Regulated Expression of Telomerase Activity in Human T Lymphocyte Development and Activation

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Summary

Telomerase, a ribonucleoprotein that is capable of synthesizing telomeric repeats, is expressed in germline and malignant cells, and is absent in most normal human somatic cells. The selective expression of telomerase has thus been proposed to be a basis for the immortality of the germline and of malignant cells. In the present study, telomerase activity was analyzed in normal human T lymphocytes. It was found that telomerase is expressed at a high level in thymocyte subpopulations, at an intermediate level in tonsil T lymphocytes, and at a low to undetectable level in peripheral blood T lymphocytes. Moreover, telomerase activity is highly inducible in peripheral T lymphocytes by activation through CD3 with or without CD28 costimulation, or by stimulation with phorbol myristate acetate (PMA)/ionomycin. The induction of telomerase by anti-CD3 plus anti-CD28 (anti-CD3/CD28) stimulation required RNA and protein synthesis, and was blocked by herbimycin A, an inhibitor of Src protein tyrosine kinases. The immunosuppressive drug cyclosporin A selectively inhibited telomerase induction by PMA/ionomycin and by anti-CD3, but not by anti-CD3/CD28. Although telomerase activity in peripheral T lymphocytes was activation dependent and correlated with cell proliferation, it was not cell cycle phase restricted. These results indicate that the expression of telomerase in normal human T lymphocytes is both developmentally regulated and activation induced. Telomerase may thus play a permissive role in T cell development and in determining the capacity of lymphoid cells for cell division and clonal expansion.

The termini of eukaryotic cell chromosomes consist of special structures, telomeres, which appear to be important for the integrity of chromosomes, chromosomal positioning in the nucleus, transcriptional activation, and cellular senescence (1–4). The length of telomeres decreases with increase in age in vivo and with cell division in vitro in normal human somatic cells (5–14). This phenomenon has been attributed to the end-replication defect of conventional DNA polymerase, which results in the loss of terminal telomeric nucleotides with each chromosomal replication and may represent a mechanism for limiting the cellular replicative life span such that cells that have reached a critical minimal length of telomeres become incapable of further cell division (15, 16). Telomerase is an RNA-dependent DNA polymerase that is capable of synthesizing telomeric repeats to compensate for the loss that occurs with cell division (17–19). Analysis of telomerase expression in human cells initially suggested that germline and malignant cells, but not normal somatic cells, express telomerase, providing a basis for the selective immortality of the germline and of cancer cells (20). However, the recent finding that telomerase is expressed at a low level in normal peripheral leukocytes, including lymphocytes, and at a relatively high level in lymphoid lineage–committed progenitor cells raises the question of whether telomerase is widely expressed in somatic cells and whether telomerase expression is actively regulated in somatic cell lineages (21–23).

The function of lymphocytes is dependent on a high degree of cell division during development, differentiation, and activation. T lymphocytes, for instance, are derived from T lineage–committed bone marrow progenitor cells, which differentiate within the thymus through an ordered sequence of phenotypically distinct stages: from immature double-negative (CD4⁻CD8⁻) to double-positive (CD4⁺CD8⁺) to mature single-positive (CD4⁺CD8⁻ or CD4⁻CD8⁺) thymocytes that are released into the periphery. During intrathymic differentiation, T cells undergo a process of "education" in which T cells expressing appropriate TCR specificities are selected for survival and clonal expansion (24–26). Mature T lymphocytes reside and circulate in the periphery, where upon encounter of a specific antigen, those T cells that recognize the antigen are activated to undergo further differentiation and clonal expansion. Antigen-specific activation of T lymphocytes is triggered by the en-

gagement of the antigen-specific TCR/CD3 with antigen/ MHC ligand complexes, as well as by costimulatory signals, such as those delivered through CD28 on the surface of T cells (27-29). These receptor/ligand interactions induce signals that are mediated by a cascade of biochemical events that result in the proliferation and differentiation of T cells. Substantial cell division and clonal expansion are essential for an effective immune response. Thus, lymphocytes provide a candidate system in which to study the regulation of telomerase expression and the relationship between telomere length, telomerase activity, and cellular replicative potential.

In the present study, we have analyzed telomerase activity in T lymphocytes isolated from the thymus, tonsil, and peripheral blood, and have found that telomerase expression in the human T lymphocyte lineage is developmentally regulated. Moreover, telomerase activity is inducible in peripheral resting T cells by activation through CD3 and CD28, and is activation/proliferation dependent, but not cell cycle phase restricted. These results indicate that the expression of telomerase in normal human T lymphocytes is both developmentally regulated and activation-induced, and they suggest that telomerase activity may play a role in normal immune function.

