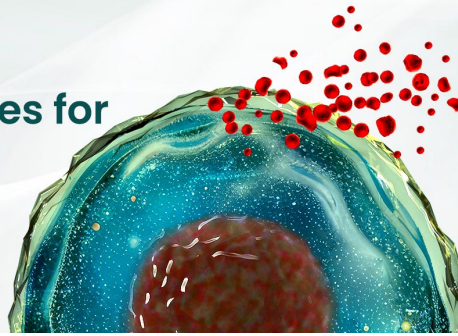




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Expression of Active Protein Kinase B in T Cells Perturbs Both T and B Cell Homeostasis and Promotes Inflammation¹

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Pamela S. Ohashi,^{2*}† and James R. Woodgett*

The molecular mechanisms that contribute to autoimmunity remain poorly defined. While inflammation is considered to be one of the major checkpoints in autoimmune disease progression, very little is known about the initiating events that trigger inflammation. We have studied transgenic mice expressing the prosurvival molecule protein kinase B/Akt under control of a T cell-specific CD2 promoter. In this study, we demonstrate that aged mice develop lymphadenopathy and splenomegaly that result from an accumulation of CD4, CD8, and unexpectedly B cells. An increased proportion of T cells express activation markers, while T cell proliferative responses remain normal. B cells are hyperproliferative in response to anti-IgM F(ab')₂ and anti-CD40, and increased IgA and IgG2a were found in the sera. In addition, a profound multiorgan lymphocytic infiltration is observed, and T cells from these mice display a defect in Fas-mediated apoptosis, which may be the mechanism underlying this phenotype. Therefore, T cell expression of active protein kinase B can alter T cell homeostasis, indirectly influence B cell homeostasis, and promote inflammation *in vivo*. *The Journal of Immunology*, 2001, 167: 42–48.

Protein kinase B (PKB³/Akt/RAC-PK) is a serine/threonine protein kinase that is activated downstream of the phosphatidylinositol 3-kinase (PI-3K) signaling pathway. PKB plays an important role in mediating the antiapoptotic effects of various cytokines, growth factors, and certain oncogenes in a variety of cell types, including hemopoietic cells (reviewed in Refs. 1–3). The mechanism by which PKB mediates its antiapoptotic effects has been the focus of intensive investigation. Evidence from multiple studies suggests PKB acts on a variety of substrates known to modulate apoptosis, such as cytochrome *c*, forkhead, NF- κ B, Bcl-2, Bcl-x_L, Bcl-x_L/Bcl-2-associated death promoter (BAD), and caspase 9 (4, 5). However, the significance of PKB interaction with BAD and caspase 9 remains controversial given the lack of conservation of PKB-dependent murine caspase 9-phosphorylation sites and studies suggesting BAD may not be a primary physiological substrate for PKB (4, 6–8).

Activation of PI-3K leads to phosphorylation of phosphatidylinositol and the generation of phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate. These phosphatidylinositides recruit PKB to the plasma membrane and promote the activation of PKB via phosphoinositide-dependent kinase 1 and phosphoinositide-dependent kinase 2 (reviewed in Ref. 9). In mature T cells, PKB is activated in response to TCR receptor

signaling and also in response to IL-2R, IL-7R, and CD28 signals (4, 10, 11–14).

The tumor suppressor gene PTEN is a phosphatase that can influence PKB activity through regulation of phosphatidylinositol 3,4,5-trisphosphate levels (15, 16). PTEN plays an important role in human oncogenesis, having effects on cell cycle arrest, cell adhesion, migration, differentiation, and programmed cell death. Indeed, somatic deletions or mutations in PTEN have been found in a large percentage of human tumors, including glioblastoma, endometrial, and advanced prostate cancer. PTEN mutations have also been found in autosomal dominant disorders such as Cowden disease (17). PTEN^{+/-} mice possess hyperplastic-dysplastic features with an increased incidence of spontaneous tumor formation. The mice also develop lymphoproliferative disorders with lethal autoimmune disease. This lymphoproliferative/autoimmune disorder has been attributed to defects in Fas/Fas ligand (FasL) signaling that could be reversed through PI-3K inhibition (18).

In this study, we have examined transgenic mice that express an active form of PKB in T cells. These studies define a molecular pathway via PKB that disrupts both T and B lymphocyte homeostasis and initiates autoimmunity *in vivo*.

Materials and Methods

Generation of transgenic mice

The generation of PKB transgenic mice has been previously described (4). Briefly, a *gagpkb* fusion, which targets PKB to the plasma membrane and promotes its activation, was cloned into a human CD2 minigene cassette. The CD2 promoter directs expression to the T cell compartment (19). DNA was injected into (B6 × DBA/2)F₂ mice, and transgenic mice were backcrossed to C57BL/6J three times. Homozygous PKB^{+/+} mice were generated by interbreeding. Southern blots, using a *gagpkb*-specific probe and *EcoRI*-digested tail DNA, were used to determine genotype.

