T cells maintain a quiescent state prior to activation. As inappropriate T cell activation can cause disease, T cell quiescence must be preserved. Despite its importance, the mechanisms underlying the "quiescent state" remain elusive. Here, we identify BTG1 and BTG2 (BTG1/2) as factors responsible for T cell quiescence. BTG1/2-deficient T cells show an increased proliferation and spontaneous activation due to a global increase in messenger RNA (mRNA) abundance, which reduces the threshold to activation. BTG1/2 deficiency leads to an increase in polyadenylate tail length, resulting in a greater mRNA half-life. Thus, BTG1/2 promote the deadenylation and degradation of mRNA to secure T cell quiescence. Our study reveals a key mechanism underlying T cell quiescence and suggests that low mRNA abundance is a crucial feature for maintaining quiescence.

Fig. 1. BTG1 and BTG2 are mainly expressed in quiescent T cells. (A) Super-enhancers are ranked by normalized H3K27ac chromatin immunoprecipitation sequencing signals. Super-enhancer signals are adopted from (12). (B) Tissue-specific expression of BTG1/2 in the immune system. RNA-seq data are adopted from Illumina Body map 2.0 project (PRJEB2445). TPM, transcripts per million. (C) Relative expression of Btg1/2 in naive and memory CD4 T cells. (D) Decrease of Btg1 and Btg2 expression upon TCR stimulation. Mouse naive CD4 and CD8 T cells were cultured with antibodies against CD3 (α-CD3) (5 μg/ml) and α-CD28 (2 μg/ml) for 5 days. The expression of Btg1/2 was quantified by reverse transcription–quantitative polymerase chain reaction (RT-qPCR) (top) and immunoblotting (bottom), respectively. Error bars represent SEM (n = 2). Each symbol represents the mean of two independent experiments with two technical replicates.

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their specific role in the immune system (Fig. 1B). The same pattern was observed in mouse tissues (fig. S1B). Next, we confirmed that Btg1/2 were mainly expressed in naïve and memory T cells rather than in other T cell subsets (Fig. 1C). This distinct pattern was similar to that of known quiescence markers, such as Foxo1, Il7r, and Klf2. Like those factors, both mRNA and protein abundances of Btg1/2 were rapidly reduced upon TCR stimulation and remained low during the activated state (Fig. 1D and fig. S1C). Thus, Btg1 and Btg2 are selectively expressed in quiescent T cells.

To understand their roles in vivo, we generated Btg1 and Btg2 conditional knockout

Fig. 2. Loss of Btg1 and Btg2 results in T cell quiescence defects. (A) Representative dot plots of naive and effector and memory T cells from the spleens of WT and DKO mice (top). Populations are represented as means ± SD. Frequencies and numbers of naive and effector memory CD4 and CD8 T cells in steady-state mice (n = 6 to 11) (bottom). (B) Ki-67 production by CD4 or CD8 T cells was measured from WT and DKO spleens (n = 6 mice per group). (C) Size of WT or DKO naive T cells was measured by flow cytometry (n = 6 mice per group). (D) Cell-cycle analyses by 4’,6-diamidino-2-phenylindole (DAPI) and Ki-67 staining from naive T cells (n = 4). (E and F) In vitro T cell proliferation assays. Naive T cells were cultured for 4 days with plate-bound α-CD3 (0, 1.25, 2.5, and 5.0 μg/ml) and α-CD28 (2 μg/ml). (E) Representative dot plots showing the production of IL-2 and Ki-67 in WT and DKO cells (top). Populations are represented as means ± SD. Cell divisions are indicated by labeling with CellTrace Violet (CTV) (bottom). Populations of >1 division are represented as means ± SD. (F) Flow cytometry plots show the cell-surface expression of IL2RA, IL2RG, CD44, and CD69 (n = 4 mice per group). (G) Weight-loss curve after adoptive transfer of naive CD4 T cells to Rag2 KO mice. Mice were monitored for 7 weeks (WT, n = 10; DKO, n = 8). (H) Colitis score was recorded by colonoscopy. A box represents the first and third quartiles, and an internal bar indicates median. Error bars in (A) and (B) represent SD, and in (D) to (G) represent SEM. P values <0.05 were considered significant (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001); unpaired Student’s t test was used for (B) to (G). Mann–Whitney U-test was used for (H).
mice using CRISPR-Cas9 technology (16). We confirmed correct insertions of two loxP sites in both Btg1/2 and checked their efficient deletion after crossing with Cd4-Cre transgenic mice (fig. S2). T cell development in the thymus and spleen of knockout (KO) mice did not differ markedly from wild-type littermate controls (WT) (fig. S3A). However, Btg1 and Btg2 double conditional KO (DKO) mice showed decreased numbers and frequencies of naïve CD4 and CD8 T cells compared to WT in the periphery (Fig. 2A). Single-KO mice did not show any significant changes, suggesting that Btg1/2 are functionally redundant (fig. S3B). Btg1/2 are indeed more closely related to each other than to their family members on the basis of their phylogenetic tree (fig. S4) (13, 14).

