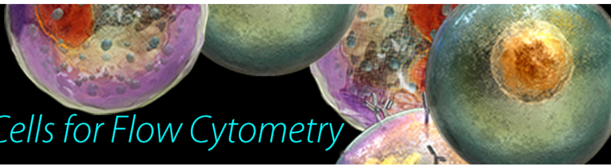

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Class I_B-Phosphatidylinositol 3-Kinase (PI3K) Deficiency Ameliorates I_A-PI3K-Induced Systemic Lupus but Not T Cell Invasion[1]

Domingo F. Barber,* Almira Bartolomé,* Carmen Hernandez,* Juana M. Flores,† Cristina Fernandez-Arias,* Luis Rodríguez-Borlado,* Emilio Hirsch,‡ Matthias Wymann,§ Dimitrios Balomenos,* and Ana C. Carrera[2]*

Class I PI3K catalyzes formation of 3-poly-phosphoinositides. The family is divided into I_A isoforms, activated by Tyr kinases and the I_B isoform (PI3Kγ), activated by G protein-coupled receptors. Mutations that affect PI3K are implicated in chronic inflammation, although the differential contribution of each isoform to pathology has not been elucidated. Enhanced activation of class I_A-PI3K in T cells extends $CD4^+$ memory cell survival, triggering an invasive lymphoproliferative disorder and systemic lupus. As both I_A- and I_B-PI3K isoforms regulate T cell activation, and activated pathogenic $CD4^+$ memory cells are involved in triggering systemic lupus, we examined whether deletion of I_B could reduce the pathological consequences of increased I_A-PI3K activity. I_B-PI3Kγ deficiency did not abolish invasion or lymphoproliferation, but reduced $CD4^+$ memory cell survival, autoantibody production, glomerulonephritis, and systemic lupus. Deletion of the I_B-PI3Kγ isoform thus decreased survival of pathogenic $CD4^+$ memory cells, selectively inhibiting systemic lupus development. These results validate the PI3Kγ isoform as a target for systemic lupus erythematosus treatment. *The Journal of Immunology,* **2006, 176: 589–593.**

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The PI3K are dual-specific lipid and protein kinases that participate in numerous cellular responses. The class I-PI3K are subdivided into class I_A and I_B. Class I_A-PI3K consists of three catalytic subunits, p110α, p110β, or p110δ, which form complexes with the p85 regulatory subunits, and are activated by tyrosine kinase receptor signaling. Class I_B is composed of the catalytic subunit p110γ, and is activated mainly by G protein-coupled receptors (GPCR)[3] (1, 2). Mutations in the PI3K pathway are involved in tumor generation, as well as in chronic inflammatory lupus-like disease (3–5). Although PI3K is a promising therapeutic target, knowledge of isoform-specific functions remains limited.

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease, characterized at early stages by an increase in autoreactive/memory $CD4^+$ cells (6–12). Deregulated T cells help trigger polyclonal B cell activation, giving rise to generalized B cell expansion, hypergammaglobulinemia, and increased autoantibody production. Circulating anti-DNA Ab form complexes that are captured in kidney, activating the complement cascade. As disease progresses, T cells and macrophages infiltrate the kidney and amplify the local inflammatory response (8). At advanced stages, mesangial proliferation, vascular collapse, and immune complex accumulation in kidney result in glomerulonephritis (GN) and renal failure (6–9).

Deregulation of T cell homeostasis is a critical early event in SLE (10–12). Both I_A- and I_B-PI3K regulate T cell differentiation, activation, and survival. I_B-PI3Kγ-deficient mice show T cell differentiation defects and reduced mature T cell activation (13–15). Deletion of the I_A isoform PI3Kδ reduces T cell activation (16); the I_A isoforms PI3Kα and β may also affect T cell activation, although this remains untested because deficiency in these isoforms is lethal in embryos (17, 18). Enhanced activation of I_A isoforms by $p65^{PI3K}$ transgene expression in T cells increases $CD4^+$ cell differentiation (15). In mature T cells, $p65^{PI3K}$ transgene expression enhances survival, triggering an invasive lymphoproliferative disease and systemic lupus (3). Deletion of the negative regulator of the class I PI3K pathway, the tumor suppressor phosphatase and tensin homolog deleted on chromosome 10, also induces SLE-like disease, confirming the capacity of PI3K to trigger this pathology (5).

