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A Deficiency in Nucleoside Salvage Impairs Murine Lymphocyte Development, Homeostasis, and Survival

Onjee Choi,* Dean A. Heathcote,*† Ka-Kei Ho,‡,† Phillip J. Müller,* Hazim Ghani,* Eric W.-F. Lam,† Philip G. Ashton-Rickardt,* and Sophie Rutschmann*

The homeostasis of the immune system is tightly controlled by both cell-extrinsic and -intrinsic mechanisms. These regulators, not all known to date, drive cell in and out of quiescence when and where required to allow the immune system to function. In this article, we describe a deficiency in deoxycytidine kinase (dCK), one of the major enzymes of the nucleoside salvage pathway, which affects peripheral T cell homeostatic proliferation and survival. As a result of an N-ethyl-N-nitrosourea–induced mutation in the last α helix of DCK, a functionally null protein has been generated in the mouse and affects the composition of the hematopoietic system. Both B and T lymphocyte development is impaired, leading to a state of chronic lymphopenia and to a significant increase in the number of myeloid cells and erythrocytes. In the periphery, we found that mutant lymphocytes adopt a CD44highCD62Llow memory phenotype, with high levels of proliferation and apoptosis. These phenotypes are notably the result of a cell-extrinsic–driven lymphopenia-induced proliferation as wild-type cells transferred into DCK-deficient recipients adopt the same profile. In addition, DCK also regulates lymphocyte quiescence in a cell-intrinsic manner. These data establish dCK as a new regulator of hematopoietic integrity and lymphocyte quiescence and survival. The Journal of Immunology, 2012, 188: 000–000.

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tivation of these compounds to cytotoxic nucleoside triphosphate derivatives (19–21).

In this study, we report a null murine dCK mutant strain, memory IL-7R phenotype (memi), which presents, in addition to lymphocyte developmental deficiencies, changes in peripheral hematopoietic populations and a very high rate of cellular proliferation associated with an increased level of cell death in peripheral lymphocytes. Our data indicate that this increased proliferation is due to both cell-extrinsic and -intrinsic effects. Our mouse model therefore supports an unexpected nonredundant role for dCK in homeostasis and survival of immune cells.

Materials and Methods

Mice, mutagenesis, and lymphocytic choriomeningitis virus infection

The memi mutant mice were generated in a C57BL/6J background (C57BL/6J, Thy1.1, and 129SvImJ purchased from Charles River, Lille, France) and bred in Central Biomedical Services (Imperial College London, London, U.K.). Mice were kept under specific pathogen-free conditions in individually ventilated cages. All animal procedures have been authorized in a British Home Office Animals Act License (PPL:70/6490). The N-ethyl-N-nitrosourea (ENU) mutagenesis was performed as previously described (22). For the infection, mice were injected i.p. with 2 × 10^7 PFU lymphocytic choriomeningitis virus (LCMV) Armstrong. At days 8 and 21 after the injection, lateral vein blood was analyzed by flow cytometry.

Cell preparation and flow cytometry

Spleen and blood cells were incubated 5 min at room temperature in an RBC lysis buffer (0.85% [w/v] NH4Cl, 50 mM Tris, pH 7.2). All Abs were from eBioscience (Hatfield, U.K.) except CD8 and TCRVβ derivatives (19–21). Three to 5 million cells from those donor groups were injected i.v. to nonirradiated Lane Bisley, U.K.). Three to 5 million cells from those donor groups were injected i.v. to nonirradiated

The 7dCK exons were amplified from genomic DNA by PCR using the JumpStart REDTaq ReadyMix (Sigma). PCR samples were purified by Qiagen PCR purification kit (Qiagen, Crawley, U.K.), sequenced (Genomic Biotech). The BM cells were then mixed at a 1:1 ratio, and 10^6 were transplanted to lethally irradiated (900 rad) non-TBI C57BL/6J mice. These were analyzed 5 wk after transplantation. Mice were given freshly prepared 0.8 mg/ml BrdU (Sigma, Waltham, MA) and survival of immune cells. mouse model therefore supports an unexpected nonredundant role for dCK in homeostasis and survival of immune cells.

Statistical analysis

All results are given as means with SD. Nonparametric unpaired t test (two-tailed) was applied: *p < 0.05, **p < 0.01, ***p < 0.001. All statistical calculations were performed with the Prism5 software (GraphPad).