Consistent with the reduced numbers and proportions of naïve T cells in DKO mice, the amounts of Ki-67 were significantly increased (Fig. 2B). Moreover, there was a significant increase in cell size and a concomitant reduction in the proportion of cells in G0 phase (Fig. 2, C and D, and fig. S5), indicating that T cell quiescence is compromised in the absence of Btg1/2.

To determine if DKO T cells are more prone to escape from quiescence, we performed in vitro T cell proliferation assays (Fig. 2E and fig. S6). Even under weak TCR stimulation conditions, DKO naïve T cells readily underwent clonal expansion as evidenced by the production of interleukin-2 (IL-2) and Ki-67, and dilution of a cell division–tracking dye (Fig. 2E). By contrast, WT T cells barely proliferated under the same conditions. There were also substantial changes in the amounts of IL2RA, IL2RG, CD44, and CD69, indicating enhanced proliferation and activation (Fig. 2F). DKO naïve T cells could proliferate even in the presence of tonic cytokines (IL-2 and/or IL-7) alone in the absence of any TCR stimulation (fig. S7). Thus, DKO T cells appear to be overly sensitive to activation signals and can easily overcome the activation threshold.

In support of the proclivity of DKO cells to exit quiescence, DKO naïve T cells adoptively transferred to Rag2-deficient mice significantly exacerbated colitis (Fig. 2, G and H). DKO naïve T cells underwent more activation and proliferation with accompanying...
pathogenic T cell responses in vivo (fig. S8). BTG1/2 appear to play an indispensable role in limiting spontaneous activation of T cells to prevent autoimmune disease. Similarly, upon *Listeria monocytogenes* infection, the number of pathogenic T cells in DKO mice increased more than in WT, which in turn resulted in the rapid clearance of *Listeria* (fig. S9). Thus, BTG1/2 maintain T cell quiescence through controlling proliferation and activation in vivo and in vitro.

To gain insight into the molecular mechanism underlying T cell quiescence, we carried out total RNA sequencing (RNA-seq) on naïve T cells from WT or DKO mice. Intriguingly, we observed a modest but global increase [5610 out of 6317 (88.8%)] in mRNA abundance in DKO T cells (Fig. 3A and table S1). This shift in the entire transcriptome may imply an alteration in the general machinery, such as deadenylases. We validated the global increase by normalizing cytoplasmic mRNA abundance to that of mitochondrial encoded mRNAs (mtRNAs) and spike-in RNAs (materials and methods) (17, 18). Intriguingly, we confirmed a similar increase in mRNA abundance by both methods, whereas mtRNAs remain unaffected (Fig. 3, B and C, and fig. S10). Because the vast majority of transcripts were up-regulated, we next asked which processes were modulated. Consistent with earlier data (Fig. 2), pathways related to activation and proliferation of the immune system were particularly enriched (Fig. 3D and fig. S11). Thus, mRNA and protein abundance was reflected in an increased number of functional proteins, we measured mean fluorescence intensity (MFI) by flow cytometry. The fluorescent intensity (MFI) by flow cytometry. The proteins that we tested were significantly increased in abundance in both CD4 and CD8 T cells (Fig. 3E). Notably, the protein abundance of stimulatory genes whose mRNAs were relatively profuse in naïve T cells was increased in abundance in both CD4 and CD8 T cells (Fig. 3E). Notably, the protein abundance of stimulatory genes whose mRNAs were relatively profuse in naïve T cells was increased, implying a lowered threshold for activation (fig. S11). Thus, mRNA and protein abundance was up-regulated in the absence of BTG1/2, which likely results in the activation of naïve T cells.

The transcriptome-wide increase in mRNA abundance hinted that BTG1/2 may play a role in mRNA stability control. Because BTG1/2 have been shown to associate with poly(A)-binding protein (PABP) and subunits of CCR4–NOT (CNOT) deadenylase complex in vitro (19–23), we examined whether BTG1/2 interact with PABP and/or the core deadenylase machinery in naïve T cells. By communoprecipitation and in situ proximity ligation assay, BTG1/2 were shown to be physically associated with PABP and CNOT7 in naïve T cells in an RNA-independent manner (Fig. 4, A and B). Furthermore, deletion mutants of BTG1 that lost the ability to interact with either PABP or CNOT7 failed to inhibit proliferation (fig. S12). Thus, BTG1/2 functions are critically dependent on the interaction with PABP and CNOT7.