Western blotting

Nylon mesh was used to make single cell suspensions of splenic lymphocytes. T cells and B cells were sorted by labeling splenocytes with anti-CD3 biotin and anti-B220 biotin, respectively, and purified using MACS separation columns (Miltenyi Biotec, Auburn, CA). Approximately 1 × 10⁶ T cells and B cells were lysed, and the amount of protein loaded normalized using Bradford assay to determine protein content. Anti-PKB and anti-473 PKB Abs (New England Biolabs, Beverly, MA) were used to

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³ Abbreviations used in this paper: PKB, protein kinase B; ALPS, autoimmune lymphoproliferative syndrome; BAD, Bcl-x_L/Bcl-2-associated death promoter; FasL, Fas ligand; PI-3K, phosphatidylinositol 3-kinase.

assess levels of total PKB expression and activation, respectively. Anti-actin Ab was used to confirm equal protein loading.

Flow cytometry

B cell and T cell suspensions were isolated from lymphoid organs following passage through 70- μ m nylon mesh into 1% BSA/PBS. Lymphocytes were stained with appropriate conjugated Abs (BD PharMingen, San Diego, CA), while viable cell gates were established based on a combination of forward and side scatter plots in addition to the cell viability dye 7-amino actinomycin D. Analysis was conducted using a FACStar^{plus} flow cytometer (BD Biosciences, Mountain View, CA) and analyzed using CellQuest software.

Lymphocyte proliferation assays

Spleens, lymph nodes, and Peyer's patches were collected and dispersed into single cell suspensions in IMDM complete media (10% FCS). CD4⁺ and CD8⁺ T cells or B220⁺ B cells were isolated via magnetic cell sorting using MACS separation columns (Miltenyi Biotec). Cell preparations were enriched >85–90% for T cells or B cells, as determined by flow cytometry. T cells were cultured in flat-bottom 96-well plates (10⁵ cells/well) in a total volume of 200 μ l in the presence of various concentrations of anti-CD3 Ab (145-2C11) or anti-CD3 plus anti-CD28 (clone 37.51) (BD PharMingen). B cells were stimulated with anti-CD40 Ab (BD PharMingen), anti-IgM (whole or F(ab')₂) (Jackson ImmunoResearch, West Grove, PA), or LPS (Sigma, St. Louis, MO). Cells were incubated for 48 h at 37°C in 5% CO₂, at which time 0.5 μ Ci [³H]thymidine was added. The cells were harvested 8 h later, and cell-associated radioactivity was determined.

Serum Ig detection

An alkaline phosphatase ELISA-based detection assay (Southern Biotechnology Associates, Birmingham, AL) was used to measure relative levels of various Ig classes. Briefly, goat anti-mouse Ig capture Ab in PBS (5–10 μ g/ml) was bound to high binding 96-well plates and incubated for 12 h at 4°C. Plates were then washed three times with PBS/Tween (0.05%) and blocked with PBS/BSA (1%) for 1 h at room temperature. Serum was obtained from mice using serum separators (Becton Dickinson) and diluted 1/500 in PBS/BSA (1%). Serial dilutions were plated and allowed to sit overnight at 4°C. Plates were then washed three times and incubated with the various classes of alkaline phosphatase-conjugated anti-mouse Abs at their recommended dilutions for 1 h at room temperature. Plates were then washed five times, substrate was added, and plates were read at 405 nm.

Anti-dsDNA Abs

Calf thymus DNA (10 mg/ml) was sheared by sonication and loaded into high binding 96-well plates (100 μ l/well) and incubated at 4°C overnight. Plates were blocked for 1 h at room temperature with 1% BSA/PBS and washed three times with PBS/Tween (0.05%). Serum was serially diluted, incubated overnight at 4°C, and washed three times with PBS/Tween (0.05%). Anti-mouse IgG alkaline phosphatase (Southern Biotechnology Associates) was added at 1/250 dilution, and plates were incubated for 1 h at room temperature. Plates were then washed five times with PBS/Tween (0.05%), and substrate was added as per instructions from Southern Biotechnology Associates. Plates were read on a Titertek multiscan ELISA plate reader.

Immunohistochemistry

Freshly removed organs were immersed in PBS and snap frozen in liquid nitrogen. Tissue sections of 5 μ m thickness were cut and fixed in acetone for 10 min. Sections were then incubated with primary Ab for 30 min at room temperature. Abs used included anti-CD8 (mAb YTS169) and anti-IgA. Primary Abs were followed by a two-step indirect immunoenzymatic staining procedure. First, alkaline phosphatase-labeled goat Abs to rat Ig were applied for 30 min. Alkaline phosphatase was then detected by a red color reaction using naphtho-AS-BI phosphate and New Fuchsin as substrate. Endogenous alkaline phosphatase was blocked by Levamisol. Sections were counterstained with Mayer's hemalum for 2 min.