Results

The ENU-induced mutation memi impairs the immune response to viral infection

To identify new regulators of immune homeostasis, we have undertaken a germline mutagenesis and used the CD8^+ T cell response to LCMV infection as a model to screen individual mutant mice. To this end, we have generated a library of third-generation ENU-induced germline mutants in a pure C57BL/6J background. A total of 1480 individual mice were screened for modifications of immune responsiveness to LCMV. Their CD8^+ response was assessed by measuring the percentage of Ag-specific CTL (gp33^+ CD8^+) and of effectors and memory precursors in the blood at the peak of expansion (day 8 postinfection) and at the end of contraction (day 21). Four independent germline transmissible mutations that modify responsiveness to LCMV were isolated, of which a purely recessive one, memi, was bred to homozygosity. After LCMV infection, homozygous memi mutants mount an Ag-specific CD8^+ T cell response, which is highly variable between individuals (Fig. 1A). The Ag-specific population in memi mice is significantly enriched in effector cells (KLRG1^+IL-7Rα^+CD8^+) and of effectors and memory precursors in the blood at the peak of expansion (day 8 postinfection) and at the end of contraction (day 21).

To identify the causative memi mutation, we used a meiotic positional cloning strategy (25). Mice carrying the memi mutation were crossed to the 129SvImJ strain to obtain first-generation hybrids, which when intercrossed generated 21 second-generation hybrids. These 42 meiotic events were analyzed for the linkage between 358 single nucleotide polymorphisms and the deficiency in CD4^+ and B220^+ blood lymphocytes, mapping the memi mutation to a 13.8-Mb region on chromosome 5 with a logarithm of odds score of 5 (data not shown). This region...
contains 112 genes and pseudogenes (ensembl version 37.2), notably dCK encoding DCK, which was considered a strong candidate based on the T cell phenotype described for the knockout mutant (dCK<sup>−/−</sup>) (18). Sequencing of dCK at the genomic DNA level in memi mice revealed a single nucleotide transversion (G to T) at nucleotide 739 (Fig. 2A). This modification changes the glutamic acid 247 into an early translational terminating codon, removing the last 14 aa of DCK.

To define the nature of the memi mutation, we measured DCK-specific enzymatic activity in dCK<sup>mem/mem</sup>, dCK<sup>mem/+</sup>, and dCK<sup>+/+</sup> splenocytes. L1210 and L1210-10K cell lines were used as positive and negative controls (26). The graph is representative of two independent experiments in which DCK activity was analyzed in triplicate with samples pooled from three mice per genotype.

B and T lymphocyte development defects in dCK<sup>mem/mem</sup> mice

The analysis of dCK<sup>−/−</sup> mice implicated a role for the kinase in T and B cell development (18). The analysis of B cell development showed a significant increase in the pro-B cell fractions A–C, correlating with a significant decrease in the pre-B cell fraction D (Supplemental Fig. 2). dCK<sup>mem/mem</sup> thymuses were found to be reduced in size and cellularity (Fig. 3A). In the thymus, T cell precursors develop via the following chronological stages: double-negative 1 (DN1; CD4<sup>−</sup>CD8<sup>−</sup>CD44<sup>−</sup>CD25<sup>−</sup>), DN2 (CD44<sup>−</sup>CD25<sup>−</sup>CD44<sup>−</sup>CD25<sup>−</sup>), DN3 (CD44<sup>−</sup>CD25<sup>−</sup>CD25<sup>−</sup>).
population (n intracellular TCR-V populations, CD4+ T and B220+ B cells were consistently de-


gindicate that the mutant peripheral lymphocytes (Fig. 3E). Taken together, our data


ys to the

pre-B and DN3 to DN4 T lymphocyte development in a manner

only slightly reduced levels of extracellular TCRb

expression (27, 28). The expression of CD25 was similar to wt

3C), a phenomenon observed in mice with defective pre-TCR

signaling (27, 28). This impairment is,

mice have profound defects in lymphocyte development. (A) dCKmem/mem mice have reduced thymic size and cellularity. Thymi of two

representative mice are indicated by the white lines. The graph is representative of four independent experiments; n ≥ 3. (B) dCKmem/mem T cells development is blocked at the transition between the DN3 and DN4 stages. The dot plots are from two representative mice, the graph representative of three independent experiments; n = 5. Proliferation of mutant DN3 and DN4 cells was assessed by measuring Ki67 expression (middle graph) or BrdU incorporation (right graph). The graphs are representative of two independent experiments; n ≥ 4. (C) The mean fluorescence intensity (MFI) obtained for CD25 expression in the DN3 population (left panel) and in peripheral cells (right panel) is indicated. These data are representative of three independent experiments; n ≥ 4. (D) Expression of intracellular TCR-Vß8.1/8.2 in DN3 thymocytes. The histograms are of two representative mice with TCRb− DN1 control (gray) and DN3 cells (black). The graph is representative of two independent experiments; n ≥ 4. (E) Surface expression of TCRb on peripheral T lymphocytes. The histograms are of two representative mice and the data representative of two independent experiments; n ≥ 4. In all experiments, error bars represent SD; *p < 0.05, **p < 0.01, ***p < 0.001.

