Cutting Edge: Antigen-Dependent Regulation of Telomerase Activity in Murine T Cells

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Telomeres, structures on the ends of linear chromosomes, function to maintain chromosomal integrity. Telomere shortening occurs with cell division and provides a mechanism for limiting the replicative potential of normal human somatic cells. Telomerase, a ribonucleoprotein enzyme, synthesizes telomeric repeats on chromosomal termini, potentially extending the capacity for cell division. The present study demonstrates that resting T cells express little/no activity, and optimal Ag-specific induction of telomerase activity in vitro requires both TCR and CD28-B7 costimulatory signals. Regulation of telomerase in T cells during in vivo Ag-dependent activation was also assessed by adoptive transfer of TCR transgenic T cells and subsequent Ag challenge. Under these conditions, telomerase was induced in transgenic T cells coincident with a phase of extensive clonal expansion. These findings suggest that telomerase may represent an adaptive response that functions to preserve replicative potential in Ag-reactive lymphocytes. The Journal of Immunology, 1998, 160: 5702–5706.

The ends of linear eukaryotic chromosomes are capped by specialized DNA-protein structures called telomeres. Both human and mouse telomeres are composed of hexanucleotide repeats, (TTAGGG)$_n$ (1, 2). However, the average number of repeats differs substantially between these species; at birth, normal human somatic cell chromosomes contain about 10 to 12 kb of tandem TTAGGG repeats whereas telomeres from Mus musculus contain greater than 35 kb of TTAGG repeats (3–5). In normal human somatic cells telomere shortening has been observed both in vitro with cell replication and in vivo with aging. Telomere length has been shown to predict replicative potential of human somatic cells, since, in the absence of compensatory mechanisms, telomeres shorten with each cell division until reaching a critical length where further replication cannot occur (6–9).

Telomerase is a ribonucleoprotein enzyme that is capable of synthesizing telomeric repeats at the ends of chromosomes and thus may play a critical role in maintaining telomere length during cell division (1). Initial analysis of normal and malignant human tissues revealed that telomerase activity is expressed by most immortalized and transformed cells, as well as germline cells, cells which do not undergo telomere shortening with cell replication. In contrast, telomerase was not detected in normal human somatic cells, which show progressive telomere shortening with in vivo aging or in vitro cell replication (6, 10). On the basis of these studies it was proposed that telomerase protects immortalized cells and germline cells from the progressive loss of telomere length normally associated with cell division and thus permits unlimited clonal expansion (7, 11). More recent studies have demonstrated that telomerase activity can be detected in normal human somatic cells. Specifically, it has been reported that normal human peripheral blood lymphocytes express low levels of telomerase activity and that lymphoid stem cells express higher levels (8, 12–13). In addition, it has recently been shown that freshly explanted human thymocytes, subpopulations of tonsillar B and T cells, and in vitro activated B and T cells express high levels of telomerase activity (14–16).

While important aspects of telomere biology have been elucidated by recent studies, the physiologic regulation of telomerase activity in T cells in response to Ag-specific stimulation has not previously been characterized. The present report analyzes the regulation of telomerase activity in vitro and in vivo in murine T cells in response to Ag-specific stimulation.

Materials and Methods

Mice

Female C57BL/6 (B6) mice were obtained from Frederick Cancer Research Facility (Frederick, MD), and BALB/c mice were purchased from either Frederick Cancer Research Facility or The Jackson Laboratory (Bar Harbor, ME). V$b$8.1 TCR transgenic (V$b$8.1 Tg)$^3$ mice (17) and BALB.Mls$^e$ mice (18) were bred at Bioqual, Rockville, MD. DO11.10 TCR Tg mice (19) were bred at the University of Minnesota, Minneapolis, MI. All mice were used at 6 wk to 8 mo of age.

Abbreviation used in this paper: Tg, transgenic.
Preparation of T and B cell suspensions

Single cell suspensions were prepared from spleen and lymph nodes, as previously described (20). For in vitro cultures, splenic Vβ8.1 TCR Tg T cells were enriched by passage over rabbit anti-mouse Ig-coated (Organon Teknika, West Chester, PA) tissue culture plates. APCs were prepared either from BALB/c.Mlsα (H-2d, Mlsα-positive) or BALB/c (H-2d, Mlsα-negative) mice that were injected with goat-anti-IgD serum (21), treated with anti-Thy-1.2 (HO-13-4 mAb, American Type Culture Collection (ATCC), Manassas, VA, TIB-99) and complement (C) (Pel-freez Biologics, Rogers, AK), and inactivated by mitomycin C treatment (Sigma, St. Louis, MO).