Materials and Methods

Isolation of Thymocytes and Peripheral Blood T Cells. Human thymi were obtained during pediatric cardiac surgery on 6-mo- to 11-yr old children. Thymi were minced and pressed through a stainless steel sieve. Thymocytes were separated by Ficoll gradient centrifugation (Organon Teknika, Durham, NC). Subsets of thymocytes were further isolated by a combination of immunomagnetic beading and fluorescent cell sorting. In brief, CD4⁻CD8⁻ cells were isolated by the removal of CD4⁺ and/or CD8⁺ thymocytes, as well as B cells and monocytes, by incubating with antibodies against CD4 (516), CD8 (B9.8), CD19 (FMC63), CD11b (NIH11b-1), and CD14 (63D3) (antibodies generously provided by Dr. S. Shaw, National Institutes of Health, Bethesda, MD) at 4°C for 1 h, followed by goat anti-mouse IgG-conjugated magnetic beads (PerSeptive Diagnostics, Cambridge, MA) for another 2-3 h at 4°C as previously described (30). CD4⁺CD8⁺ thymocytes were isolated by positive selection using detached anti-CD4 or anti-CD8 Ab-coated magnetic beads (Dynal, Great Neck, NY), following the manufacturer's instructions. CD4⁺CD8⁻ or CD4⁻CD8⁺ thymocytes were enriched separately by the removal of CD8⁻- and CD4⁺-positive thymocytes, respectively, followed by positive selection with either anti-CD4 or anti-CD8 Ab-coated magnetic beads or by fluorescent cell sorting (FACStar®, Becton-Dickinson & Co., Mountain View, CA). Tonsils were obtained from tonsillectomies for chronic tonsillitis on children and adolescents 7-16 yr old. Tonsil cells were isolated by the same method as isolation of thymocytes, and subpopulations of tonsil T cells were further isolated by immunomagnetic beading as described previously (30). Peripheral blood and buffy coat cells were obtained from 17 normal donors 9-58 yr old from the blood bank at NIH Clinical Center. CD4⁺ and CD8⁺ T cells were isolated by immunomagnetic beading.

Flow Cytometry Analysis of Thymocytes and Peripheral T Cells. 10⁶ thymocytes, tonsil, or peripheral T lymphocytes were stained

with FITC-conjugated anti-CD8 and PE-conjugated anti-CD4 antibodies (Caltag Laboratories, San Francisco, CA) according to the manufacturer's instructions. Stained cells were then analyzed by either FACStar® or FACScan® (Becton-Dickinson). The purity of isolated subsets of thymocytes, tonsil, and peripheral T lymphocytes was generally >95%.

Culture, Activation and Inhibition of Peripheral T Cells In Vitro. Culture of normal CD4⁺ T cells was carried out as previously described (31, 32). In brief, 5-10 × 10⁶ peripheral blood CD4⁺ T cells (10⁶ cells/ml in RPMI 1640, 10% fetal bovine serum) were stimulated in vitro with a combination of bead-bound anti-CD3 and -CD28 mAbs or other specified conditions. For telomerase assays, cells were collected as indicated at days 0.5, 1, 2, 3, 6, or 12 after stimulation. For experiments assessing the inhibition of stimulation, inhibitors were added at the initiation of culture stimulation, and cells were collected at days 1, 2, and 3. The concentrations of the inhibitors used were as follows: 10 μg/ml actinomycin D (Sigma Immunochemicals, St. Louis, MO), 10 μg/ml cycloheximide (Sigma), 1 μM herbimycin A (Calbiochem-Novabiochem, La Jolla, CA), 0.5 μg/ml cyclosporin A (Sandoz, East Hanover, NJ), and 10 μg/ml rapamycin (Calbiochem-Novabiochem). In some experiments, 1.9 nM PMA (Sigma) and 0.08 μg/ml ionomycin (iono) (Calbiochem-Novabiochem) were also used to stimulate CD4⁺ T cells.

Proliferation Assay. Cell proliferation was assayed by [³H]thymidine incorporation as previously described (31, 32). In brief, 2-5 × 10⁵ cells were cultured in triplicate samples in flat-bottomed 96-well microtiter plates in RPMI 1640 medium containing 10% fetal bovine serum. Cells were incubated for 2 d, 1 μCi of [³H]thymidine was added to each well, and incubation was continued for another day before harvest. [³H]thymidine (New England Nuclear Corp., Boston, MA) incorporation was measured by liquid scintillation counting.

PCR-based Telomerase Assay. The telomerase assay used here was modified from the telomeric repeats amplification protocol (TRAP) described previously (20). Cell extracts were prepared at the concentration of 50 μl CHAPS (cholamidopropyl-dimethylammonio-propanesulfonate; Calbiochem-Novabiochem) lysis buffer per 10⁶ cells. The telomerase assay and PCR amplification steps were carried out in separate tubes with different buffers to improve the telomerase efficiency. The telomerase assay was carried out in an Eppendorf tube containing cell extracts from 2 × 10⁵ cells with incubation at 22°C for 1 h according to a described method (33). The amplification of telomere product was modified by the addition of TaqStart (CLONTECH, Palo Alto, CA) to the reaction to increase the specificity of amplification. In general, one tenth of telomerase products, equivalent to the extracts from 10⁴ cells, were used for PCR at described conditions with 27-30 cycles of amplification in a DNA Thermal Cycler (model 9600; Perkin Elmer, Norwalk, CT). The results shown used 27 cycles of amplification unless otherwise indicated. The amplified products were then separated on a 12% acrylamide gel (Novex, San Diego, CA), and the results were analyzed on a Phosphor-Imager (Molecular Dynamics, Inc., Sunnyvale, CA). To estimate telomerase activity, serial dilutions of cell extracts and different PCR cycle numbers were used to determine the linearity of the reaction. The controls of the TRAP assay included RNase (Boehringer Mannheim Corp., Indianapolis, IN) treatment of cell extracts, single primer (Ts or Cx alone), and water as templates for PCR as described previously (20). PCR analysis performed with mixtures of cell lysates from inhibitor-treated and telomerase-positive cells demonstrated that inhibitors did not interfere with telomerase activity or PCR amplification.

