Reduced Lymphocyte Longevity and Homeostatic Proliferation in Lamin B Receptor-Deficient Mice Results in Profound and Progressive Lymphopenia

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The lamin B receptor (LBR) is a highly unusual inner nuclear membrane protein with multiple functions. Reduced levels are associated with decreased neutrophil lobularity, whereas complete absence of LBR results in severe skeletal dysplasia and in utero/perinatal lethality. We describe a mouse pedigree, Lym3, with normal bone marrow and thymic development but profound and progressive lymphopenia particularly within the T cell compartment. This defect arises from a point mutation within the Lbr gene with only trace mutant protein detectable in homozygotes, albeit sufficient for normal development. Reduced T cell homeostatic proliferative potential and life span in vivo were found to contribute to lymphopenia. To investigate the role of LBR in gene silencing in hematopoietic cells, we examined gene expression in wild-type and mutant lymph node CD8 T cells and bone marrow neutrophils. Although LBR deficiency had a very mild impact on gene expression overall, for common genes differentially expressed in both LBR-deficient CD8 T cells and neutrophils, gene upregulation prevailed, supporting a role for LBR in their suppression. In summary, this study demonstrates that LBR deficiency affects not only nuclear architecture but also proliferation, cell viability, and gene expression of hematopoietic cells. The Journal of Immunology, 2012, 188: 000–000.

The nuclear envelope comprises the outer and inner membranes, separated by luminal space, and is traversed by nuclear pore complexes. The inner nuclear membrane is lined with a scaffolding structure known as the lamina, which engages the nucleoplasm. The lamina is made up of filamentous proteins called lamins and several lamin-interacting inner nuclear membrane proteins, including the lamin B receptor (LBR). Although the structural role of the lamina in regulating many nuclear functions, including mitosis and meiosis, has long been recognized, there is increasing evidence that the tethering of heterochromatin to the nuclear periphery and interaction of the nuclear lamina with epigenetic modifiers and transcription factors is important for transcriptional repression (1–5). Key studies in Droso-sophila and humans have demonstrated low gene expression is correlated with lamina-associated domains (6, 7). In humans, these domains have also been shown to have a lower gene density than non-nuclear lamina tethered regions.

The LBR is an inner nuclear membrane protein with dual modality (8–11). It has an N-terminal region, comprising two globular domains, including a Tudor-like DNA binding domain, joined by a linker region, which protrudes into the nucleoplasm where it engages proteins of the nuclear lamina, including lamin B1/B2, heterochromatin protein 1 (HP1), heterochromatic methyl binding protein McCP2, and histones, and directly engages heterochromatin (4, 12–15). Through these interactions, LBR is thought to control nuclear architecture and to have a role in gene silencing. The distinct LBR C-terminal region is composed of eight putative transmembrane domains encompassing a conserved sterol reductase domain, which has been shown to possess enzymatic activity. The evolution and conservation of LBR in vertebrates as a protein with divergent functions has led to speculation that physical linkage of the two functional domains may have some significance in coordinating nuclear events.

Two rare conditions, Pelger–Huët anomaly (PHA) and Greenberg/HEM dysplasia, independently described in the early and late 1900s, respectively, were in 2002–2003 shown to arise from mutation of the same gene, the LBR gene (9, 11, 16–18). Greenberg dysplasia is a recessive in utero lethal disorder presenting as severe hydrops, short-limbed dwarfism, and disorganization of chondro-osseous (bone–cartilage) calcification. This condition has been attributed to defective cholesterol biosynthesis, resulting in an accumulation of intermediate sterol metabolites, and bears some hallmarks of diseases arising from malfunction of different enzymes within the cholesterol biosynthetic pathway. There is still dispute, however, over whether the phenotype is consistent with cholesterol deficiency or laminopathy, the later supported by redundancy with another δ 14 sterol reductase, DHCR14/Tms7sf2.
PHA, which occurs in individuals heterozygous for mutations of the LBR gene, was originally described as the presence of abnormal blood neutrophil morphology, visible as reduced lobularity, without any health implications (8, 9). “Pseudo PHA” can also result transiently from unrelated health conditions including infections, although whether this indicates a transient reduction in LBR expression or LBR modification is unknown. In rare cases, individuals with PHA have been described that have varying degrees of skeletal defects and mental retardation, reflecting homozygosity of hypomorphic alleles.

In mice, homozygous null mutations of the Lbr gene (ic-J) are responsible for underlying defects in the ichthyosis mouse that include severe skeletal abnormality, in utero/perinatal lethality, absence of hair, and scaly skin (19). Like in humans, reduced neutrophil lobularity is observed in both heterozygote and homozygote mice, often as bilobed or ovoid nuclei, respectively. Complementation studies have suggested that the defects in ichthyosis mice reflect laminoopathies rather than deficiencies in cholesterol bio-synthesis; whereas mice deficient for DHCR14 are normal, DHCR14(−/−):LBR (−/−) mice display growth retardation and neurologic abnormalities that are distinct from those observed in ichthyosis mice and likely represent true deficiencies in cholesterol biosynthesis (20, 21).

The LBR is essential for normal embryonic development as indicated by the severe defects that occur in its absence. Its selectively high expression in hematopoietic cells, [array data by Su et al. (22)], however, suggests that its primary function postnatally may be in regulation of these cells. To date, studies have focused primarily on LBR-deficient neutrophils. The highly lobulated nucleus of neutrophils is thought to facilitate their movement through blood vessel walls and tissues (8). LBR-deficient neutrophils have been found to be deficient in chemotactic migration, possibly relating directly to reduced nuclear lobularity (7, 23–25). Reduced respiratory burst activity and proliferative responses have also been reported. Altered nuclear morphology in the form of heterochromatin clumping has been described for lymphocytes devoid of LBR, although how this affects lymphocyte survival, proliferation, or function has not been investigated.

The mouse pedigree, lymphocyte affected 3 (Lym3), was identified in a forward genetic screen for recessive mutations perturbing lymphocyte composition. It harbors a novel mutation in the Lbr gene, encoding a nonconservative amino acid substitution that dramatically reduces protein expression. Lym3 mice (LbrLym3/Lym3) have normal thymic and bone marrow (BM) development but display significant and progressive T and B cell lymphopenia in secondary lymphoid organs. Reduced cellular life span and impaired homeostatic proliferation of LBR-deficient T cells in vivo were found to contribute to lymphopenia. TCR signaling in vitro appeared unimpaired in Lym3 T cells with similar phospho-tyrosine induction and proliferative responses after CD3 cross-linking. Lym3 T cells also displayed robust survival responses to IL-7 in vitro, although viability was slightly less than that of equivalent wild-type (WT) cultures. Despite profound changes in nuclear morphology, there were minimal perturbations in gene expression observed in LBR-deficient neutrophils and CD8 T cells. There were, however, some common genes differentially expressed in both LBR-deficient cell types, and for the majority of these gene upregulation was observed, supporting a role for the LBR in their suppression.