Induction of Fas-mediated apoptosis

Splenocytes from wild-type and *gagpkb* transgenic mice were isolated and cultured with plate-bound anti-CD3 (10 μ g/ml) and anti-CD28 (5 μ g/ml) for 24 h, followed by culture in media containing murine rIL-2 (50 U/ml; PeproTech, Rocky Hill, NJ) for 4 days. Viable, activated lymphocytes were treated with human rCD8/murine FasL (gift from M. Bray, Amgen Institute, Toronto, Canada) for various time points. Treated cells were then analyzed for apoptosis by annexin V and propidium iodide staining (R&D Systems, Minneapolis, MN).

Results

T and B cell hyperplasia in PKB^{+/+} transgenic mice

To examine whether PKB expression in T cells plays a role in lymphocyte homeostasis, transgenic mice were made that express an active form of the prosurvival molecule PKB under control of the human CD2 promoter (4). By examining these animals over time, we found that T cell-specific expression of PKB led to a significant increase in morbidity, which was upward of 50% for PKB^{+/+} (homozygous) and 24% for PKB^{+/-} (heterozygous) transgenic mice aged 6–18 mo. This was accompanied by lymphadenopathy and splenomegaly. Therefore, we set out to characterize this lymphoid hyperplasia. In relatively young PKB^{+/+} transgenic mice (0–4 mo), few mice showed signs of lymphoid hyperplasia. However, PKB^{+/+} transgenic mice between 4 and 18 mo of age generally showed progressive increases in lymphoid cellularity. The cellularity of lymphoid organs for both early stage (4–8 mo) and late stage (8–14 mo) PKB^{+/+} transgenic mice was compared with age-matched wild-type controls. Spleen, lymph node, and Peyer's patches showed moderate increases in cellularity for early stage and more substantial increases in cellularity for late stage PKB^{+/+} transgenic mice (Fig. 1A). Flow cytometry analysis revealed that lymphocyte expansion in older PKB^{+/+} transgenic mice surprisingly involved an increased number of B cells. In addition, both CD8⁺ and CD4⁺ T cell compartments were expanded, but skewed toward CD4⁺ T cells (Fig. 1, B and C). The disruption of lymphocyte homeostasis in PKB^{+/+} transgenic mice is reminiscent of the phenotype observed in Fas^{-/-}/FasL^{-/-} mice, which show an expansion of normally rare CD4⁻/CD8⁻/ α β TCR⁺ cells (20). However, PKB^{+/+} mice did not show an increase in these cells (data not shown).

Although *gagpkb* expression was directed to the T cell compartment using the human CD2 promoter, PKB^{+/+} transgenic mice clearly developed alterations in B cell number. To confirm that activated PKB was not expressed in B cells, both T cells and B cells from PKB^{+/+} transgenic and wild-type control mice were sorted and probed with Abs specific for total PKB and activated PKB (anti-phospho 473-PKB). No change in total PKB or activated PKB was evident in B cells from PKB^{+/+} transgenic mice compared with wild-type B cells. As expected, significantly increased levels of total PKB and activated PKB were seen in PKB^{+/+} T cells (Fig. 2). Also, dendritic cells did not express elevated PKB activity (data not shown). Therefore, overexpression of activated PKB in T cells causes alterations in both B and T lymphocyte cellularity.

T cells and B cells are activated in Peyer's patches and lymph nodes

Since there was a marked increase in lymphoid cellularity for PKB^{+/+} transgenic mice, it was important to determine whether these cells were activated and possessed increased proliferative potential. This was done by analyzing the expression of T cell activation markers (CD69 and CD44) and B cell activation markers (CD23 and CD44) on lymphocyte populations from PKB^{+/+} and wild-type control animals. Interestingly, there was an increase in the percentage of activated T cells in the Peyer's patches and peripheral lymph nodes of PKB^{+/+} transgenic mice, as determined by increased surface expression of CD69 and CD44 (Fig. 3A, and data not shown). Similarly, there was an increase in the number of activated B cells found in the Peyer's patches and peripheral lymph nodes, as indicated by increased CD23 and CD44 expression (Fig. 3B, and data not shown). Surprisingly, there was no difference in spontaneous proliferative responses observed in T or