Cell Synchronization and G1, S and G2/M Phase–specific Cell Isolation. CD4$^+$ T cells were stimulated with anti-CD3 plus anti-CD28, cultured for 3 d, and then subjected to G1 phase synchronization (34). Cells first were grown in the presence of 2.5 mM of thymidine (Sigma) for 24 h, washed twice with Ca^{2+}- and Mg^{2+}- free PBS (BioWhittaker, Inc., Walkersville, MD), resuspended in conditioned medium with a ratio of fresh/conditioned media at 2:1, and incubated for 8 h. Cells were then incubated for 14 hours in the presence of 2 mM hydroxyurea (Sigma), at which point the majority of cells were arrested in G1 phase. To obtain cells that were enriched in S and G2/M phases, cells were washed out of hydroxyurea with PBS, resuspended in the same fresh/conditioned medium, and incubated for another 5–6 h at 37°C. To obtain a high purity of cell cycle–specific cells, G1, S, and G2/M phase enriched cells were stained with 5 µg/ml Hoechst 33342 and 0.3 µg/ml DiOC$_5$ (3) (3,3'-dipentyloxacarbocyanine iodide) (Molecular Probes, Eugene, OR) at 37°C for 1 h (35) and sorted by a Coulter Epics Elite (Coulter Corp., Hialeah, FL) under UV laser light. The purity of sorted cells was generally >95%.

Results

Telomerase Activity in Human T Lineage Cells. To assess the expression of telomerase in normal human T cells, we examined T lymphocytes isolated from the thymus, tonsil, and peripheral blood. A high level of telomerase activity, comparable to that observed in transformed 293 cells, was detected in unseparated thymocytes, intermediate levels in tonsil T cells, and low to undetectable levels in quiescent peripheral blood T cells (Fig. 1). The levels of telomerase
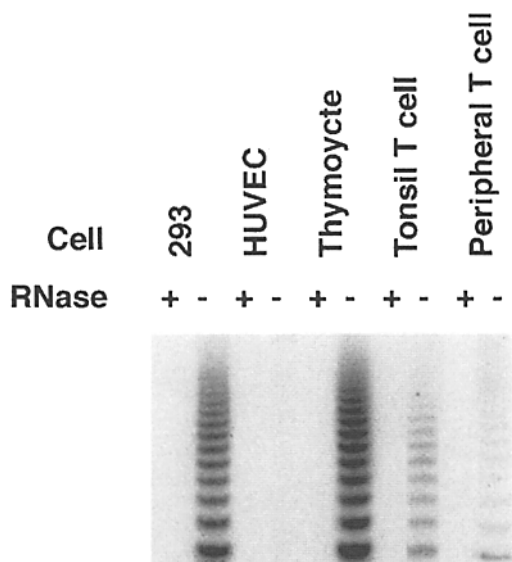

Figure 1. Telomerase activity in normal human T lymphocytes. Unseparated thymocytes, tonsil, and peripheral T lymphocytes were analyzed for telomerase activity. The ages of the donors in the experiment shown are: thymus, 11 yr old; tonsil, 12 yr old; peripheral blood, 10 yr old. *293*, a transformed human kidney cell line that expresses high levels of telomerase and is used here as a positive control; *HUVEC*, normal epithelial cells isolated from a human umbilical vein used here as a negative control. RNase treatment of cell extracts is used here to demonstrate the requirement of RNA for telomerase activity. Other negative controls included PCR using either a single primer or PCR using water instead of cell extracts (data not shown).

activity in thymocytes were comparable among five thymi from 6-mo- to 11-yr-old donors, and the levels in tonsil T cells were comparable among three 7–16-yr-old donors. The thymus contains identifiable subpopulations that constitute an ordered differentiative pathway. To compare the telomerase activity in subpopulations of thymocytes, we isolated and analyzed double-negative (CD4$^-$CD8$^-$), double-positive (CD4$^+$ CD8$^+$), and single-positive CD4$^+$ and CD8$^+$ populations (Fig. 2). It was found that double-negative, double-positive, and CD4$^+$ single-positive subpopulations of thymocytes expressed similar high levels of telomerase activity that were ~27-fold higher than that of peripheral blood CD4$^+$ T cells, while CD8$^+$ single-positive thymocytes expressed consistently lower telomerase activity (Fig. 3). Tonsil CD4$^+$ and CD8$^+$ T lymphocytes expressed an intermediate level of telomerase activity that was three- to nine-fold higher than that of peripheral blood CD4$^+$ T cells. No difference in telomerase activity was apparent between CD4$^+$ and CD8$^+$ T lymphocytes that had been isolated from peripheral blood (data not shown).