Materials and Methods

Generation and screening of mutant mice

Male Mpl−/− C57BL/6 mice were treated with ethylisotourea (ENU) as described (26–31). ENU-treated mice were mated with isogenic females to yield first-generation (G1) progeny. G1 mice were intercrossed to yield second-generation (G2) progeny, which were brother–sister mated to produce third-generation (G3) mice. G3 mice were bled at 7 wk of age, and peripheral blood cell values were determined using an Advia 120 automated hematology analyzer (Bayer). The percentage of B and T lymphocytes in peripheral blood was determined using flow cytometry. Upon isolation in a G1 pedigree, Lym3 was bred to Mpl−/− C57BL/6 mice. All animals used in the studies described were on an Mpl−/− C57BL/6 genetic background. Unless otherwise indicated, analyses involve comparison of WT and LbrLym3/Lym3 mice, with homozygous mutants referred to as Lym3. Mice were routinely housed in clean, conventional facilities and were used for experiments at 7–10 wk of age, unless otherwise described. Experiments were approved by the animal ethics committees of St. Vincent’s Hospital and The Walter and Eliza Hall Institute.

Genetic mapping

To map the Lym3 mutation, male C57BL/6 Lym3/Lym3 mice were mated to WT C57/129/SV females. F1 mice were then intercrossed to yield F2 progeny, a quarter of which were affected. DNA from 9 affected and 20 unaffected F1 mice was genotyped for 64 single nucleotide polymorphism spaced evenly throughout the genome, localizing the Lym3 mutation to the distal region of chromosome 1. The candidate interval was refined via analysis of additional MIT and in-house SSLP markers in the region to a 1-Mbp region encompassing 14 genes. Those candidate genes and/or gene transcripts normally expressed in T cells (information derived from publicly available gene atlas profiles) were sequenced.

Sequencing and genotyping

Genomic DNA was prepared from tail biopsies, and the coding exons and splice junctions of candidate genes were PCR amplified. In some cases, gene transcripts were PCR amplified by RT-PCR from total RNA of PBMC. PCR products were treated with ExoSap-IT (USB Corporation), sequencing performed using BigDye Terminator chemistry (Applied Biosystems), and sequencing reaction mixes resolved and analyzed on an Applied Biosystems 3730 Genetic Analyzer at the Australian Genome Research Facility. A single point mutation, C to A, was identified within exon 10 of the LBR gene that encodes a nonconservative glutamic acid to alanine substitution at amino acid position 418.

Genotyping of the Lym3 mutation was subsequently performed by PCR amplification of exon 10 of the LBR gene with forward (5’-GCCCTT-TGAGAAGCACTGTG-3’) and reverse (5’-CAAGTGGGATGATTCA-CA-3’) primers to create a 441-bp product that was digested with restriction enzyme BsaD1 (NEB). The Lym3 mutation creates an RFLP, an additional BsrD1 site that results in generation of three fragments of 241, 164, and 34 bp compared with two fragments of 277 and 164 bp from WT samples. WT, heterozygote, and homozygote mutants were distinguished on 2% agarose gels (50% high-resolution agarose, 50% standard agarose).

Generation and screening of additional mouse strains

LbrLym3/Lym3 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). A PCR/sequencing protocol was used for genotyping of the ic-J mutation. The affected gene region was PCR amplified using forward (5’-ATG TAG CCC CAG GAT GAG TG-3’) and reverse (5’-TGT GCA TGA TGG AAC TG-3’) primers and sequenced. This ic-J mutation involves an insertion of an additional two Cs in a string of four Cs, and sequencing profiles are disrupted beyond this point in heterozygotes.

For complementation experiments, Lym3 (Lym3/Lym3 and Lym3+/+) mice were crossed with LbrLym3/Lym3 mice and littermates genotyped for the Lym3 and ic-J mutations as described earlier. The experimental data for these analyses were compiled from mice housed in both clean and unclean facilities (always with filter-top boxes). Identical trends were observed regardless of housing.

Hematopoietic cell analysis

Leukocytes were enriched in blood samples by lysing erythrocytes twice (37°C, 3 min) in red cell lysis buffer (156 mM ammonium chloride, pH 7.3). Single-cell suspensions were prepared from pooled lymph nodes (brachial, axillary, mesenteric, inguinal, cervical) and spleens using a 100 μM cell strainer (BD Falcon). RBCs were removed from splenocyte suspensions using red cell removal buffer. BM cells were flushed from femurs and filtered. For flow cytometric analysis, cells were incubated with a blocking Ab against FcγR, 2-4G2, prior to staining with fluorochrome-conjugated Abs specific for a wide range of cell surface markers. The Abs used were CD45R/B220 (clone RA3-68B2), CD90.2/Thy-1.2 (53-2.1), CD3 (17A2), Ly-6G/Gr-1 (RB6-8C5), CD4 (RM5-5), CD8 (53-6.7), CD11b/Mac-1 (M1/70), IgM (II/41), CD44 (IM7), CD69 (H1.2F3), CD25 (PC6), LAMIN B RECEPTOR DEFICIENCY CAUSES LYMPHOPENIA.
CD62L (CD44lo, CD62L+) and central memory (CD44hi, CD62L+) CD8 T cells (in 0.2 ml PBS) or with a 1:1 mix of CFSE-labeled CD45.1 WT and CD45.2 mutant cells) and tissues harvested 2 and 7 d later for flow cytometric analysis.