The adoptive transfer system using CD4+ DO11.10 Tg T cells has been described previously (22, 23). Briefly, spleen and lymph node cells from DO11.10 mice were treated with anti-CD8 mAb (83.12.5, ATCC) and rabbit C (Accurate Chemical and Scientific, Westbury, CT) and adoptively transferred into BALB/c mice. CD4+ DO11.10 Tg cells were isolated from the draining lymph nodes of recipient BALB/c mice by immunomagnetic selection using Dynabeads (Dynal, Oslo, Norway) precoated with rat anti-mouse IgG2a mAb and then with the Tg TCR-specific mAb KJ1.26 (24). The purity of the Tg+ cells was greater than 92%. Following exhaustive depletion of KJ1-26+ cells, recipient CD4+ Tg+ T cells were isolated by treatment with anti-CD8 mAb, rabbit C (Accurate Chemical and Scientific), and passage over a Cellect T cell column (Biotex Laboratories, Edmonton, Canada) to remove B cells. Greater than 85% of the Tg+ cells were CD4+.

In vitro stimulation cultures

The in vitro stimulation culture utilized in this report has been described previously (20).

In vivo stimulation of T cells

BALB/c mice were injected i.v. with 5 × 10^6 CD8-depleted syngeneic T cells from DO11.10 Tg Tg mice and, within 24 h of cell transfer, immunized s.c. with 100 μg OVA (Sigma) in CFA (Sigma). As described above, CD4+ Tg+ and Tg+ T cells were isolated from pooled draining lymph node (LN) on days 0, 3, 5, and 15 after Ag immunization.

Reagents

Anti-CD8-2 mAb (GL1 mAb, rat IgG2a) (25), anti-CD4-1 mAb (16/10 mAb, hamster IgG,) (26), control rat IgG2a mAb (III/10 mAb) (20), and biotin-KJ1-26 were prepared by conventional methods. Goat-anti-IgD serum and anti-FcR-IgG2 (2.4G2 mAb) were the kind gifts of Dr. F. Finkelman (University of Cincinnati Medical Center, Cincinnati, OH) and J. Titus (National Institutes of Health, Bethesda, MD), respectively. ChromPure Hamster IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). FITC-anti-CD4, phycoerythrin-anti-CD4, biotin-anti-CD8, and biotin-anti-CD69 were purchased from PharMingen. SA-FITC and FITC-goat-anti-mouse IgG were purchased from Caltag Laboratories (Burlingame, CA). FITC-anti-Leu 4, biotin-anti-Leu 4, and biotin-anti-CD8 were purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA) and Texas-Red-conjugated streptavidin (TRA) was purchased from Life Technologies (Gaithersburg, MD).

Telomeric repeat amplification protocol (TRAP)

The telomerase products were generated by a modification of the TRAP assay (10) described by Weng et al. (14). To quantify telomerase activity, serial dilutions of telomerase extension products were assayed in the PCR reaction and used to determine linearity of the amplification reaction.

Cell cycle analysis

Cell cycle analysis of propidium iodide (PI)-stained (Sigma) cells was performed according to established methods (27).

Results

In vitro Ag-specific and costimulation-dependent induction of telomerase activity

The regulation of telomerase activity in T cells has not previously been analyzed in responses to “physiologic” stimulation by Ag and costimulatory ligands. Therefore, to assess the ability of Ag-specific stimulation to induce telomerase in T cells, Mlsα-reactive Vβ8.1 TCR Tg T cells were stimulated with BALB.Mlsα APC and cultured for 1 to 3 days. Freshly isolated, resting T cells were found to have little or no detectable telomerase activity (data not shown). Vβ8.1 TCR Tg T cells stimulated with BALB.Mlsα APC in the presence of control or B7-1- and B7-2-specific mAb. A. Cells were cultured for 1, 2, or 3 days, and 2 × 10^5 cell equivalents were analyzed for telomerase activity. B. Relative telomerase activity detected by serial threefold dilutions of telomerase extracts prepared from 2 × 10^5 cells harvested after 3 days of culture.
TCR signaling (data not shown). Taken together these results demonstrate that optimal induction of telomerase activity and proliferative responses by T cells appear to require both TCR signals and costimulatory signals that can be provided by CD28-B7 interactions.