Induction of Telomerase Activity in Peripheral Blood CD4$^+$ T Cells In Vitro. The observed difference in telomerase activity in tonsil and peripheral blood CD4$^+$ T cells suggested that telomerase activity may be related to the activation state and/or cell cycle stage of T lymphocytes, since tonsil T lymphocytes are likely to be more highly activated than predominantly resting peripheral blood T lymphocytes. To determine whether activation of T lymphocytes could induce telomerase expression, CD4$^+$ T lymphocytes were isolated from peripheral blood and stimulated with anti-CD3/ CD28 or PMA/ionomycin in vitro. Activated lymphocytes were collected at different time points, and telomerase activity was analyzed. Activation induced a substantial increase in telomerase expression in T lymphocytes under both stimulation conditions (Fig. 4). After anti-CD3/CD28 or PMA/ionomycin stimulation, telomerase activity was detected as early as day 1, reached a peak at days 3–6, and gradually decreased thereafter. Based on serial dilution analysis, the peak level of telomerase activity induced was at least 27-fold greater than that in unstimulated T cells, and was comparable to that found in freshly isolated unseparated thymocytes (Fig. 5). Stimulation with anti-CD3 alone was sufficient to induce telomerase activity with response kinetics that were somewhat slower than those induced by anti-CD3/CD28, while nonmitogenic stimulation with anti-CD28 alone did not induce telomerase expression (Fig. 6).

Metabolic and Signaling Requirement for Telomerase Induction. To determine the metabolic and signaling requirements of telomerase induction, CD4$^+$ T lymphocytes were stimulated with anti-CD3/CD28 in the presence of metabolic inhibitors (Fig. 6). Telomerase induction was completely blocked by actinomycin D or cycloheximide, suggesting that RNA and protein syntheses are essential for telomerase induction. Herbimycin A, an inhibitor of *src* protein tyrosine kinase and NF-κB (36–38), almost completely blocked the induction of telomerase, suggesting that TCR-mediated induction of telomerase requires *src* kinase and/or NF-κB activity.
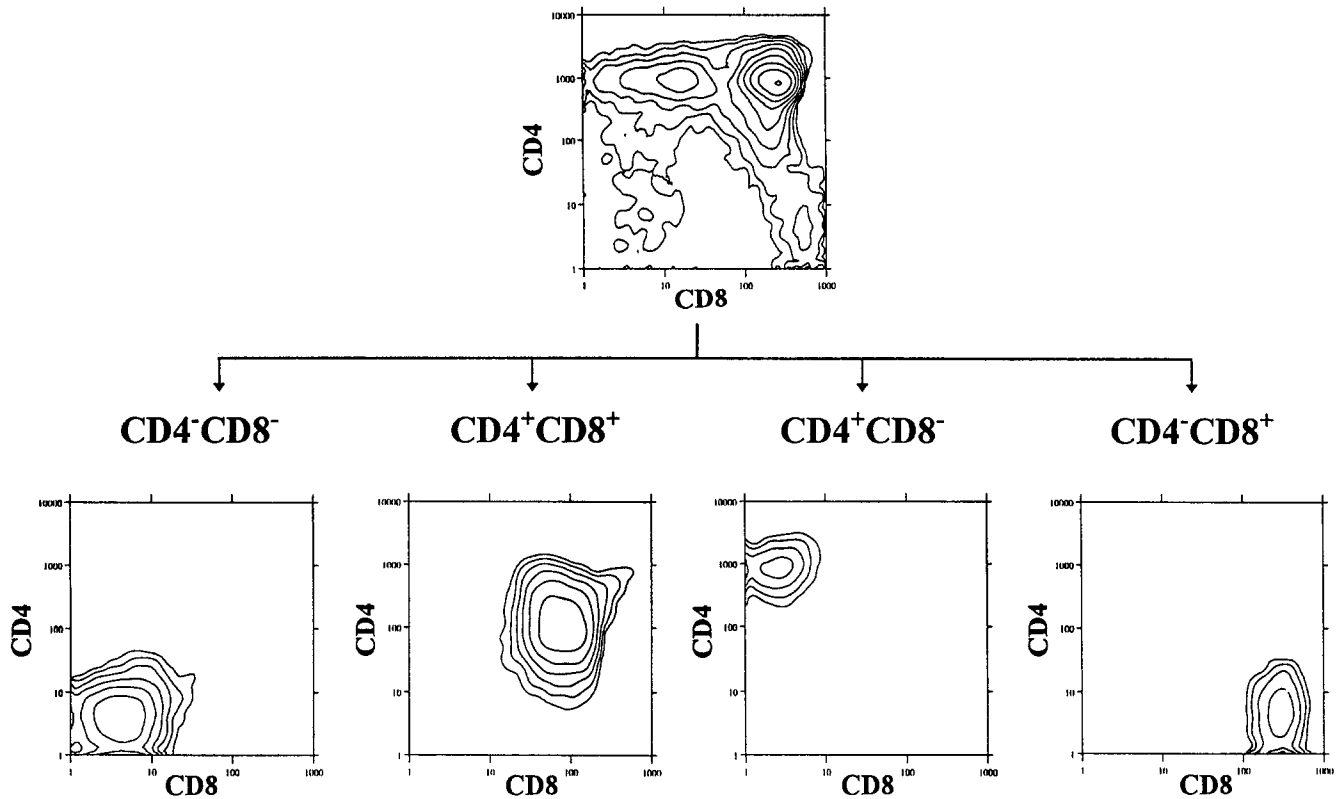
Unseparated thymocytes