In vitro proliferation of T cells

Total T cells were isolated by negative selection (to >95% purity) from LN suspensions (brachial, axillary, mesenteric, inguinal, cervical) using CD3+ T cell enrichment columns (MTCC-500; R&D Systems). These cells were further sorted into naive (CD45RA+CD62L+) and CD4 and CD8 T cells and central memory CD8 T cells (CD45RA–CD62L+) after staining with appropriate fluorochrome-labeled Abs using a MoFlo sorter (Beckman). The cells were suspended at 10^5 cells/ml in PBS/0.1% BSA/0.4 mM EDTA and labeled for 10 min with 5 μM CFSE at 37°C. They were washed three times in culture medium (RPMI 1640 supplemented with 10% FCS, 50 μM 2-mercaptoethanol, 1% penicillin-streptomycin, and 2% glutamine) and cultured (0.5 × 10^5 to 1 × 10^5 cells/well in 0.1 ml) at 37°C, 5% CO₂, in flat-bottom 96-well plates that were precoated overnight at 4°C with CD3-specific Ab-OKT3 (5 and 10 μg/ml in PBS) or with PBS alone. IL-7 (5 ng/ml) was added to some cultures. The cells were examined by flow cytometry for proliferation (CFSE) and cell viability (propidium iodide) after 48 h. Modelled data of CFSE peaks to determine percent divided (percentage of the original cells that divided, assuming no cell death), division index (average number of divisions of cells from original starting population), and proliferative index (average number of divisions calculated only from those cells that divided) was performed using the FlowJo application program.

Microarray experiments

For CD8 T cell gene expression analyses, two separate array experiments were conducted, each involving three WT and three Lym3/Lerm3 RNA samples, derived from individual 7- to 8-wk-old male mice. In the first array, CD8 T cells were isolated from LNs (brachial, axillary, mesenteric, inguinal) by positive selection using CD8a microbeads (130-049-401, MACS; Miltenyi). Purity was in excess of 97%. In the second array, CD8 T cells were isolated by negative selection using a CD8a T cell purification kit (130-095-236, MACS; Miltenyi) from LNs (brachial, axillary, mesenteric, inguinal, plus cervical). Purity was in excess of 94%.

Analysis was conducted on RNA from neutrophils that were independently purified from the BM of three WT C57BL6, three LbrLym3/+ and three LbrLym3-/+ male mice. To isolate neutrophils, BM cells were resuspended in 5 ml 1.091 g/cm³ Nycodenz, layered onto 5 ml 1.091 g/cm³ Nycodenz (Axis Shield), layered onto 5 ml 1.091 g/cm³ Nycodenz, and overlaid with 2 ml FCS. The Nycodenz preparation was spun at 2000 rpm for 15 min at 4°C with low brake. The interface cell fraction was recovered by centrifugation at 1700 rpm for 7 min at 4°C. Enrichment for neutrophils was further achieved by lineage depletion of B cells and erythrocytes using the Abs RA6-8C3, B220 (B cells), and Ter-119 (erythrocytes) together with BioMag beads (Qiagen). The enriched population was then prepared for sorting by flow cytometry. FcR blocking was performed by initially incubating cells with rat Ig (chromo pure rat IgG, cat. no. 012 000 003; Jackson ImmunoResearch). Cells were then stained for markers Mac-1 (CD11b)–allophycocyanin, F4/80–PE, Ly6G–PE Cy7, and IL-5Ra (CD125)–Alexa 488. Unstained and single-stain controls were included for establishing compensation settings. Neutrophils were sorted by flow cytometry on an Aria cell sorter using the following strategy. Step 1: Cells were first gated on forward scatter (FSC)-W versus FSC-H, gating those above the main line of cells (P1) leaving out the doublets that were above the mainstream. Step 2: The P1 population was then selected on side scatter (SSC)-W versus SSC-H with gating tightly established around the main line of cells (P2). Step 3: The P2 population was further analyzed on SSC-A versus FSC-A and gated establishing around the medium to large cells (P3). Step 4: P3 was then examined by PI (FL-3) versus SSC, with gating around the lower population that was PI negative (P4). Step 5: The P4 population was then examined for F4/80–PE versus Mac-1–allophycocyanin, and the F4/80– PE to lo, Mac-1hi cells selected (P5). Step 6: The P5 population was examined for IL-5Ra–Alexa 488 versus Ly6G–PE Cy7, and gating was fixed around the IL-5Rahi, Ly6Ghi cells (P6), representing those cells that were sorted and collected. A small sample was removed for cytospin analysis and the remaining recovered by centrifugation at 400 rpm for 5 min. Total RNA for microarrays was purified using RNasey Micro columns (Qiagen) before hybridization to Illumina MouseWG-6 v2.0 Expression Bead Chips at the Australian Genome Research Facility.
Microarray data were analyzed in R using the limma, lumi, gplots packages of the Bioconductor software project (32). Each data set was analyzed as an independent data set. Raw intensities were normalized with the normexp background correction followed by quantile normalization across the whole data set. Differential expression was assessed using linear modeling and empirical Bayes moderated t-statistics (33). Within each array experiment, the samples were found to be of equivalent quality. Data are available at ArrayExpress (http://www.ebi.ac.uk/arrayexpress) under accession number E-TABM-1134.

To investigate gene clustering, differentially expressed probes, selected with unadjusted p < 0.005, were collapsed to unique genes. To obtain p values, 10,000 sets of genes, equivalent to the number differentially expressed, were randomly sampled and tested for clustering. The directionality of genes within clusters was investigated using the number of genes expected by chance in a cluster. Two differentially expressed genes clustered with each other can either be both upregulated, both downregulated, or changing in different directions. The relative proportion expected of each of these clusters depends on the proportion of upregulated and downregulated genes that were differentially expressed across the whole genome. A χ² test was used to see if the distribution of each type of cluster was significantly different to what was expected.

Results

The Lym3 mouse pedigree was isolated in a forward genetic screen in C57BL/6 mice for recessive phenotypes caused by mutagenesis with ENU. ENU creates random germline mutations in male mice with an expected frequency of 1:10⁶ bp that may result in truncation or altered amino acid composition of the gene product (28–31). Examining blood at 2 mo of age, Lym3 mice displayed a reduction in WBC count (Advia) and a decreased rate of neutrophils (Fig. 2A). Further reductions in WBC count were observed at 4 mo.

The Lym3 mutation was mapped to a 1-Mbp region within the distal region of chromosome 1 using established mating/mapping strategies (Fig. 1B). Those candidate genes (or gene transcripts) known to be expressed in T cells were sequenced, and a single point mutation (C to A) was identified within exon 10 of the Lbr gene (Fig. 1C) resulting in a glutamic acid to alanine substitution at residue 418 of the encoded protein. This highly conserved residue is normally part of a charged region between two hydrophobic/putative transmembrane stretches, and mutation causing an altered charge might be expected to affect organization of these transmembrane domains and/or protein stability (Fig. 1D, 1E). Lym3 mice appeared outwardly normal with no obvious skeletal abnormalities or skin defects. Examination of spleen extracts, however, revealed a severe reduction in LBR protein expression (Fig. 1F). In subcellular fraction analyses of WT and Lym3 spleen cells, LBR was concentrated in the last fraction that includes cytoskeletal, nuclear matrix, and all remaining proteins not solubilized in previous steps. Although clearly demonstrated in WT samples, using two different LBR-specific Abs, mutant LBR was only just detectable with extended exposure (Fig. 1F, long exposure). Similarly, when transiently expressed in 293T cells as a C-terminally Flag-tagged protein, LBR E418A was only very weakly expressed. In spleen fraction IV samples from LbrLym3/- mice, LBR expression was approximately half that of the WT control (Fig. 1G).