**In vivo induction of telomerase activity**

Regulation of telomerase activity in T cells in response to defined antigenic challenge in vivo has not previously been evaluated. This issue was therefore addressed in an adoptive transfer system employing CD4 T cells from DO11.10 TCR Tg mice expressing a TCR specific for OVA peptide (22, 23). CD4 Tg and Tg T cells were isolated from draining lymph nodes of adoptively transferred mice 0, 3, 5, and 15 days after OVA + CFA immunization. The number of CD4 Tg T cells recovered 3 to 5 days after immunization was increased five- to ninefold over starting numbers, and a high proportion of the Tg cells recovered on day 3 were in S, G2, and M phases of the cell cycle (Table II), indicating that substantial clonal expansion of Tg cells was induced by in vivo Ag challenge. This result is consistent with previous reports demonstrating that Ag induced adoptively transferred DO11.10 cells to proliferate and to express cell surface activation markers through a pathway that is dependent on CD28-B7 interactions (23, 28). As shown in Figure 2, Tg CD4 T cells isolated at day 0 expressed low or undetectable levels of telomerase activity. In contrast, adoptively transferred Tg CD4 T cells isolated 3 or 5 days after Ag stimulation expressed levels of telomerase activity at least 18- to 36-fold higher than that observed in unimmunized cells. By day 15, telomerase activity in these Tg T cells had diminished. In contrast to the substantial induction of telomerase activity in Tg T cells, Tg CD4 T cells isolated from these same mice had no significant increase in telomerase activity over the low level seen at day 0 (Fig. 2B). Thus, the in vivo expression of telomerase activity in T cells was dependent both upon expression of Tg TCR and Ag activation. In addition, these results demonstrate that in vivo Ag-specific stimulation of CD4 Tg T cells concomitantly induces high levels of telomerase activity as well as extensive cell division and clonal expansion.

**Discussion**

During the course of an immune response to antigenic challenge, lymphocyte function is dependent on extensive cell proliferation

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**Table I. Activation of Mls-reactive Vij8.1 Tg T cells**

<table>
<thead>
<tr>
<th>Stimulator Cells&quot;</th>
<th>BALB.Mls&quot;</th>
<th>BALB/c (Mls-neg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb Added&quot;</td>
<td>IL-2 Added</td>
<td>CPM</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>39,017</td>
</tr>
<tr>
<td>B7-1 + B7-2</td>
<td>–</td>
<td>13,212</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>44,357</td>
</tr>
<tr>
<td>B7-1 + B7-2</td>
<td>+</td>
<td>26,660</td>
</tr>
</tbody>
</table>

Vij8.1 TCR Tg T cells were stimulated with mitomycin-treated APC. This table indicates the percentage of CD4 Tg T cells that were CD69 or IL-2Ra after 18 h of culture. Unstimulated CD4 Tg T cells were 5.0% CD69 and 4.3% IL-2Ra. B7-1-specific mAb was used at a concentration of 0.5 μg/ml.

"% Inhibition = [(CPM in control culture Mls Nam APC – CPM in control culture Mls-neg APC) – (CPM in test culture Mls APC – CPM in test culture Mls-neg APC)]/(CPM in control culture Mls APC – CPM in control culture Mls-neg APC).

**Table II. Ag activation of Tg and Tg T cells**

<table>
<thead>
<tr>
<th>Days After Immunization</th>
<th>% Cycling CD4 Cells</th>
<th>% CD4 Cells in Draining LN&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(S + G2 + M)&quot;</td>
<td>Tg</td>
</tr>
<tr>
<td>0</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>20.4</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>15</td>
<td>1.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

CD4 DO11.10 Tg T cells were adoptively transferred into BALB/c mice. Mice were immunized with OVA + CFA, and Tg and Tg Tg CD4 cells were isolated from LN at the indicated days after Ag immunization. The data in this table and in Figure 2 were generated in the same experiment.

"Cycling cells were detected by propidium iodide incorporation.