Figure 2. Flow cytometric analysis of thymocytes. These FACS® profiles of unfractionated and fractionated thymocytes from an 11 yr-old boy are representative of five thymi studied. Cells were stained with FITC-conjugated anti-CD8 and PE-conjugated anti-CD4.

Cyclosporin A is an immunosuppressive drug that has been shown to prevent the induction of T cell proliferation and cytokine production in response to PMA/ionomycin by interfering with the normal function of calcineurin (39–41). In contrast, stimulation through the CD28 costimulatory receptor induces cyclosporin-resistant proliferation and cytokine production (31, 42, 43). The induction of telo-

merase in T cells by PMA/ionomycin or anti-CD3 was also blocked by cyclosporin A treatment, whereas cyclosporin A treatment had no detectable inhibitory effect on telomerase induction when T cells were stimulated by anti-CD3 plus anti-CD28 (Fig. 7).

Telomerase Activity, Cell Proliferation, and Cell Cycle Phase.
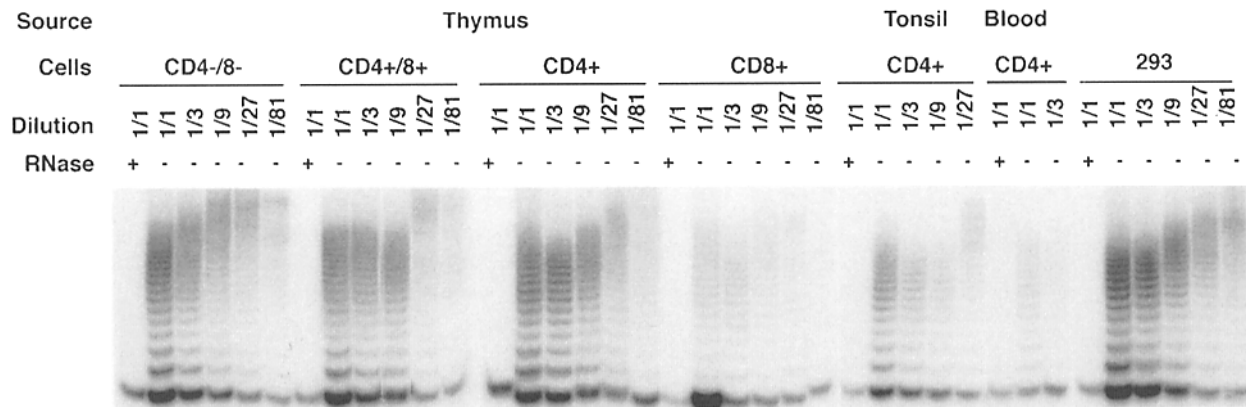CD4+ T lymphocytes isolated from peripheral blood were

Figure 3. Comparison of telomerase activity among the subpopulations of thymocytes, tonsil and peripheral T lymphocytes. Subpopulations of thymocytes (CD4⁻CD8⁻, CD4⁺CD8⁺, CD4⁺CD8⁻, and CD4⁻CD8⁺) and subsets of tonsil and peripheral blood CD4⁺ T cells were isolated as described. Telomerase activity was normalized based on equal cell number equivalents, and serial threefold dilutions of cell extracts were assayed to determine the magnitude of telomerase activity in these cells. 30 rounds of PCR amplification were used in this experiment.