Reduced neutrophil lobularity in Lym3 mice, including some bilobed and ovoid nuclei (Fig. 2A), was consistent with an intermediate phenotype between homozygotes and heterozygotes of null alleles (19). Although less profound, reduced neutrophil lobularity was also detected in LbrLym3/+- mice (Fig. 2A). Altered lymphocyte nuclear morphology in the form of nuclear clumping was only evident in homozygotes where lymphocytes with a pyknotic appearance and signs of membrane blebbing, consistent with apoptosis or necrosis, were also detected.

To validate further the role of the LBR gene mutation in lymphopenia, we conducted complementation experiments with another LBR mutant mouse line, Lbric-3 (Fig. 2B). The ic-3 allele is a null allele, and homozygosity causes in utero or perinatal lethality, skeletal defects, reduced size, and scaly skin (ichthyosis) (19). LbrLym3/ic-3 compound heterozygote mice were, however, viable and healthy but, like LbrLym3/Lym3 mice, exhibited reduced blood lymphocyte numbers, providing additional evidence that LBR deficiency causes lymphopenia. At 4 mo of age, lymphopenia was also detected in Lbric-3/+- and LbrLym3/+ mice, although this was not statistically significant for LbrLym3+/+ mice until 6 mo of age (data not shown).

Analyses of hematopoietic compartments found thymic and BM composition/cellularity to be normal in Lym3 mice (Fig. 3A, 3B). In stark contrast, however, significant reductions in total CD4, CD8, and B cell numbers were observed at 2 mo, in both LN and spleen, with further reductions in organ cellularity at 4 mo of age (Fig. 3C, 3D). As initially observed in blood (Fig. 1A), lymphopenia was more pronounced for T cells than B cells. The representation of nonlymphocyte blood cell populations—neutrophils, eosinophils, monocytes, RBCs (data not shown), and platelets (data not shown)—in 2-mo-old Lym3 mice was normal (Fig. 3E). Lymphopenia did not appear to be driven by antigenic exposure as it was also detected in TCR transgene-bearing (OTI and OTII) Lym3 mice in the absence of OVA exposure (Fig. 3F).

An increase in the expression of activation markers, particularly CD44, was noted on LN Lym3 CD8 T cells from 2-mo-old mice (Fig. 3G), which was also observed on splenic and blood Lym3 CD8 T cells and blood Lym3 OTI CD8 T cells (data not shown). The proportion of both Lym3 CD4 and CD8 T cells that were CD44+CD62L+ was also increased. (Fig. 3G). With lymphopenic progression, further increases in CD44 expression were observed, and, at 4 mo, the proportion of Lym3 CD4 T cells that were CD44+ was now also significantly higher than that for WT CD4 T cells (LN, Fig. 3G, blood and spleen, data not shown). CD44 is expressed on activated and memory T cells and on T cells undergoing homeostatic proliferative expansion (HPE). After HPE, naive T cells are known to adopt a CD44+CD62L+ central memory profile (34–36). Although CD44 and CD62L expression patterns on T cells in Lym3 mice were consistent with HPE induced under lymphopenic conditions, progressive lymphopenia indicated that any cellular expansion was outweighed by cell death.

Lym3 mice also develop B cell lymphopenia, and increases in surface IgM [median fluorescence intensity (MFI) 242 ± 48 versus 174 ± 12, Lym3 versus WT, n = 4, p < 0.05], CD21 (MFI 70 ± 6.3 versus 58.7 ± 4.8, n = 4, p < 0.05), and CD40 (MFI 38 ± 3.2 versus 31 ± 0.8, n = 4, p < 0.05) expression on splenic CD23 gated T cells from 4-mo-old mice suggest also a change in B cell activation/proliferative status.

Lymphopenia is frequently associated with the development of autoimmune disease. There was, however, no obvious indication of autoimmune infiltration of tissues (liver, LNs, kidneys, spleen) or other health disorders in aged Lym3 mice (9–12 mo old) (data not shown).

Hematopoietic reconstitution experiments (Fig. 4) demonstrated lymphopenia to be a defect intrinsic to Lym3 lymphocytes. Curiously, Lym3 BM was also found to be deficient in restoring neutrophils, eosinophils, and RBCs to lethally irradiated WT recipients, evident at 2, 4, and 6 mo post-reconstitution, suggesting a stem cell defect and/or reduced cellular half-life not evident in Lym3 mice under normal circumstances. Detailed stem cell analysis of BM from mice 6 mo post-reconstitution (Supplemental Fig. 1) was conducted. No significant differences in the breakdown of Lineage-Scalε-Kit+ stem cells into long-term stem cells (LT), short-term stem cells (ST), and multipotent progenitor populations (MPP) were observed for Lym3 and WT BM recon-
stituted mice (Supplemental Fig. 1A). Of lineage depleted c-Kit+Sca12 (LK) cells, there were similar proportions of common myeloid progenitors (CMPs) or granulocyte macrophage progenitors (GMPs) (Supplemental Fig. 1B). Furthermore, there were no differences in absolute numbers of Lineage-Sca1+c-Kit+ or LK cells (data not shown) from mice reconstituted with WT or mutant mice.