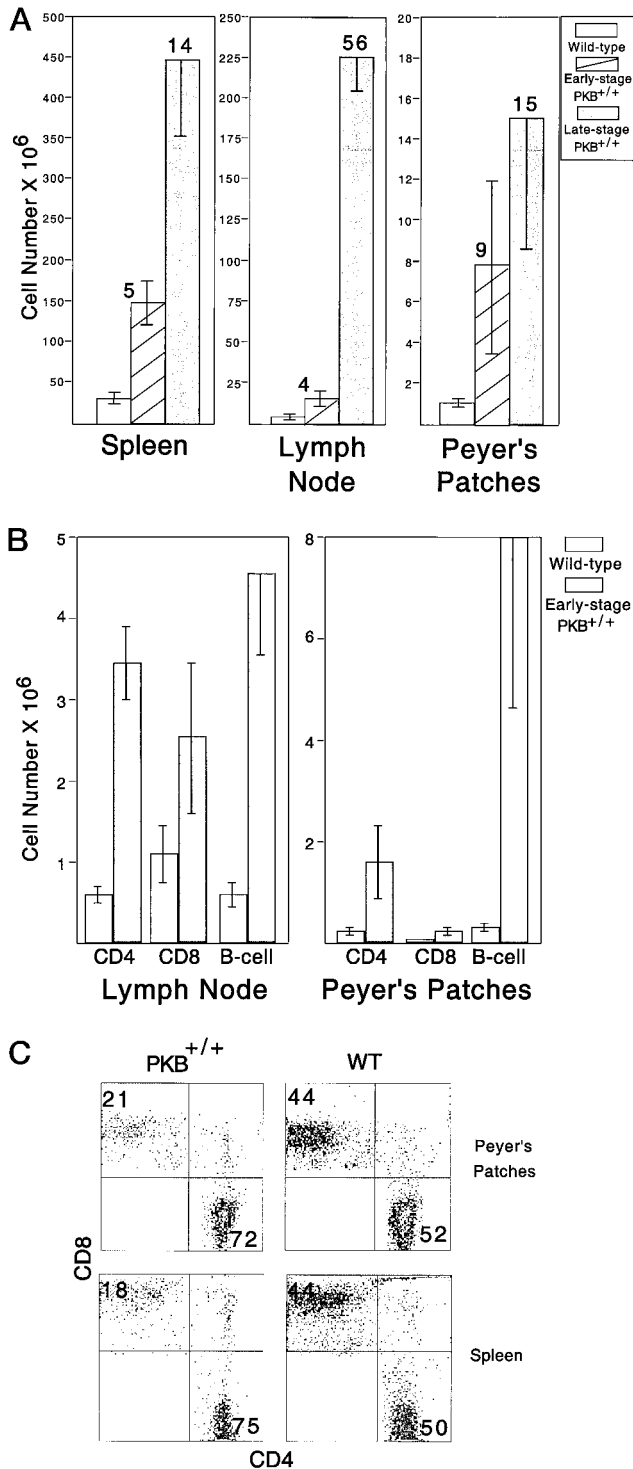


FIGURE 1. Substantial increase in lymphocyte populations in PKB^{+/+} mice. **A**, Increased lymphocyte cellularity in the spleen, inguinal lymph nodes, and Peyer's patches of early stage, late stage PKB^{+/+} and age-matched wild-type controls. Spleens were treated with lympholyte before counting, and cell counts were obtained using a hemacytometer in conjunction with trypan blue staining. An average of four mice per histogram is shown, with ages between 4 and 8 mo for early stage, and 8 and 14 mo for late stage mice. Numbers above histogram bars indicate fold increase above wild type. **B**, Total number of CD4⁺, CD8⁺ T cells, and B cells (CD19⁺) in the lymph node and Peyer's patches of early stage PKB^{+/+} and age-matched wild-type control mice (average of at least four mice per histogram). **C**, Dot plot showing the relative percentages of CD4⁺ and CD8⁺ T cells in the Peyer's patches and spleens of PKB^{+/+} and age-matched wild-type control mice.

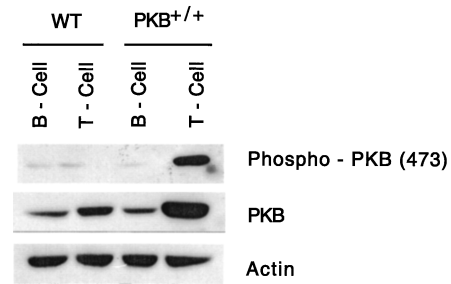


FIGURE 2. B cells do not overexpress active PKB. Purified T and B cells from PKB^{+/+} and wild-type (WT) control mice were analyzed by Western blot using anti-473 PKB, anti-PKB, and anti-actin Abs.

B cells, despite the expression of these activation markers. Peripheral T cells from the lymph node showed normal proliferative responses after 48-h stimulation with anti-CD3 or anti-CD3/CD28 compared with wild type controls (Fig. 3C). In contrast, lymph node B cells had strong proliferative response to anti-IgM F(ab')₂ and anti-CD40 (Fig. 3D). Collectively, these data indicate that T cell-restricted expression of activated PKB leads to increased proportions of activated T and B cells, as well as hyper-B cell responses to mitogenic stimuli.