Next, to assess whether mRNA turnover is impaired in DKO cells, we measured the decay rates of mRNAs. Notably, decay rates of cytoplasmic mRNAs, but not mtRNAs, were retarded in DKO T cells compared to WT (Figs. 4, C and D; figs. S13 and S14; and table S3). mRNA decay rates in activated T cells, where BTG1/2

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**Fig. 4.** BTG1 and BTG2 promote mRNA deadenylation and decay in naïve T cells. (A) Communoprecipitation with antibodies against PABP, CNOT7, BTG1, BTG2, and control immunoglobulin G (R, rabbit; M, mouse) with or without ribonuclease A (RNase A) treatment. (B) Direct interactions between BTG1 or BTG2 with CNOT7 or PABP were detected as red dots in WT naïve T cells, but not in DKO cells. Scale bars indicate 2 μm. (C) Mean poly(A) tail length distribution determined by mTAIL-seq from WT and DKO naïve CD4 T cells. The median poly(A) length of each sample is shown in parentheses and marked by vertical lines. (D) Half-lives are calculated from (C) by linear fitting of the log-transformed exponential decay function. (E) Global poly(A) length distribution determined by mTAIL-seq from WT and DKO naïve CD4 T cells. The median poly(A) length of each sample is shown in parentheses and marked by vertical lines. (F) A scatter plot showing geometric mean poly(A) length. Transcripts supported by >20 mean poly(A)+ tags are shown (n = 3). Red dots represent mRNA transcripts. (G) Schematic illustration of how naïve T cells maintain the quiescent state.**
are no longer expressed, showed no difference in mRNA degradation between WT and DKO cells (fig. S15), suggesting that BTG1/2 contribute to lowering mRNA amounts through mRNA turnover in naïve but not activated T cells.

To examine if poly(A) length is indeed elongated in DKO cells, we performed mTAIL-seq (fig. S16A) (24). In line with the stabilization of mRNA, the poly(A) tail length of DKO T cells was longer than that of WT (Fig. 4, E and F, and table S4). Again, mtrNA poly(A) tail lengths were nearly unchanged (Fig. 4F), suggesting that BTG1/2-mediated deadenylation was responsible for degrading the bulk of cytoplasmic mRNA in naïve T cells. Notably, the difference in poly(A) tail length in naïve T cells was diminished in activated T cells (fig. S16, B and C, and table S5).

In conclusion, we propose a model to explain how the quiescent state in T cells is maintained (Fig. 4G). BTG1 and BTG2 are highly and specifically expressed in quiescent T cells and promote mRNA deadenylation and degradation. Unlike BTG1/2, deadenylases appear to be ubiquitously expressed at a low level, implying a specialized function of BTG1/2 in immune cells (fig. S17). Because BTG1/2 interact with PABP and the CNOT complex, which bind nonspecifically to mRNAs (25), BTG1/2 can direct the down-regulation of mRNAs at a global level. This mechanism is seemingly inefficient in terms of cost, but the availability of presynthesized mRNA provides a benefit to quiescent T cells of a rapid response to activation signals. Given that naïve T cells differentiate into multiple lineages, having such a primed state on a hair trigger would be ultimately beneficial. Upon activation, BTG1/2 quickly disappear, which results in an accumulation of mRNAs and exit from the quiescent state (Figs. 1D and 4G).

Here, we show the functional consequences of BTG1/2-mediated deadenylation in vivo. B cells may also use a similar mechanism to maintain quiescence through BTG1/2, as BTG1/2 are commonly dysregulated in leukemia and lymphoma (15–28). Likewise, other cofactors, in addition to BTG1/2, may recruit and enhance mRNA deadenylation and degradation in certain cell types. Thus, the mechanism that we propose may be broadly applicable to other cells and tissues, serving as a general system to secure the quiescent state.

REFERENCES AND NOTES

SUPPLEMENTARY MATERIALS
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Data and materials availability: Sequenced reads have been deposited in the NCBI Gene Expression Omnibus (GEO) database (accession no. GSE125890).
mRNA destabilization by BTG1 and BTG2 maintains T cell quiescence
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Deadenylate or activate?
When cells are quiescent, they undergo reversible cell cycle arrest and evince low basal metabolism. Naïve T cells are normally quiescent until they recognize cognate antigens through T cell receptor–costimulatory molecule signaling. T cell quiescence appears to be an active process, but the mechanistic details are poorly understood. Hwang et al. report that the transcription factors BTG1 and BTG2 are selectively expressed in quiescent T cells. In mice, T cells conditionally knocked out for both factors showed enhanced proliferation and a lowered threshold of activation both in vitro and in response to Listeria monocytogenes infection. Deficiency of BTG1 and BTG2 resulted in increases in global messenger RNA half-life, suggesting that messenger RNA deadenylation and degradation are important processes for maintaining T cell quiescence.

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