expression was detected in several stages of the differentiation pathway of T cells, as shown in Figure 2, but clearly peaked in the immature single positive (ISP) T cells. This compartment represents a subpopu-

Figure 1. Expression of *TRIDENT* in cell lines. Northern blot analysis of total RNA prepared from a panel of human cell lines. 1, REH (pro-pre B); 2, NALM-6 (pre B); 3, APD; 4, BJAB; 5, DAUDI (blasts); 6, U266; 7, ARH (plasma); 8, CEM; 9, H-9; 10, JURKAT; 11, PEER (T); 12, HL-60 (myeloid); 13, KG-1 (myeloblast); 14, THP-1 (monocyte); 15, U937 (monocyte); 16, K562 (erythroid); 17, colon 320 (colon carcinoma); 18, HeLa (cervix carcinoma); 19, HEPG-2 (liver carcinoma); 20, GLC-8 (lung carcinoma).

Figure 2. *Trident* expression in differentiating thymocytes as revealed by Northern blotting. Cells were sorted based on the expression of surface markers as described (8). 1, Non-lymphoid cell line P815; 2, CD25⁻/CD44⁺; 3, CD25⁺/CD44⁺; 4, CD25⁺/CD44⁻; 5, CD25⁻/CD44⁻; 6, Immature single positives (CD8⁺); 7, Double positives CD4⁺/CD8⁺; 8, Single positives (CD4⁺/CD3⁺ or CD8⁺/CD3⁺).

lation of very actively dividing cells in the thymus (9). These data indicated a role for *Trident* in cycling T cells and prompted us to study *Trident* expression in resting and dividing lymphocytes.

Figure 3. Expression of *TRIDENT* is induced in cells entering the cell cycle. **a.** Northern blot of total RNA derived from PBL harvested 0, 1, 2 and 3 days after PHA stimulation as indicated. Lane B contains control RNA derived from a B cell line. **b.** Northern blot of total RNA from CLL cells harvested 0, 1 and 2 days after PMA stimulation as indicated.

TRIDENT expression is induced in lymphocytes entering the cell cycle

We used two systems to test *TRIDENT* expression in lymphocytes entering the cell cycle. In the first system, peripheral blood lymphocytes (PBL), which are essentially G0 cells, were isolated from the blood of normal donors and stimulated with the T cell mitogen phytohemagglutinin (PHA). On a Northern blot, no expression was detected in total RNA isolated from these cells on day 0 (Fig. 3a). Expression of *Trident* was induced within a day after PHA stimulation and reached its maximum on day 2.

A comparable expression pattern was observed in the second system. Malignant cells were obtained from a chronic lymphocytic leukemia patient. These non-dividing cells from a monoclonal population of B lymphocytes can be isolated in high numbers from the blood. After these B cells were induced to enter the cell cycle using the phorbol ester PMA, expression of *TRIDENT* mRNA could be detected on day 1 and 2 (Fig. 3b).

This study implies a role for the winged-helix/fork head transcription factor *Trident* in cycling lymphocytes. *Trident* was expressed in a large number of cells of different ancestry, including lymphoid cells. Expression of *Trident* peaked in the immature single positive T cells during T cell differentiation. When lymphocytes were isolated from the blood, expression was found only after stimulation with mitogens *in vitro*. The data presented here indicate that *Trident* does not seem to function as a regulator of lymphocyte differentiation, but rather has a role in cell cycle regulation of lymphocytes.

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