**FIGURE 1.** Lym3 mice have a missense mutation within the Lbr gene. A, A decreased blood T (Thy1+) to B (B220+) cell ratio and reduced WBC count was detected in ENU mutant pedigree Lym3 (m/m). B, Through mapping crosses with SV/129 mice (see Materials and Methods), the causative mutation was localized to chromosome 1, bound by markers JCCA246 and JCCA208. Defining recombination events (gray), C57BL/6 homozygosity (black), and C57BL/6:129/Sv heterozygosity (divided) are indicated. C–E, Mutation (A to C) within exon 10 of the Lbr gene in Lym3 mice (C) encodes an E418A substitution, reducing charge in the conserved region as indicated in the Kyte–Doolittle hydrophobicity plot (D) and in protein sequence alignment (E) of the relevant region from different species (mouse, Mu; human, Hu; zebrafish, Da). F, LBR E418A is expressed weakly. Subcellular fractions (I, cytosolic; II, membrane/organelle; III, nuclear; IV, cytoskeletal/matrix/residual) from spleens (2 WT, +/+; 2 Lym3, m/m) examined by immunoblotting with two LBR-specific antisera (i, Cosmo, short and long exposure given; ii, Abcam). WCL of 293T cells untransfected (U) or transfected with plasmids encoding Flag–LBR WT or mutant (L3) protein shown. Position of LBR indicated by arrows. Asterisk denotes nonspecific bands detected in 293T cells. Blotting for β-tubulin indicates equal loading. G, Fraction IV from +/+, m/+, and m/m spleens examined for LBR (antisera i) and β-tubulin.
Lymphopenia results from LBR deficiency. A, Blood cell morphology in Lym3 mice is consistent with LBR deficiency. Neutrophils (top panels) from heterozygote and homozygote mutants (m+/m, m/m) have reduced lobularity, with bilobed and ovoid nuclei commonly observed in homozygote mutant mice. Lymphocyte morphology (bottom panels) is normal in WT and heterozygotes, but in homozygote mutant mice, heterochromatin clumping is evident, and some cells display pyknosis and signs of membrane blebbing (May–Grünewald stain, original magnification ×1000).

B, Blood analysis (2 and 4 mo) of mice from LbrLym3/ic-J and Lbric-J/Lym3 matings to test genetic complementation. Lymphopenia evident in LbrLym3/ic-J and Lbric-J/Lym3 mice at 2 mo of age, with further development at 4 mo. Lymphopenia also evident in LbrLym3 mice at 4 mo of age. Statistical significance determined by unpaired t test. *p < 0.05, **p < 0.01, ***p < 0.001.

BM. Of more mature BM populations, a small increase in the proportion of Gr1+Mac1+ cells was observed in mice reconstituted with Lym3 BM, possibly indicating a homeostatic response to neutropenia, whereas mature B cells numbers were reduced, reflecting systemic and progressive lymphopenia (Supplemental Fig. 1C). These data provide no evidence for a stem cell defect in Lym3 mice. It is, however, still possible that there is a deficiency in transition from less mature BM to fully mature blood cell and/or that blood neutrophils, eosinophils, and RBCs of mutant origin have a reduced cellular half-life only evident in extreme circumstances, such as in emergency hematopoiesis. In a previous study, LBR-deficient immortalized neutrophils homozygous for the ic-J mutation were shown to be less sensitive to proliferation induced by low concentrations of GM-CSF whereas multipotent precursors proliferated normally to stem cell factor (23). This also suggests LBR deficiency affects mature cells but has little or no effect on precursor cells.

The contribution of donor cells to different blood cell populations was examined at various times postreconstitution. At 10 mo postreconstitution, B220 B cells and Mac1+Gr1+ blood cells were determined to be predominantly donor cell derived (CD45.2+) regardless of whether mice were reconstituted with WT or Lym3 BM (Fig. 4B). The vast majority of CD4 and CD8 T cells also remained donor derived in WT reconstituted mice. In contrast, an increasing contribution of host-derived T cells was noted for Lym3 reconstituted mice over time constituting >60% of total CD8 T cells and >30% of total CD4 T cells after 10 mo. This suggested a strong competitive advantage of WT T cells compared with Lym3 CD8 T cells in HPE.

To examine further the proliferative capacity of T cells in vivo, CFSE-labeled LN cells from WT and Lym3 mice were injected into sublethally irradiated WT recipients. LN and spleen were examined 5 d after. Although proliferation of CD8 T cells was clearly evident in all samples, there was a reduced proportion of CD8 T cells in the divided fraction of harvested Lym3 CFSE-labeled donor cells compared with WT T cells (Fig. 5A, i and ii). For those cells that had undergone division, however, the proliferative index, reflecting the number of cycles completed, was at least as high as for WT cells, and sometimes higher. CD4 T cells from both WT and Lym3 mice had undergone much less proliferation than CD8 T cells at 5 d after injection, but, as with CD8 T cells, the division index of mutant CD4 T cells was significantly lower than that of WT CD4 T cells. The recovery of Lym3 T cells was also found to be markedly reduced compared with that of WT T cells.

To control internally for variation in injection efficiency, we also compared proliferation and recovery of CFSE-labeled T cells from irradiated hosts receiving a 1:1 ratio of CFSE CD45.1 and CD45.2 Lym3 LN cells. Gated CFSE-labeled CD4 or CD8 cells were further separated into CD45.1 positive and negative populations. Again, the percentage of divided cells and the division index was reduced for Lym3 (CD45.1 negative) T cells compared with WT controls (data not shown). In addition, the contribution of WT (CD45.1) CFSE-positive cells to recovered total, CD4, and CD8 populations from LN, spleen, and blood at day 5 was significantly increased compared with starting donor populations (day 0) across all samples (Fig. 5B). Although superior HPE of WT T cells could account for a small increase in representation of WT cells (<5%) based on division index determinations, it did not account for the differences observed (>10%), indicating selective loss of Lym3 T cells.

Because the higher representation of CD44hiCD62L+ cells within the Lym3 CD8 population might impact in vivo proliferative responses, we also examined isolated and CFSE-labeled naive (CD44hiCD62L+) and central memory (CD44hiCD62L−) CD8 T cells for their proliferation in vivo. Homeostatic proliferation of Lym3 naive CD8 T cells was severely impaired compared with WT naive CD8 T cells (Fig. 5C). Whereas WT and Lym3 memory T cells proliferated strongly in vivo, we were consistently unable to obtain even labeling of Lym3 memory T cells that would enable modeling and direct comparison of these cells.