Condition **Anti-CD3/28** **PMA/IONO.**

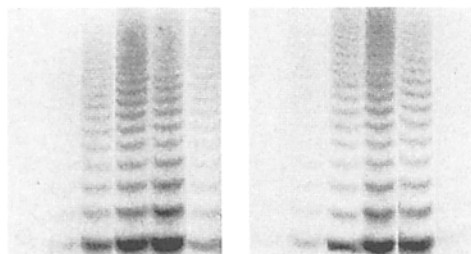**Day** 0 1 2 3 6 12   0 1 2 3 6 12

Figure 4. Telomerase induction in peripheral blood CD4$^+$ T lymphocytes by in vitro stimulation with anti–CD3/CD28 and PMA/Ionomycin. This is a representative result of a total of four independent experiments. Peripheral blood CD4$^+$ T lymphocytes isolated from the same individual were stimulated with anti-CD3 plus anti-CD28 or with PMA/ionomycin.

mainly resting G$_0$/G$_1$ cells that exhibited low telomerase activity. Activation through CD3/CD28, CD3 alone, or PMA/ionomycin induced extensive T cell proliferation (31, 44), and as shown above, high levels of telomerase activity. To analyze the relationship between cell proliferation and telomerase induction, T cells grown under various culture conditions were pulsed with [^{3}H]thymidine, and cell proliferation was compared with telomerase activity after stimulation. The induction of T cell DNA synthesis by a variety of stimuli and inhibition of proliferation by inhibitors of metabolism and signal transduction closely paralleled the induction and inhibition of telomerase activity by these agents (Table 1), suggesting that telomerase expression is linked to cell proliferation and entry into the cell cycle.

To further determine whether telomerase expression in CD3/CD28–activated CD4$^+$ T lymphocytes is cell cycle phase restricted, a combination of synchronization and flow cytometric sorting was used to isolate highly purified populations of G1, S, or G2/M cell after 3 d of culture. These fractionated populations were then assayed for telomerase activity. All fractions of CD3/CD28–activated T cells had equivalently high levels of telomerase, with no significant

enrichment in telomerase activity detected among G1, S, and G2/M populations (Fig. 8).

Discussion

Studies of telomere length in a number of human somatic cell lineages have demonstrated that the reduction of telomere length occurs progressively with age in vivo and with cell divisions in vitro, leading to the hypothesis that telomeres serve as a biological clock of the replicative life span of normal somatic cells (5–14). Thus, in the absence of telomerase expression, somatic cells would undergo progressive telomere shortening with each cell division until the reduction of telomere length to a critical level resulted in exhausting the capacity for cell replication. In contrast, replicative potential is not limited in germline and malignant cells because the expression of telomerase in these cells is capable of extending telomeric repeats to compensate for replication-associated telomere shortening. In an extensive survey, telomerase activity has been reported in 86% (906/1,056) of all malignant tumors and 0% (0/78) of a panel of normal somatic cells that did not include hematopoietic and lymphoid cells (19). The findings presented here indicate that telomerase is expressed in normal somatic cells of the human T lymphocyte lineage, and that this expression is developmentally regulated. Moreover, these results indicate that telomerase activity can be induced in vitro by stimulation through TCR/CD3 complexes and presumably in vivo in peripheral T cells. These results are consistent with two recent reports of telomerase expression in human peripheral blood T lymphocytes (22, 23), and with the demonstration that telomerase activity is inducible in unfractionated PBMC with mitogen stimulation (23). Telomerase may thus have a broad role in the process of lymphocyte development, as well as in the process of clonal expansion and the differentiation of lymphocyte effector functions.

When human CD4$^+$ naive or memory T cells were stimulated cyclically with anti-CD3/CD28 under conditions that were identical to those used in the present study, it was found that telomere length was reduced progressively at a rate of 50–100 bp per cell division, and that this reduction
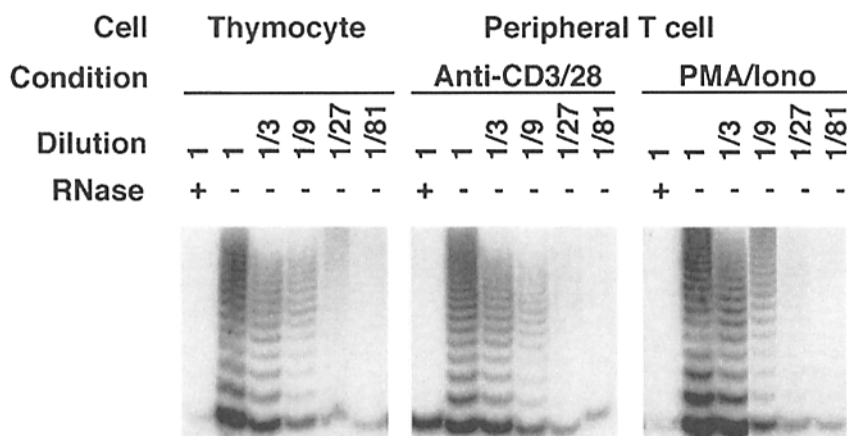
Cell **Thymocyte** **Peripheral T cell**