CD25+) DN4 (CD4−CD25−), double positive (CD4+CD8+), and

finally, single positive (CD4+ or CD8+). In dCKmem/mem thymus, we observed a developmental blockage between the DN3 and

DN4 stages (Fig. 3B). Proliferation was significantly higher in mutant cells as shown by the increased expression of Ki67, a protein expressed in all cycling/nonquiescent cells. Interestingly, when assessed by BrdU incorporation over a 5-d period, proliferation was found to be increased only in DN3 cells and wt in

DN4 cells. Remarkably, the IL-2Rα-chain CD25 was expressed at

significantly higher levels by the mutant DN3 population (Fig. 3C), a phenomenon observed in mice with defective pre-TCR

signaling (27, 28). The expression of CD25 was similar to wt

levels on peripheral cells. Finally, we were able to measure wt

levels of intracellular TCRβ in mutant DN3 cells (Fig. 3D) and

only slightly reduced levels of extracellular TCRβ expression on

mutant peripheral lymphocytes (Fig. 3E). Taken together, our data indicate that the memi mutation dramatically impairs both pro-

to pre-B and DN3 to DN4 T lymphocyte development in a manner

similar to the dCK−/− knockout model (18). This impairment is,

however, incomplete, because some B and T cells are found in the periphery (Fig. 1C).

Splenomegaly and spleen composition in dCKmem/mem mice

In the periphery, we found that dCKmem/mem mice had much en-
larged spleens (Fig. 4A), with a significant increase in the absolute number of Ter119−erythrocyte precursors and of myeloid cells (CD11b+, CD11c+, Gr1+, or Ly-6A+; Fig. 4B). In the lymphocytic populations, CD4+ T and B220+ B cells were consistently de-

creased in numbers, whereas CD8+ T lymphocytes were present in

absolute cell numbers equivalent to wt controls.

Increased proliferation of dCKmem/mem peripheral T lymphocytes

Our observation that LCMV-specific CD8+ T cells expressed high

levels of KLRG1, a marker associated with replicative senescence (Fig. 1B), prompted us to investigate the proliferative status of

dCKmem/mem peripheral T cells. BrdU incorporation was signifi-

cantly increased in both mutant T cell subpopulations (Fig. 5A)

and confirmed by the increased expression of Ki67 (Supplemental

Fig. 3). In vivo, this increase correlated with a significantly greater

cell with an effector memory phenotype, CD4+high

CD62Llow (Fig. 5B), and with an increased level of activation

(Supplemental Fig. 3) (29). This memory phenotype in the ab-

sence of Ag could imply a role for DCK in homeostatic prolif-

eration.

To further confirm the change in proliferative status, we analyzed

splenocytes ex vivo for their cell-cycle progression pattern (Fig. 5C). We observed a significant decrease in the G0/G1 population,

correlating with a significant increase in S-phase cells (Supplemental

Fig. 4). We investigated cell-cycle regulators in whole-

spleen extracts from dCKmem/mem, dCKmem/+ mice, and dCK+/+ mice (Fig. 5D). In dCKmem/mem cells, both cyclin D3 and cyclin A were expressed at higher levels than in wt controls, whereas p27Kip1 and pRb levels were decreased or undetectable. Protein extracts from lymph nodes confirmed these data. Finally, we found that the transcription factor Foxo3a was constitutively inactivated in

dCKmem/mem splenocytes. These data indicate that the decreased

quiescence observed in dCKmem/mem peripheral lymphocytes correlates with changes in the expression of cyclins and their co-

regulators.
we observed a significant augmentation in Thy1.1;dCK+/+ donor cells in donor and endogenous cells 35 d after injection (Fig. 6C). We was confirmed when we measured Ki67 expression in splenic cell ratio in the CD4+ compartment (Fig. 6B), suggesting an in-
crease in nonlymphoid cells. (Supplemental Fig. 4), confirming that

A

![Graph](image)

**FIGURE 4.** dCKmem/mem mice present an important splenomegaly because of an increase in nonlymphoid cells. (A) Spleens from two representative dCKmem/mem and dCKmem/+ mice. The relative spleen weights were obtained by dividing individual spleen weights by the corresponding total body weight; n = 5. (B) Spleen composition in dCKmem/mem and dCKmem/+ mice. Graph is representative of three independent experiments; n ≥ 3. Similar data have been obtained for B cells with a CD19-specific Ab.