Having demonstrated reduced Lym3 T cell survival in a lymphopenic environment, we were interested in determining whether there is also reduced cellular longevity of nondividing mutant T cells that might contribute to the initial development of lymphopenia in Lym3 mice. For these experiments, CFSE-labeled WT (CD45.1) and Lym3 (CD45.2) donor LN cells were combined but instead injected into nonirradiated (nonlymphopenic) WT hosts. Mice were killed 2 or 7 d postinjection, and the proportion of WT to Lym3 CFSE-labeled cells recovered from LN, spleen, and blood was examined (Fig. 5D). At 2 d after injection in a nonlymphopenic environment, an increase in the contribution of WT (CD45.1) CFSE-labeled cells relative to the starting donor mix
was detected for CD4 and CD8 T cells recovered from LN and
blood, although not from spleen. At 7 d, further increases were
observed. In spleen also, there was now evidence for increased
WT T cell representation, although falling below significance.
Increases in WT contribution to total splenic CFSE-labeled cells,
which includes B cells, was however observed at both 2 and

FIGURE 3. Hematopoietic analyses of Lym3 (m/m) mice. Total cellularity (A, right panel, B–D, top panels) and specific cell populations (B–D, bottom panels) within (A) thymus, (B) BM, (C) LN, and (D) spleen were determined by flow cytometry with marker-specific Abs. E, Blood composition comparison of 2-mo-old WT and Lym3 mice. F, Blood lymphocyte and T cell comparison of 2- to 3-mo-old OTI and OTII TCR transgenic mice on WT and Lym3 background. G, Activation and memory marker expression on LN CD4 and CD8 T cells from 2-mo-old mice (left panels) and CD44 expression profiles on LN CD4 (bottom panels) and CD8 (top panels) T cells at 2 and 4 mo (right panels). Results show the mean ± SEM (n = 4–6 mice for each genotype). Statistical significance determined by unpaired t test. *p < 0.05, **p < 0.01, ***p < 0.001.
7 d postinjection. These data demonstrate a shortened life span for nondividing Lym3 lymphocytes. The greater differences between WT and Lym3 T cell recoveries from blood and LN, compared with spleen, could also indicate impaired recirculation and LN homing of Lym3 T cells.

HPE is mostly driven by increased availability of cytokines such as IL-7 and IL-15, although signals via low-affinity TCR engagement with MHC-self peptide also contribute (34, 36–38). We examined the expression of additional cell surface molecules that might impact in vivo homeostasis and proliferation (Fig. 6). TCRβ expression was normal on Lym3 naive CD4, naive CD8, and memory CD8 T cells but was reduced on Lym3 CD4 memory T cells, whereas there were no significant differences in CD122 (IL-2/15 common β receptor chain) expression by Lym3 T cell populations. A slight but consistently observed downregulation of CD127 (IL-7R) was observed on naive CD4 and CD8 Lym3 T cells (Fig. 6A, 6B).

To examine TCR signaling biochemically, CD8 LN T cells were purified by negative selection and stimulated by soluble CD3 cross-linking (Fig. 7A). The induction of phospho-tyrosine of activated T cells was remarkably similar between the WT and mutant cells in both kinetics and intensity with no reproducible differences observed. TCR-mediated proliferation of CFSE-labeled T cells activated by CD3 cross-linking in vitro was also examined. Although initial experiments with enriched mixed T cell cultures indicated hyperproliferation of Lym3 CD8 T cells (Supplemental Fig. 2), subsequent investigation indicated this was largely due to the increased prevalence of CD44hi memory cells within the mutant T cell population. In contrast, the proliferation of purified naive CD44lo CD62L+ CD8 or CD4 Lym3 T cells in response to 10 μg/ml anti-CD3 was indistinguishable from co-CFSE-labeled and cocultured CD45.1 WT counterparts (Fig. 7B).

We also examined the viability of Lym3 T cell cultures. In the absence of any stimulation, the viability of Lym3 naive (CD44lo CD62L+) and memory (CD44hiCD62L+) CD8 T cells was consistently less than that of their WT counterparts, whereas the reverse was true for Lym3 naive CD4 T cells (Fig. 7C, data representative of three experiments). All Lym3 T cell populations displayed strong survival enhancement in the presence of IL-7, although viability was typically less than that of equivalent WT cells. This may reflect the slightly lower levels of IL-7R expression, especially for naive Lym3 T cells, although the reduced viability of unstimulated Lym3 CD8 T cells limits this conclusion somewhat. Similar trends were observed for non-CFSE-labeled cultures, although overall viability across all cultures was higher. Lym3 T cells did not appear to be more prone to activation-induced cell death after CD3 cross-linking, displaying similar or only slightly reduced viability than that of WT cells.

LBR has an important role in nuclear architecture and is thought to be involved in coordination of events during cell mitosis, particularly in disassembly and reassembly of the nuclear envelope. There is also increasing evidence for regulation of gene expression...
by the nuclear lamina, and although the role of LBR in this process is unclear, its known interaction with heterochromatin and heterochromatin associated factors, such as HP1, have raised the possibility of a role in gene silencing. LBR is highly expressed in hematopoietic cells, and deficiency has a clear impact on their nuclear architecture. To explore whether this also affects gene

FIGURE 5. Defective homeostatic proliferation and a reduced life span for Lym3 T cells contribute to lymphopenia in Lym3 mice. A, Recovery and analysis of CFSE-labeled WT and Lym3 CD4 and CD8 T cells from the LNs and spleens of sublethally irradiated C57BL6 recipients 5 d after i.v. injection with donor cells (CFSE-labeled LN cells of either WT or Lym3 origin). Ai, Modeling of proliferative peaks on gated CD8 CFSE-positive LN cells illustrated in blue, with raw data in black. Aii, Summary of data from WT and mutant spleen (Spl) and LN CD4 and CD8 T cells indicating percent divided, division index, and proliferative index (see Materials and Methods). Data are mean ± SEM of three biological replicates for each genotype. Significance determined by two-tailed unpaired t test. B, As above, except mice received a mix of CFSE-labeled Lym3 (CD45.2) and WT (CD45.1) LN cells combined at a 1:1 ratio. Contribution of WT (CD45.1) CFSE-positive cells to recovered total, CD4, and CD8 populations from LN, Spl, and blood (PB) at day 5 (D5) compared with composition of donor mix at day 0 (D0). Starting ratio of CD45.1 (WT):CD45.2 (Lym3) CD4 and CD8 T cells was higher due to a reduced T/B cell ratio within Lym3 donor LN suspensions. Results (mean ± SEM) are from four biological replicates (two mice per independently prepared LN mix of distinct mouse origin). Paired two-tailed t test analysis conducted. C, In vivo proliferation of WT and Lym3 CD44CD62L− (naive) CD8 and CD44CD62L+ (memory) CD8 T cells. Cell populations, purified from LN suspensions, were CFSE labeled and injected into sublethally irradiated recipients. Representative profiles of donor cells harvested from LNs 5 d after injection are shown. Proliferative measurements (mean ± SEM, n = 3–4) for WT and Lym3 naive CD8 T cells were as follows: percent divided (37 ± 3 versus 20 ± 3, p < 0.01), division index (0.50 ± 0.04 versus 0.26 ± 0.04, p < 0.01), and proliferative index (1.33 ± 0.02 versus 1.27 ± 0.02, p = 0.05). Corresponding values for CD62L−CD45.2 WT CD8 T cells were 72.6 ± 0.9, 1.54 ± 0.06, and 2.13 ± 0.07, whereas uneven CFSE labeling did not permit proliferation modeling for Lym3 memory CD8 T cells. D, Reduced cellular longevity of Lym3 T cells. CFSE-labeled Lym3 (CD45.2) and WT (CD45.1) LN cells were combined at a 1:1 ratio and injected into C57BL6 recipients (nonirradiated). Mice were sacrificed at 2 (D2) and 7 (D7) d postinjection, and contribution of CFSE-labeled WT (% CD45.1) to total recovered CFSE-labeled cells from LN, Spl, and PB was determined. Results (mean ± SEM) are from three biological replicates (recipients receiving different donor cell mixes) per time point. Paired two-tailed t test analysis conducted for comparison of day 2 and day 7 mixes with day 0 starting mixes. *p < 0.05, **p < 0.01, ***p < 0.001.
expression, we conducted microarray analysis of total RNA from LBR-deficient and WT LN CD8 T cells and BM neutrophils.