Condition   **Anti-CD3/28** **PMA/Iono**

Dilution 1 1 1/3 1/9 1/27 1/81   1 1 1/3 1/9 1/27 1/81   1 1 1/3 1/9 1/27 1/81

RNase + - - - - -   + - - - - -   + - - - - -

Figure 5. Comparison of telomerase activity in thymocytes and in vitro–activated T cells. Telomerase activity was analyzed from cell lysates of unseparated thymocytes and of peripheral blood CD4$^+$ T lymphocytes 6 d after in vitro activation by anti-CD3 plus anti-CD28 or PMA/ionomycin.
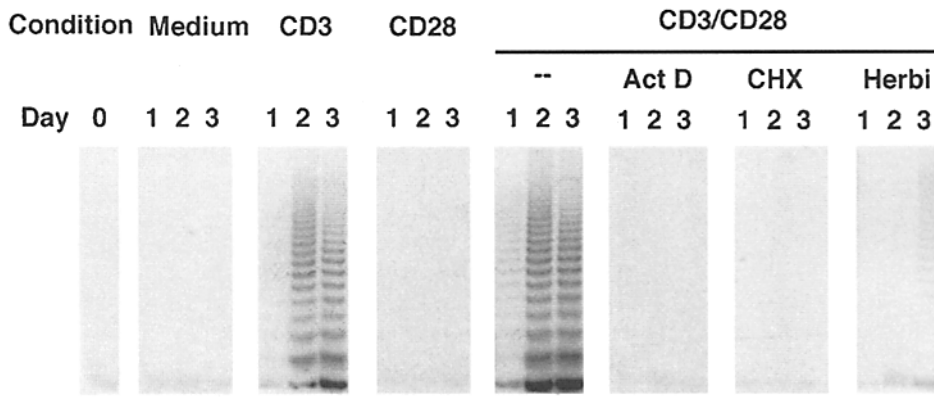
Figure 6. Induction and inhibition of telomerase in CD4+ T lymphocyte in vitro. The results presented are a compilation of several experiments, each of which was carried out at least twice on independent donors. Cells grown under the various conditions were collected at days 1, 2, and 3, and the telomerase activity was measured by normalization on equal cell number equivalents. *Act. D*, actinomycin D; *CHX*, cycloheximide; *Herbi*, herbimycin A.

continued up to the time when cells would no longer proliferate in response to this stimulation (13). Cells harvested at various times during this long-term culture have subsequently been analyzed for telomerase activity, and it has been observed that telomerase activity is indeed detectable at multiple points during the culture process (data not shown); thus, paradoxically, telomere shortening occurs under conditions in which telomerase activity is detected. It is possible that telomerase provides partial compensation for loss of telomeres during cell division, which would proceed at a more rapid rate in the absence of telomerase. However, these findings highlight the need for a definitive determination of the role of telomerase in normal somatic cell development and function. Such a determination will require the identification of the genes that encode components of telomerase, as well as the ability to manipulate or target these genes in vivo as well as in vitro. The recent cloning of genes that encode the RNA template components of human and mouse telomerase will allow the study of the regulation of telomerase RNA template expression, as well as the determination of the telomerase function in lymphocytes (45, 46).

Under the conditions of T cell activation and response inhibition that were analyzed in the present studies, the regulation of telomerase activity closely paralleled that of entry into the cell cycle and proliferation. Stimulation with anti–CD3/CD28, anti-CD3 alone, or PMA/ionomycin induced high levels of telomerase activity, and these same stimuli are also strong inducers of cell proliferation (31, 44). Moreover, the induction of telomerase activity by anti–CD3/

CD28, and the induction of proliferation by the same stimulus are inhibited by the blockade of RNA synthesis or protein synthesis, as well as the *src* kinase inhibitor herbimycin A. Perhaps most strikingly, the induction of proliferation and telomerase activity by anti–CD3/CD28 is resistant to cyclosporin A, whereas the induction of both proliferation and telomerase activity by anti-CD3 alone or PMA/ionomycin is sensitive to cyclosporin A. Thus, although anti-CD28 alone is not sufficient to induce telomerase expression, signaling through CD28 is distinct and complementary to CD3 signaling, and CD28 provides a cyclosporin A–resistant contribution to telomerase induction as well as proliferation. These results suggest that regulation of telomerase expression in T lymphocytes may be closely linked to entry into cell cycle, despite a previous report that cell proliferation per se is not sufficient to induce telomerase in normal human fibroblasts (18). In the present study, strong telomerase activity was also observed in CD4−CD8−, CD4+CD8+, and CD4+CD8− human thymocytes in the absence of in vitro stimulation, where the level of cell cycling is relatively low. The nature of the stimuli that might induce or regulate telomerase expression in vivo is unclear, but the appearance of telomerase activity in multiple developmental fractions of thymocytes, tonsil, and peripheral blood T cells suggests that multiple signals may be capable of influencing telomerase expression.