PKB^{+/+} mice have characteristics of autoimmune disease

Since lymphoid hyperplasia is often accompanied by autoimmunity, we looked for indications of autoimmune disease in PKB^{+/+} transgenic mice. Serum from PKB^{+/+} transgenic and age-matched control mice was analyzed for levels of various Ig classes. IgA levels were consistently and significantly elevated (2- to 7-fold) in seven of seven PKB^{+/+} mice tested (Fig. 4). In addition, we observed slight increases (2- to 2.5-fold) in serum IgG2a or IgG2b from some PKB^{+/+} transgenic mice. Also, two of ten PKB^{+/+} mice had elevated levels of anti-dsDNA Abs relative to wild-type controls (Fig. 4). Immunohistochemical analysis showed that PKB^{+/+} mice have striking Ig deposition and lymphocytic infiltration in a variety of organs. Large accumulations of IgA were deposited in the kidney glomeruli of PKB^{+/+} mice (Fig. 5, A and B). In severely affected PKB^{+/+} animals, IgA deposits could also be found throughout other target organs, such as the lungs, liver, and salivary glands (data not shown). In addition, CD8⁺ lymphocytic infiltration was readily detected in organs such as the liver (Fig. 5C) and salivary gland (Fig. 5D), causing gross enlargement of those organs. Infiltration of CD4⁺ cells was also seen, most notably in the kidney (data not shown). In addition, a significant number of PKB^{+/+} mice developed lymphomas and, occasionally, thymomas.

Active PKB inhibits FasL-induced T cell death

Lymphoproliferative disorders in mice and humans have been correlated with a defect in Fas-mediated apoptosis (18, 20–22). Therefore, Fas-mediated apoptosis was examined in T cells from PKB^{+/+} transgenic mice. Splenocytes from PKB^{+/+} transgenic or wild-type mice were activated with anti-CD3 and anti-CD28 Abs and expanded in culture in the presence of IL-2. Following culture, viable T cells were subjected to Fas-mediated killing through the addition of rFasL. T cells expressing the *gagpkb* transgene displayed increased viability in response to challenge with FasL relative to control cells (Fig. 6). In response to 100 ng/ml, FasL PKB^{+/+} T cells showed approximately 2.5-fold greater viability over wild-type T cells. Thus, impaired Fas-mediated T cell death in PKB^{+/+} animals may prevent normal T cell homeostasis, leading to development of lymphoid hyperplasia and autoimmunity in PKB^{+/+} transgenic mice.