**Increased apoptosis of dCKmem/mem peripheral lymphocytes**

The significant increase in proliferation observed in dCKmem/mem CD4+ T cells was not sufficient to restore absolute cell numbers equivalent to those found in wt controls, suggesting that survival might also be affected by the mutation. We therefore measured the population of Annexin V+/PI- lymphocytes and found that both CD4+ and CD8+ T cells displayed a significant increase in apoptosis (Fig. 5E). This correlated with an increase in the amount of active caspase-3 (Supplemental Fig. 4), confirming that memi mutant T cells undergo a greater rate of apoptotic cell death in the periphery. dCKmem/mem increases proliferation and apoptosis in both a cell-intrinsic and -extrinsic manner

We next asked whether the proliferation, the cell death, or both resulted from a cell-intrinsic or -extrinsic effect of the mutation. To do so, we transferred either Thy1.1;dCK+/+CD4+ T cells into Thy1.2; dCKmem/mem recipients or Thy1.2; dCKmem/mem CD4+ T cells into Thy1.1;dCK+/+ recipients (Fig. 6A). At various time points, recipient blood was analyzed for the ratio of donor cells in the CD4+ compartment. In memi hosts 6 d after injection onward, we observed a significant augmentation in Thy1.1;dCK+/+ donor cell ratio in the CD4+ compartment (Fig. 6B), suggesting an increase in wt donor cell proliferation. This increase in proliferation was confirmed when we measured Ki67 expression in splenic donor and endogenous cells 35 d after injection (Fig. 6C). We indeed found that Ki67 expression was significantly higher in Thy1.1;dCK+/+ donor cells in memi recipients than in Thy1.1; dCK+/+ endogenous wt recipients.

In wt hosts, Thy1.2; dCKmem/mem donor cell ratios remained very low (Fig. 6B), indicating that either memi cells have reverted to a quiescent naive phenotype or their level of proliferation is compensated by a high level of cell death. Interestingly, the analysis of Ki67 expression in the splenocytes of wt recipients (Fig. 6C) indicated that the level of proliferation in Thy1.2; dCKmem/mem donor cells was equivalent to the Thy1.2; dCKmem/mem+ endogenous cells in memi recipients and significantly higher than the Thy1.1;dCK+/- endogenous cells of the wt host. These data would indicate that even 35 d after transfer into a wt environment, the DCK-deficient memi cells are still highly proliferative. We therefore analyzed cell death in the spleen of recipient mice 35 d after injection (Fig. 6D). We found that the level of apoptosis in Thy1.1;dCK+/+ donor cells in memi recipient was significantly greater than in both Thy1.2; dCKmem/mem and Thy1.1;dCK+/+ endogenous cells. As expected, the rate of apoptosis in Thy1.2; dCKmem/mem donor cells in wt recipients was high and equivalent to the one in Thy1.2; dCKmem/mem+ endogenous cells in the memi recipients.

The increased proliferation and maturation of wt CD4+ T cells in the memi host strongly supports a model of homeostatic proliferation in response to the lymphopenic DCK-deficient environment. This is accompanied by a high rate of apoptosis, as has been previously described for cells undergoing lymphopenia-induced proliferation (LIP) (30, 31). Interestingly, the data obtained with Thy1.2; dCKmem/mem+ donor cells in wt recipients seemed to indicate that the absence of functional DCK could also control proliferation and cell death in a cell-intrinsic manner. memi’s cell-intrinsic effect was further investigated in 1:1 chimera mice. Lethally irradiated wt recipient mice were injected with a 1:1 Thy1.2; dCKmem/mem+; Thy1.1;dCK+/+ mixture of BM cells depleted of their mature T cells. Five weeks after the injection, at least 95% of thymic and splenic cells were derived from wt progenitors, indicating that memi cells are outcompeted by wt cells during reconstitution (data not shown). In the thymus, the development of Thy1.2; dCKmem/mem+ thymocytes was impaired at the transition between DN3 and DN4 stages (Fig. 7A). In the periphery, both proliferation and apoptosis were significantly increased in Thy1.2; dCKmem/mem+ cells as compared with coinjected Thy1.1;dCK+/+ controls (Fig. 7B). Altogether, these data indicate that memi impairs thymocyte development and induces excessive proliferation and apoptosis of peripheral T lymphocytes in a cell-intrinsic manner.