"Cell suspensions were analyzed by flow cytometry after staining with phycoerythrin anti-CD4 and biotin-KJ1-26, LN = lymph node.

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**FIGURE 2.** Comparison of telomerase activity induced in vivo in Tg and Tg CD4 T cells. A, Telomerase activity in in vivo Ag-stimulated Tg CD4 T cells. Adoptively transferred CD4 Tg T cells were isolated from mice that had been immunized with OVA + CFA 0, 3, 5, or 15 days previously. Telomerase activity was detected by serial dilutions of telomerase extracts. The 1:1 dilution shows telomerase activity present in 2 × 107 cell equivalents. B, Relative telomerase activity detected by serial threefold dilutions of telomerase extracts prepared from 2 × 107 Tg and Tg CD4 T cells isolated 0, 3, 5, and 15 days after Ag immunization.
and differentiation. One mechanism hypothesized to regulate and extend the replicative capacity of normal somatic cells is the control of telomerase length through the activity of telomerase. A limited number of studies have examined the expression of telomerase activity in mouse tissues and reported low levels of activity in a variety of mouse tissues (29, 30); and Ogoshi et al. (31) reported that telomerase activity was induced in murine T cells cultured in vitro with mitogenic stimuli. However, the regulation of telomerase activity during defined immune responses has not been characterized. The studies presented here analyzed, for the first time, the expression of telomerase activity in murine T cells in response to both in vitro and in vivo Ag-specific stimulation.

To investigate the signals that regulate telomerase induction in lymphocytes under conditions of Ag-specific stimulation, we examined the relative contributions of Ag-specific TCR signals and CD28 costimulatory signals to telomerase induction in T cells. T cells are activated by the engagement of their Ag-specific TCR with MHC complexes on the APC in concert with costimulatory signals provided through the engagement of CD28 by its ligands B7-1 (CD80) and/or B7-2 (CD86) on the APC (32, 33). To date, controversy exists in the literature as to whether TCR signals are sufficient for optimal telomerase induction or whether CD28-B7 costimulatory signals are also necessary (14, 34). The results reported in the present study demonstrate for the first time that CD28-B7 costimulatory signals are required for the optimal induction of telomerase activity in T cells that are activated under APC-dependent conditions in response to an Ag-specific TCR stimulus. Since, in the absence of CD28-B7 costimulation, addition of exogenous IL-2 can restore telomerase activity, this result suggests that the telomerase induction may result from altered IL-2R signals generated by CD28-dependent IL-2 production (31). CD28-B7 stimuli, which regulate telomerase activity in vitro, may play a similar regulatory role during immune responses in vivo.

The studies presented here examined for the first time the regulation of telomerase activity in T cells challenged in vivo with an Ag-specific stimulus. Whereas unstimulated TCR Tg+ T cells expressed low or undetectable amounts of telomerase activity, Ag-specific in vivo stimulation of Tg+ CD4+ T cells resulted in substantial induction of telomerase activity in these cells 3 to 5 days after antigenic challenge, with activity substantially reduced by 15 days after Ag challenge. Further, the kinetics of induction of telomerase activity correlated with in vivo cellular expansion and the appearance of cycling Ag-reactive Tg+ CD4+ cells. Tg+ CD4+ cells isolated from these same mice had low levels of telomerase activity at day 0, perhaps corresponding to cycling CD4+ cells activated by endogenous Ags, but this low level activity was not significantly enhanced by Ag administration. These results suggest that induction of telomerase activity in Ag-reactive T cells may represent an adaptive response that functions to preserve or extend the replicative potential of Ag-specific lymphocyte populations.

The regulation of immune responses to antigenic challenge depends upon expansion and differentiation of responsive cells. One mechanism hypothesized to modulate the proliferative capacity of normal somatic cells is the regulation of telomere length by telomerase. The results presented here are consistent with a model in which stimuli that result in the proliferation and expansion of normal lymphocytes during an immune response also result in the induction of telomerase activity, serving to maintain telomere length and replicative potential. Thus, interventions designed to inhibit telomerase activity in vivo, for example treatment of malignant tumors, may have significant inhibitory effects on beneficial immune responses as well. A direct analysis of the role of telomerase in normal immune function may be facilitated by investigating genetically engineered mice with altered telomerase activity (35) or by transfection of telomerase components into somatic cells (36).

Acknowledgments

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References