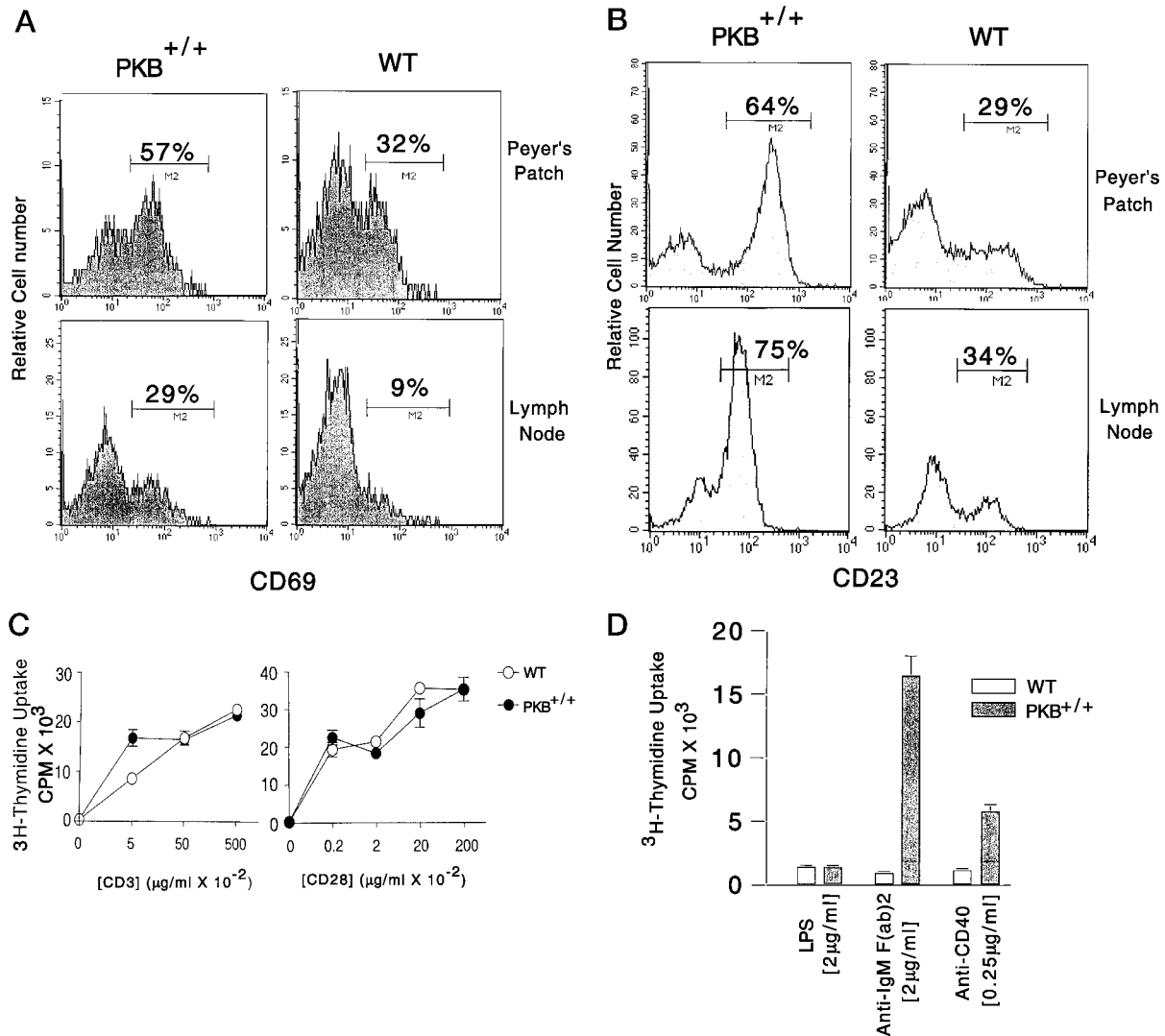


FIGURE 3. Active PKB leads to increased expression of B and T cell activation markers in addition to enhanced B cell proliferation. *A*, Increased percentage of CD69⁺ T cells in Peyer's patches and lymph nodes from PKB^{+/+} mice. Cells were stained with anti- β TCR and anti-CD69 Abs. CD69 expression is shown after gating on β TCR⁺ T cells. *B*, Increased CD23-positive B cells in Peyer's patches and lymph nodes from PKB^{+/+} mice. Cells were stained with anti-CD19 and anti-CD23 Abs, and CD23 expression is shown on CD19⁺ B cells. *C*, T cells from PKB^{+/+} and wild-type (WT) mice display similar proliferative responses after 48 h following stimulation with increasing concentrations of anti-CD3 or anti-CD3 (2 μ g/ml), with increasing concentrations of anti-CD28. *D*, Proliferation of purified lymph node B cells stimulated with LPS, IgM (F(ab)₂), and anti-CD40. Spontaneous proliferation of T cells and B cells in media alone was below 1 and 0.5 cpm \times 10³, respectively. These data represent three independent experiments.

Discussion

This study has demonstrated that T cell-specific expression of active PKB resulted in lymphoid hyperplasia involving CD4, CD8, and primarily B lymphocyte populations. Activated T and B lymphocytes were readily detected, and B cell hyperproliferation was observed in response to certain stimuli. Prominent IgA hypergammaglobulinemia was seen with IgA deposits and T cell infiltrates found in various organs. Consequently, a large proportion of PKB transgenic mice eventually succumbed to complications arising from lymphoproliferative/autoimmune diseases.

A similar condition has been reported in mice heterozygous for the PTEN gene, which have elevated PKB activity. The PTEN^{+/-} mice also develop splenomegaly and lymphadenopathy. IgA hypergammaglobulinemia was not reported, although increased IgG, anti-DNA Abs, and organ infiltration were routinely observed (18). It is surprising that the phenotype of PTEN^{+/-} mice is similar to the PKB^{+/+} mice since PTEN has the potential to influence a variety of downstream targets. In this regard, our data clearly dem-

onstrate that T cell-restricted expression of active PKB can alter lymphocyte homeostasis. The phenotype observed in PTEN^{+/-} mice was largely attributed to a defect in T and B cell Fas-induced death (18). The T cells from PKB^{+/+} transgenic mice are also refractory to FasL-induced death, directly demonstrating that PKB can alter lymphocyte homeostasis through inhibition of Fas/FasL signaling. This is consistent with earlier reports using transient transfections in cell lines that found Fas-induced cell death could be prevented by activated PKB (23, 24). Taken together, the data provide the first in vivo evidence that enhanced PKB activity in T cells can antagonize Fas-mediated cell death, and support the role of PKB as one of the main effector molecules downstream of PTEN that alters Fas-mediated apoptosis.