**Discussion**

Our study describes a new point mutation in dCK and shows that it qdoes not only impair lymphocyte development but also reduces quiescence and survival of peripheral hematopoietic cells. Our dCKmem/mem null mutation affects lymphocyte development in a manner similar to that reported for deletional deficiency in the dCK-/− knockout mouse (18). The phenotypes of both dCK+/− and dCKmem/mem mice indicate that DCK is dispensable for non-hematopoietic developmental programs, but demonstrate an essential and nonredundant role for the protein in lymphopoiesis. In addition, our point mutation memi reveals an unexpected role for the nucleoside salvage pathway in the regulation of T cell ho-

In the BM, memi B cell development is impaired at the transition between pro-B B220+B220−CD43+ and pre-B B220−CD43− cells. These are the stages at which Ig heavy V(D)J rearrangement takes place. In T cells, V(D)J recombination and TCRβ rearrangement take place at the DN3 stage. Cells with TCRβ that cannot rearrange properly die of apoptosis. The TCRβ-chain covalently couples to the pre-T α-chain and CD3 to form the pre-TCR complex. In the absence of pre-TCR signaling, thymocyte development is severely affected at the DN3 to DN4 transition.

In their article, Toy et al. propose two major hypotheses that could account for the T lymphocyte development defects observed in dCK-/− mice (18). The first one would be that a deficiency in...
DCK and deoxynucleotide salvage impairs the considerable proliferation occurring at the DN3 stage, therefore reducing the DN4 population. However, in our hands with Ki67 expression, proliferation is significantly higher in mutant thymocytes (Fig. 3B). It therefore seems unlikely to us that impaired proliferation is the sole reason for the failings in lymphocytes development. The less pronounced increase in proliferation obtained with BrdU could be because of a high level of cell death in the mutant and the resulting loss of some BrdU+ cells. Toy et al. also suggest that a defect in DCK could impair V(D)J recombination (18). In our mutant, we observe a significantly higher level of expression of the CD25/IL-2R α-chain in DN3 cells (Fig. 3C), as observed in cells from mice lacking pre-TCR signaling (27, 28). This could therefore indicate that a defect in dCK might impair TCRβ production or signaling. We were able to measure wt levels of intracellular TCRβ in mutant DN3 cells (Fig. 3D), supporting a normal V(D)J TCRβ recombination in DCK-deficient thymocytes, and therefore pointing toward an impaired TCR signaling in DCK-deficient cells. The phenotypes observed in memi mice are comparable with pre-Tα-deficient mice in which development is partially blocked between DN3 and DN4 stages and which present increased proliferation, apoptosis, and memory cells in the periphery (32). Under ADA-deficient conditions, the accumulation of adenosine and deoxyadenosine has been shown to impair TCR signaling and directly induce apoptosis in developing thymocytes, respectively (33–35). Because deoxyadenosine is one of DCK substrate, we could therefore hypothesize that a similar mechanism is operating in memi thymus whereby, in the absence of a functional DCK, deoxyadenosine and its derivatives accumulate and affect early thymocytes development through cell-intrinsic impairment of TCR signaling. However, even if severe, this developmental defect is only partial because some cells overcome the DCK deficiency, continue their development, and migrate to the periphery. These cells might, however, represent a limited repertoire of specificity; that is, they are oligoclonal populations expanded by homeostatic proliferation as exemplified with the CD8+ T cell response to LCMV (Fig. 1A). Interestingly, the apparent absence of autoimmunity in this lymphopenic environment might be because of an increase in proportion of regulatory T cells, a hypothesis that deserves further investigation.