Because telomere shortening has been attributed to the terminal nucleotide loss that occurs during DNA replication, it was of interest to determine whether the activation–
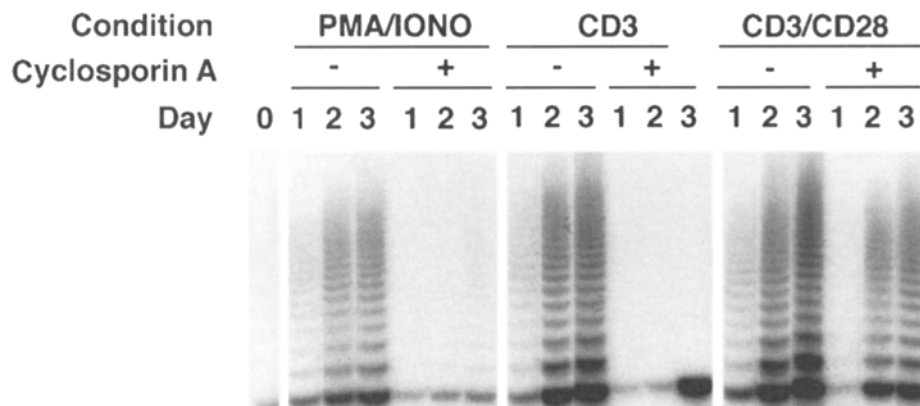

Figure 7. Selective inhibition of telomerase induction under different stimulation conditions by cyclosporin A. Cyclosporin A inhibits in vitro induction of telomerase in PMA/ionomycin and anti-CD3, but not in anti–CD3/CD28–stimulated peripheral blood CD4+ T lymphocytes. T cells were collected at days 1, 2, and 3, and the telomerase activity was measured by normalization for cell number equivalents.

Table 1. *Telomerase Induction and Proliferation of Human CD4⁺ T Lymphocytes In Vitro*

Conditions	Telomerase activity	[³H]thymidine incorporation	
		cpm	Inhibition (%)
Medium	−	168 ± 20	
CD3/28*	+	26,192 ± 3,265	
CD3/28 + Act D	−	28 ± 4‡	100
CD3/28 + CHX	−	34 ± 10	100
CD3/28 + Herbi	−	620 ± 43	98
CD3/28 + CsA	+	23,030 ± 1,146	12
CD3	+	20,538 ± 2,760	
CD3 + CsA	−	3,429 ± 403	83
CD28	−	124 ± 44	
PMA + IONO	+	2,286 ± 1,046	
PMA + IONO 1 CsA	−	1,043 ± 28	63

*CD3/28, anti-CD3, plus anti-CD28–conjugated magnetic beads; CD3 or CD28, anti-CD3 or anti-CD28–conjugated magnetic beads; Act D, actinomycin D, CHX, cycloheximide; Herbi, herbimycin A; CsA, cyclosporin A; IONO, ionomycin.

‡After treatment with actinomycin D, cyclkoheximde, or herbimycin A, the number of live cells at day 3 was 60–70% of the number of live cells in medium, based on trypan blue exclusion.

Figure 8. Telomerase activity in different cell cycle phases. In vitro–activated peripheral blood CD4⁺ T lymphocytes at different cell cycle phases (G1, S, and G2/M), were isolated after 3 d culture by a combination of G1 arrest synchronization and fluorescent cell sorting. Serial three-fold dilutions of cell extracts from all three populations were assayed for comparison of telomerase activity.

induced telomerase activity detected in human T lymphocytes is cell cycle phase restricted. When activated T cells were synchronized and fractionated on the basis of cell cycle, it was found that telomerase activity was relatively uniform throughout different phases of the cell cycle. Although it cannot be determined whether currently available assays of telomerase activity adequately reflect physiologic intracellular events, these results suggest that telomerase-mediated elongation of telomeres may not be strictly linked to DNA replication. Th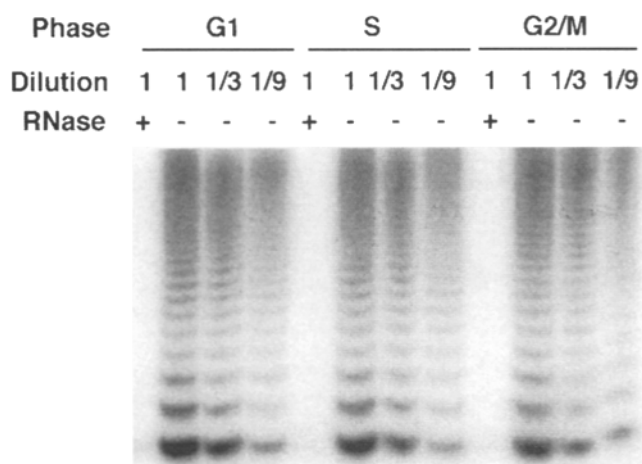is result is consistent with the previous find-ing that telomerase is active in both S and M phases of cell cycles during *Xenopus* embryogenesis (47). It is of interest that the regulation of telomerase appears to contrast in this respect with that of human DNA polymerase δ, which is tightly regulated by cell cycle (48).

The finding that telomerase expression is highly regulated, both developmentally and by TCR-mediated activation in cells of the human T lymphocyte lineage suggest that this unique reverse transcriptase may play a significant role in normal immune function. As the ability develops to experimentally manipulate telomerase gene expression and/or enzymatic activity in vivo, it will be of importance to determine what role telomerase activity might play in lymphocyte development and differentiation, as well as in cell replication, clonal expansion, and clonal exhaustion. Assessment of this possibility will be critical to efforts at exploring the potential for using telomerase inhibition as a strategy for treating malignant cells without extensive toxicity to normal somatic cell functions.

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