Fas is believed to play a critical role in maintaining immune homeostasis through activation-induced cell death as well as in establishing peripheral tolerance and tumor elimination through immune surveillance (20, 21). In light of the fact that PKB^{+/+} transgenic T cells have impaired FasL-induced death, at least two

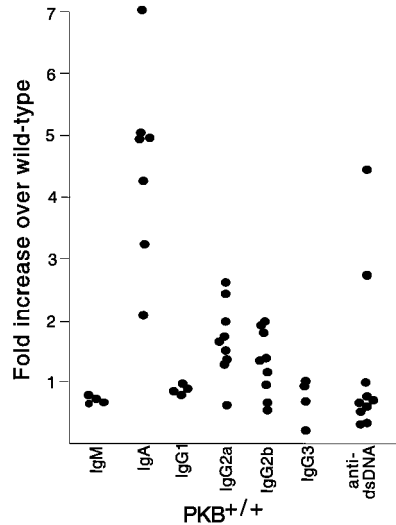


FIGURE 4. Elevated levels of serum IgA and anti-dsDNA Abs in PKB^{+/+} mice. A, Amounts of various classes of Ig and anti-dsDNA Abs from PKB^{+/+} transgenic mice. Sera from PKB^{+/+} transgenic mice or age-matched wild-type controls were analyzed for Ig isotypes. Sera from control mice were normalized to one. Each point represents an independent experiment.

possible scenarios could explain the phenotype of PKB^{+/+} transgenic mice. First, self-reactive T cells overexpressing active PKB could escape peripheral tolerance, remain activated, and persist due to a defect in Fas-induced death. Failure to delete these lymphocytes may result in accumulation of activated lymphocytes, leading to cytokine-induced B cell alterations and signs of autoimmunity. Alternatively, activated T cells may continue to express molecules such as CD40L, which enhances communication between B and T cells and may contribute to B cell hyperplasia. Another possibility is that T cells activated during normal immune responses or possibly triggered by intestinal flora within the gut mucosa expand and fail to undergo activation-induced cell death. The persistence of these lymphocytes may result in an overall accumulation over time and contribute to disease progression. Evidence in the literature currently favors a role for Fas in establishing peripheral tolerance to self Ags. Several *in vivo* models have shown that Fas can play a role in peripheral tolerance in both CD4 and CD8 populations (25–27), although the exclusive role for Fas in peripheral tolerance remains controversial (28–30). In addition, many studies have shown that Fas does not contribute to maintaining homeostasis after viral infection *in vivo* (29–34). Whether lymphocyte accumulation is due to impaired deletion after encounter with self or foreign Ags remains to be elucidated in this model.