For CD8 T cell gene expression analysis, two data sets were generated, using slightly varied procedures (see Materials and Methods), each examining RNA samples isolated from LN CD8 T cells from three WT and three Lym3 mice. The proportion of expressed probes was similar, and there was low differential expression between mutant and WT samples for both data sets overall, suggesting that LBR does not impact globally on gene expression. For genes that were differentially expressed, however, an excellent correlation between the two data sets was observed; 38 gene probes were found to be upregulated by greater than 40% (log2 0.5 fold change [FC]) in mutant samples from both data sets, and 15 probes were downregulated (Supplemental Table I).

LBR-deficiency has a particularly profound impact on neutrophil nuclear morphology as observed for blood neutrophils (Fig. 2A), and its upregulation in neutrophils to see if there were common genes upregulated (Supplemental Table II). In addition, for 43 of these probes, stepwise changes in gene expression between WT, heterozygote, and homozygote samples were observed. With 29 of the 50 gene probes upregulated, bias toward gene upregulation was less evident than in LBR-deficient CD8 T cells. Differentially expressed genes included several members of the 2'-5'-oligoadenylate synthetase (Oas) gene family, a cluster of genes on chromosome 5 that have an important role in mediating antiviral activity. A dose-dependent upregulation of their expression was observed with increased severity of LBR deficiency (Fig. 8A). Our microarray data also revealed a modest dose-dependent upregulation of the Csf2r gene (encoding the common β subunit for GM-CSF, IL-3, and IL-5) in LBR-deficient neutrophils, whereas the chemokine

LAMIN B RECEPTOR DEFICIENCY CAUSES LYMPHOPENIA
ligands 3 and 4 genes (Ccl3,4) were downregulated (Supplemental Table II).

Importantly, there were several genes for which differential expression in the same direction was observed for both the CD8 and neutrophil data sets (Fig. 8B, Table I). Strikingly, eight of nine of these genes were upregulated, strongly suggesting that LBR normally has a role in their suppression. This set included two of the Oas family members, Oas2 and Oas12, and another antiviral gene, radical S-adenosyl methionine domain containing 2 (Rsad2). Also dramatically upregulated in both cell types was the erythrocyte differentiation regulator 1 gene (Erd1, Erd). Originally identified as a factor in the supernatant of the myelomonocytic leukemia line WEHI3B that induces hemoglobin production in erythroleukemia cells, EDR has subsequently been shown to release in response to cellular stress and to enhance cell survival (39, 40). The gene for hemoglobin α1 (Hba-a1) was also elevated in Lym3 CD8 T cells and neutrophils perhaps as a direct consequence of elevated Erd. Collectively, these data provide evidence for gene perturbation in LBR-deficient hematopoietic cells and support a role for LBR in gene silencing.

LBR may directly regulate gene expression by physical interaction with regions of the genome. We were interested in the proximity of genes differentially expressed in LBR-deficient cells to each other and whether the detection of gene clusters, defined as more than one differentially expressed gene within a 1-Mbp region, was higher than would be predicted by chance. In the neutrophil data set, there were 171 unique genes that were differentially expressed (selected with an unadjusted p < 0.005). From 10,000 sets of 171 randomly selected genes, the maximum cluster number was 9, whereas 26 gene clusters were observed among the 171 differentially expressed genes (p < 0.0001). Similar analysis of the CD8 data demonstrated the presence of significantly more gene clusters than likely to occur by chance (19 gene clusters from 168 differentially expressed genes, p < 0.0001).

Any regional changes to gene expression caused by lamina perturbation in LBR-deficient hematopoietic cells supports a role for LBR in gene silencing. A. Uregulation of the 2′,5′-oligoadenylate synthetase (OAS) family of genes in LBR-deficient neutrophils. Stepwise increases in gene expression from WT (+/+ to LBR<sub>lmb3</sub> lmb3 (m/m) samples detected. Mean ± SEM of three biological replicate values given for each genotype (RNA from neutrophils isolated from different mice) hybridized simultaneously. The suffix “p2” indicates an alternative probe (May–Grünewald stain of cytospins of purified neutrophil populations shown in the top right, original magnification ×1000). B. Comparing gene expression changes in Lym3 CD8 T cells (set 1) to changes observed in Lym3 (m/m) neutrophils. Blue dots represent overlapping gene probes for which a change, in the same direction, of >0.5 log<sub>2</sub> FC was detected in the two hematopoietic cell types. Several gene probes appear in the top right-hand corner (genes upregulated in both cell types) and may represent genes normally suppressed by LBR.

![Figure 8](image-url)

**FIGURE 8.** Uregulated gene expression in LBR-deficient hematopoietic cells supports a role for LBR in gene silencing. A. Uregulation of the 2′,5′-oligoadenylate synthetase (OAS) family of genes in LBR-deficient neutrophils. Stepwise increases in gene expression from WT (+/+ to LBR<sub>lmb3</sub> lmb3 (m/m) samples detected. Mean ± SEM of three biological replicate values given for each genotype (RNA from neutrophils isolated from different mice) hybridized simultaneously. The suffix “p2” indicates an alternative probe (May–Grünewald stain of cytospins of purified neutrophil populations shown in the top right, original magnification ×1000). B. Comparing gene expression changes in Lym3 CD8 T cells (set 1) to changes observed in Lym3 (m/m) neutrophils. Blue dots represent overlapping gene probes for which a change, in the same direction, of >0.5 log<sub>2</sub> FC was detected in the two hematopoietic cell types. Several gene probes appear in the top right-hand corner (genes upregulated in both cell types) and may represent genes normally suppressed by LBR.