In the periphery, two phenomena that have not been described before in a DCK-deficient context are observed: reduced quiescence and increased apoptosis. Our transfers experiments (Fig. 6) have shown that the proliferation is notably a cell-extrinsic effect of the memi mutation as wt cells injected into a dCKmem/mem host undergo proliferation. In a normal peripheral lymphocytic environment, mature T cells remain quiescent for prolonged periods (4). This homeostatic survival relies on contact with self-peptide/MHC complex and IL-7. In an acute lymphopenic environment, these signals will become more available to the remaining naive T cells, resulting in LIP, which restores almost normal levels of T cells in the periphery. In animals chronically lymphopenic because of a complete or severe T cell defect, like TCRβ−/− or animals with SCID, some transferred T cells will adopt a form of rapid proliferation.
that is presumably driven by foreign Ags from commensal microflora (4). Finally, a third form of peripheral homeostatic proliferation has been observed when other γ-cytokines are present in excess and in which donor cells undergo a rapid proliferation (36, 37). In our immunodeficient dCKmem/mem mutants, we observe an important cell proliferation with an effector memory phenotype (CD44highCD62Llow). A similar phenotype is found on wt cells transferred into a memi environment (Fig. 6C and data not shown), clearly supporting a model of LIP. However, we also found that as late as 35 d after injection in wt hosts, dCKmem/mem cells still present a memory phenotype (CD44 high CD62Llow, data not shown), express high levels of Ki67 (Fig. 6C), and are apoptotic (Fig. 6D). If the lymphocyte proliferation and subsequent apoptosis was exclusively driven by the memi lymphopenic environment, we would expect that once in a wt immunocompetent host, dCKmem/mem cells would revert to a more naive quiescent phenotype. This is, however, not the case and the cell-intrinsic effect of memi on proliferation and cell death was further supported by the data we obtained with 1:1 chimeric mice (Fig. 7B). The link between an impaired dCK pathway and the continuous inhibition of the Foxo3a pathway that we have observed in memi cells deserves further investigation.

**FIGURE 6.** Proliferation and apoptosis are controlled by both cell-extrinsic and -intrinsic mechanisms. (A) Thy1.1;dCK +/+ CD4+ T cells were transferred into nonirradiated Thy1.2;dCKmem/mem recipients and Thy1.2; dCKmem/mem CD4+ T cells into nonirradiated Thy1.1; dCK +/+ hosts. All the panels are representative of two independent experiments with five recipients per group. (B) The percentage of Thy1.1; dCK +/+ donor cells in the memi recipient CD4 + compartment (black line) and of Thy1.2; dCKmem/mem donor cells in the wt recipient CD4 + compartment (gray line) is represented. (C) The proliferation of donor and endogenous cells was measured by Ki67 expression in recipients’ splenocytes 35 d after transfer. (D) Apoptosis of donor and endogenous cells was measured in recipients’ splenocytes 35 d after transfer. **p < 0.01, ***p < 0.001.

**FIGURE 7.** memi acts on thymocytes’ development and peripheral lymphocytes’ proliferation and apoptosis in a cell-intrinsic manner. Lethally irradiated wt recipient mice were injected with a 1:1 Thy1.2; dCKmem/mem:Thy1.1;dCKmem/mem mixture of BM cells depleted of their mature T cells. The recipient mice were analyzed 5 wk after the injection. (A) By gating on either Thy1.1+w or Thy1.2+ memi cells, thymocytes were analyzed in the recipient mice for their DN cell distribution. The histograms are from two representative mice and the graph from two independent experiments; n ≥ 4. (B) Apoptosis and proliferation were analyzed by gating splenic lymphocytes for either Thy1.1+ wt or Thy1.2+ memi populations and measuring Annexin V or Ki67 expression, respectively. The graphs are representative of two independent experiments; n ≥ 4. **p < 0.01, ***p < 0.001.
The second phenomenon observed is a significant increase in apoptosis. Cells transferred into chronic lymphopenic recipients and undergoing LIP never really manage to reconstitute a normal lymphoid cellularity (38–42). Instead, the donor cells reach a plateau after a few weeks in the host and remain significantly proliferating and susceptible to Bim- and Fas-dependent cell death (30, 31, 41). Therefore propose that the apoptosis observed in wt cells injected into chronically lymphopenic mermi recipients is a direct consequence of LIP, whereas the increased apoptosis observed in mermi cells is a cell-intrinsic effect of the mutation and could be a direct consequence of changes in their deoxynucleoside triphosphate pools (43–46).

We have presented in this article a new null-mutant allele in the gene coding for DCK, a crucial enzyme of the deoxynucleoside salvage pathway. The phenotypes described make a strong case for a vital, nonredundant, cell-intrinsic role for DCK during lymphocyte development. In addition, our study describes a crucial, nonredundant, and cell-intrinsic role for DCK in peripheral homeostatic proliferation and survival. The data presented in this article support an effect of both the DCK-deficient environment and a cell-intrinsic effect of DCK deficiency on homeostatic proliferation. Our study might also have exposed a potential important regulator of hematopoietic quiescence in human, and our mutant mouse might therefore be a valuable model of hematopoietic proliferative disorders in humans.

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Disclosures

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References