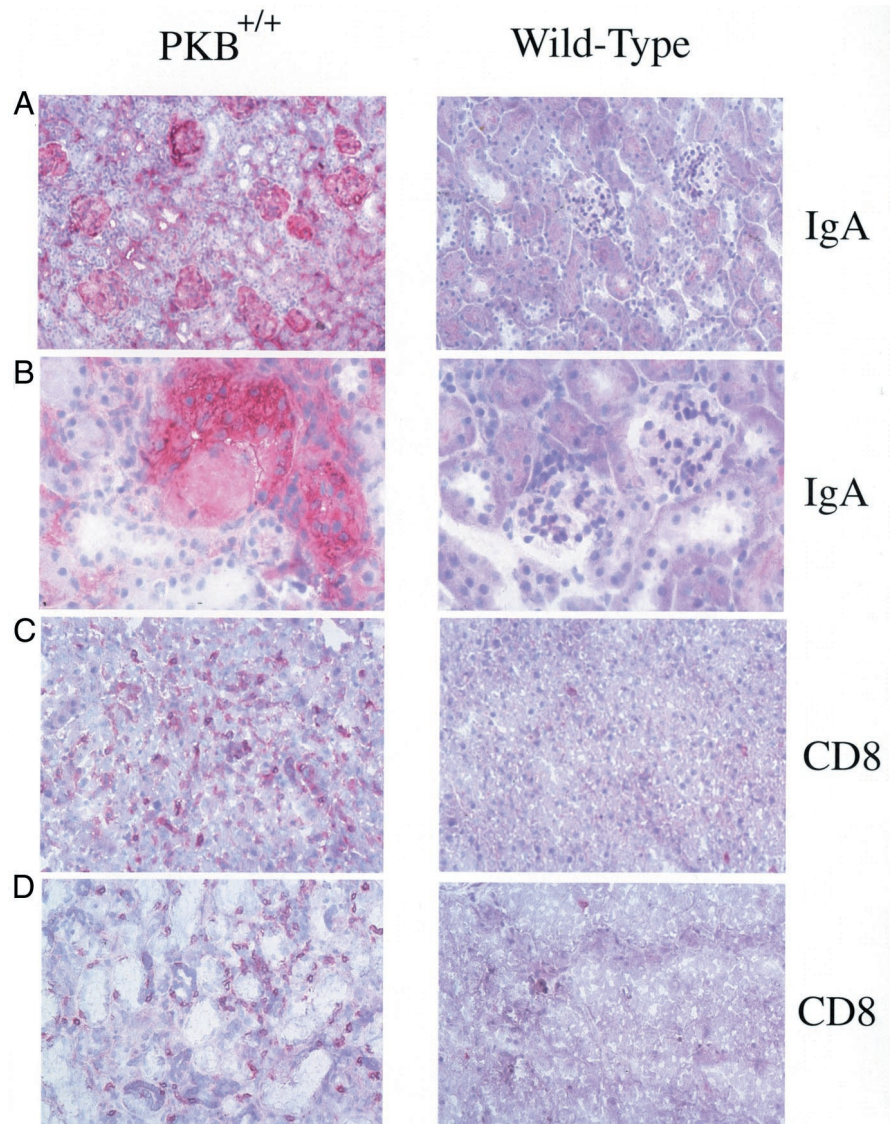


FIGURE 5. Spontaneous lymphocyte infiltration and high IgA in organs from PKB^{+/+} mice. Organs from mice with early stage disease and age-matched wild-type controls were analyzed by immunohistochemistry. A and B, Kidney sections stained with anti-IgA shown at $\times 200$ and $\times 400$ magnification, respectively. Sections from the liver (C) and salivary glands (D) of PKB^{+/+} transgenic and age-matched wild-type control mice analyzed using anti-CD8 Abs.

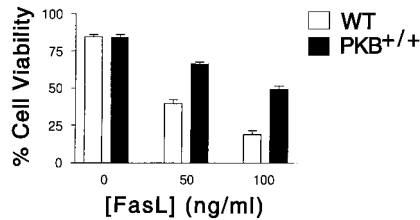


FIGURE 6. PKB^{+/+} T cells are resistant to FasL-induced cell death. Viability of PKB^{+/+} and wild-type (WT) T cells after treatment with two concentrations of FasL following CD3/CD28 stimulation.

Although it is clear that a defect in Fas-induced apoptosis alters lymphocyte homeostasis, there are subtle differences in the phenotype of diseases associated with different genetic defects (20, 22). *Lpr* (Fas^{-/-}) and *gld* (FasL^{-/-}) mice have a characteristic increase in CD4⁻/CD8⁻/B220⁺/αβT cells, which was not observed in either the PTEN^{+/-} or PKB^{+/+} mice. PTEN^{+/-}, *lpr*, and *gld* mice have been reported to experience IgG hypergammaglobulinemia (18, 35). Interestingly, while the sera from some PKB^{+/+} mice show an increase in IgG, all have substantial increases in IgA. This indicates involvement of the mucosal system and potentially points to differences between PTEN^{+/-}, Fas^{-/-}, FasL^{-/-}, and PKB^{+/+} transgenic mice. In this context, it is interesting to note that we have previously shown PKB transgenic T cells to have elevated NF-κB activity (4). NF-κB plays an important role as a *trans*-activator of a wide range of genes involved in immune and proinflammatory response, apoptosis, differentiation, and growth, and is also key mediator of mucosal inflammation (36). Indeed, NF-κB RelA-deficient lymphocytes have been shown to be deficient in IgA production, supporting the connection among PKB, NF-κB, and mucosal-initiated inflammation (37).

In humans, Fas gene mutations and consequently defects in Fas signaling result in an autosomal dominant disorder called autoimmune lymphoproliferative syndrome (ALPS) or Canale Smith syndrome. ALPS patients develop lymphoid hyperplasia and autoimmune disease, as well as exhibit a characteristic peripheral expansion of αβTCR⁺/CD4⁻/CD8⁻ lymphocytes (38, 39). Since ALPS was described, a few cases having ALPS symptoms but lacking Fas^{-/-}/FasL^{-/-} mutations have emerged. These patients were found to have mutations in caspase-10, a signaling component downstream of Fas and designated type II ALPS (40).

Interestingly, another group of patients has also been identified having splenomegaly and lymphadenopathy, but lacking both Fas/FasL mutations and a predominant CD4⁻/CD8⁻ population. Since these patients do not fit the classic criterion for ALPS type II, they were referred as having an autoimmune lymphoproliferative-like disease. It has been shown that these patients also have a defect in Fas-mediated apoptosis (22, 41). Therefore, in humans, it appears that defective Fas-induced apoptosis may be the underlying defect in lymphoproliferative syndromes with various phenotypes. Thus, it is likely that different genetic alterations that impair Fas-mediated apoptosis will have a slightly different phenotype that ultimately leads to the accumulation of lymphocytes in a variety of secondary lymphoid compartments.

These studies have defined a molecule PKB/Akt that contributes to lymphocyte homeostasis and the progression of autoimmune disease in vivo. We have shown that the expression of active PKB in T cells promotes survival (4), and has physiological relevance because it alters T cell homeostasis in vivo. In addition, PKB clearly promotes T cell inflammation, an important checkpoint of autoimmunity. Surprisingly, these studies also demonstrate that altered T cell homeostasis has profound implications on B cell homeostasis. This provides a new model that contributes to under-

standing of the physiological importance of Fas-mediated apoptosis in vivo. Although it is not yet known how activated PKB prevents Fas-mediated death, it is clear that PKB plays an important role in the exquisite balance between the PI-3K survival pathway and the death-promoting signals of Fas and potentially other members of the TNFR family.

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