<table>
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<th>Gene Symbol</th>
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<td>4933439C20Rik</td>
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*Genes with log<sub>2</sub> FC change >0.5 in both LBR-deficient (m/m) neutrophils and CD8 T cells (m/m) from both independent CD8 arrays.

*Genes with differential log<sub>2</sub> FC >0.5 in neutrophils and either CD8 array, with identical trends of >0.3 in the second CD8 array.

**Table I.** Genes differentially expressed in both LBR-deficient neutrophils and CD8 T cells

**Discussion**

In this article, we have described a mouse pedigree, Lym3, that is homozygous for a mutation within the LBR gene. Although this mutation drastically reduces protein expression, there is sufficient LBR mutant protein remaining for normal embryonic development of mice, which is perturbed in the complete absence of LBR. This novel pedigree has allowed us to explore the impact of severe LBR deficiency on hematopoietic cells and has demonstrated the importance of LBR in maintaining normal T cell proliferative responses and cell viability. As a consequence of LBR deficiency, Lym3 mice have profound and progressive lymphopenia. LBR is unusual in its possession of two very distinct domains and potentially a multitude of activities. Its N terminus includes a conserved Tudor DNA binding domain and mediates multiple interactions with components of the nuclear lamina and the heterochromatin and heterochromatin-associated proteins. The LBR C terminus has δ 14 reductase activity, enzymatic activity involved in cholesterol biosynthesis. During mitosis, and with disassembly of the inner nuclear membrane, LBR is dispersed throughout the endoplasmic reticulum. The phosphorylation of
specific residues within the LBR N terminus by kinases such as RS kinase and p34cdks facilitate this movement by disrupting LBR interactions, including interaction with chromatin (41–44). Upon completion of mitosis, reassociation of LBR with chromatin, possibly via importin β, is thought to assist in reassembly of the inner nuclear membrane, and LBR itself has been shown to stimulate nuclear envelope generation (41). It is perhaps not surprising then that a loss of LBR could result in perturbation of the cell cycle in lymphocytes as has been demonstrated in some immortalized cell lines (43).

In this study, we showed that in lymphopenia-induced homeostatic proliferative responses in vivo, LBR-deficient T cells were at a distinct disadvantage with evidence for less proliferation and decreased survival. Reduced cellular recovery of donor Lym3 T cells in nonlymphopenic recipients was also observed indicating reduced cellular longevity. Lym3 naïve T cells had slightly lower levels of IL-7R expression that may affect in vivo proliferation and survival. In vitro, Lym3 CD4 and CD8 T cells displayed robust survival responses to IL-7, although slightly reduced viability of Lym3 CD8 T cell cultures was evident both in the presence and absence of IL-7, which may reflect reduced IL-7 responsiveness and/or increased fragility of mutant cells.

The disintegration of the thymus with the onset of puberty limits further T cell development, and postthymic homeostatic proliferation is important in maintaining peripheral T cell numbers (45, 46). Progressive lymphopenia in Lym3 mice arises from decreased thymic output of T cells, which occurs normally with age, together with reduced cellular longevity and an inability to compensate due to ineffective HPE, defects specific to LBR-deficient T cells. The importance of LBR in providing an appropriate nuclear morphology that supports normal proliferation and survival is clear.

There has been much speculation over the possible role of LBR in gene suppression. The nuclear architecture of hematopoietic cells is dramatically affected by LBR deficiency, and we were interested in whether this affected gene expression. In microarray studies of both CD8 T cells and neutrophils, we found that LBR deficiency did not globally affect gene expression; the proportion of probes expressed was not significantly altered, and there was a low level of differential gene expression overall. However, we did find differential expression of a selection of genes in both arrays, with several overlapping between the two cell types. Gene upregulation prevailed especially among differentially expressed genes common to both LBR-deficient neutrophils and T cells, suggesting a role for LBR in their suppression normally.

Although many of the genes selectively upregulated in CD8 T cells could be a consequence of lymphopenia-induced proliferation in these cells, neutrophils, despite clear morphological differences, were represented normally in Lym3 mice. In examining gene expression of Lym3 neutrophils, upregulation of several antiviral genes was observed. The dose-dependent upregulation of the Oas family of antiviral genes was perhaps most striking. These molecules, normally upregulated in response to IFNs, facilitate viral RNA degradation through interaction with dsRNA and activation of RNase L. It is possible that LBR normally suppresses these genes and that gene expression is unleashed in an antiviral response. Curiously, the condition referred to as "pseudo PHA," a transient decrease in neutrophil lobularity that can occur in response to infections and other pathological conditions, may be part of the antiviral defense mechanism of neutrophils, opening up regions for transcription factor access. Whether it correlates with a transient drop in LBR expression or perhaps LBR N-terminal domain phosphorylation and disruption of heterochromatin–LBR interaction has not been examined. Alteration of nuclear architecture has been shown to occur when LBR–HP1 association is disrupted after human polyomavirus agnoprotein (Agno) interaction with HP1, and this is suggested to provide an easier nuclear exit of generated virion nuclear release (47).

The strongest argument for genes suppressed by LBR normally is those genes that were differentially regulated in both LBR-deficient CD8 T cells and neutrophils. Falling into this category were some Oas genes, and another antiviral gene, Rsad2. Another upregulated gene was Edr, a gene upregulated by cellular stress, which may be responsible for its elevated expression in LBR-deficient hematopoietic cells. The shortened life span of lymphocytes, and possibly neutrophils, is consistent with cellular stress.

Although the role of the LBR in regulating the nuclear morphology of hematopoietic cells has long been established, our knowledge of the impact this has on cellular function has been limited, largely because homozygosity of null alleles is invariably fatal. In this study, we identified profound and progressive lymphopenia as a consequence of severe LBR deficiency and showed that this arises from both reduced cellular longevity and defective proliferation. We have also, for the first time to our knowledge, investigated the effect of LBR deficiency on gene expression in hematopoietic cells and provided evidence for LBR-mediated gene suppression.

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Disclosures
The authors have no financial conflicts of interest.

References