We previously showed that enhanced activation of I_A-PI3K compensates some T cell differentiation defects in PI3K$\gamma^{-/-}$ mice (15). Using $p65^{PI3K}$ transgenic (Tg) mice, we tested whether I_B-PI3Kγ deletion could reduce the invasion, lymphoproliferation, and systemic lupus development induced by increased I_A-PI3K activity in T cells. PI3Kγ deletion did not abolish lymphoproliferation or invasion, but diminished $CD4^+$ memory cell survival, leading to amelioration of lupus and prolongation of mouse life span. As an increase in pathogenic $CD4^+$ memory cells is a hallmark of multigenic murine and human lupus (6–12), selective inhibition of

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[3] Abbreviations used in this paper: GPCR, G protein-coupled receptor; GN, glomerulonephritis; SLE, systemic lupus erythematosus; Tg, transgenic.

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memory cell survival by deletion of one PI3K isoform (I_B-PI3Kγ) suggests a treatment for this disease. These observations contribute to understanding the specific functions of I_A- and I_B-PI3K isoforms and their contribution to inflammation.

Materials and Methods

Mice

$p65^{PI3K}$Tg (C57/BL6) and PI3Kγ-deficient (129sv) mice were described previously (3, 13). $p65^{PI3K}$Tg mice were crossed with PI3Kγ-deficient mice. The F_2 generation was produced by $F_1 \times F_1$ mating: F_3 progeny were used for experiments. Lupus-like disease in $p65^{PI3K}$Tg PI3K$\gamma^{+/-}$ mice was indistinguishable from that of $p65^{PI3K}$Tg mice (3). As controls, we used littermates that did not express the transgene and were PI3K$\gamma^{+/-}$ or PI3K$\gamma^{+/+}$, which presented no obvious differences. Offspring were analyzed by PCR. Mice were bred and maintained under specific pathogen-free conditions at the Centro Nacional de Biotecnología animal facility. The Consejo Superior de Investigaciones Cientificas ethics committee approved the protocols used for experiments with mice.

Flow cytometry and cell death analysis

Spleen and lymph node cell suspensions were prepared; erythrocytes were lysed, and cells were counted. For surface staining, Abs were FITC, PE, or biotin conjugated, and cells were stained with saturating concentrations (4°C). Biotinylated Abs were developed with streptavidin-Spectral Red (Southern Biotechnology Associates). Abs used were CD3 (145-2C11), CD4 (L3T4, H129.19), CD8 (Ly-2, 53-6,7), CD44 (pgp1, IM7), and CD62L (all from BD Pharmingen). The Annexin V^{FITC} kit was from Corixa. Cells were analyzed on an EPICS XL with System II software (Beckman Coulter). Statistics analyses were performed using the StatView 512^+ program and the χ^2 test (⟨www.physics.csbju.edu⟩).

Biochemical and serological analyses and histology

Serum Ig and isotype-specific anti-dsDNA Ab were measured, as described (3). Urine protein levels were assessed with Medi-test Protein 2 Strips (Macherey-Nagel) every 15 days. Mice were examined daily; when severe SLE symptoms appeared, affected mice were killed and organs were collected for histology or flow cytometry analysis. Tissues were fixed in 4% Formalin in PBS until processing, as described (3).

Results

Prolonged life span in $p65^{PI3K}$Tg/PI3Kγ-deficient mice

We previously showed that the increase in I_A-PI3K activity induced by $p65^{PI3K}$ transgene expression in T cells causes accumulation of $CD4^+$ memory cells, an invasive lymphoproliferative disorder, and development of SLE-like disease; $p65^{PI3K}$Tg mice die of renal failure (3). I_A- and I_B-PI3K isoforms exhibit a partial functional compensation during thymic development (15). We thus examined whether I_B-PI3Kγ deletion could reduce the disease generated by enhanced I_A-PI3K activity in mature T cells. $p65^{PI3K}$Tg mice were crossed with PI3Kγ-deficient mice, and resulting progeny were examined. Mice were euthanized when they showed signs of disease (descamation and proteinuria; data not shown). Histological examination showed that 80% of $p65^{PI3KTg}$/PI3K$\gamma^{+/-}$ mice developed renal disease symptoms by 16 mo of age. In contrast, ~70% of $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ mice showed no symptoms at this age and ~30% remained healthy at 20 mo, near the end of their natural life span (Fig. 1*A*; Table I). Compared with $p65^{PI3K}$Tg/PI3K$\gamma^{+/-}$ littermates, $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ mice showed a significantly prolonged life span.

As $p65^{PI3K}$Tg mice show lymphocyte accumulation with age, we examined spleen and lymph node cell suspensions. $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ and $p65^{PI3K}$Tg/PI3K$\gamma^{+/-}$ littermates had significantly higher splenocyte numbers than control littermate mice, although numbers were slightly lower in $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ mice (Fig. 1*B*). In flow cytometry, the cell composition of $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ spleens was comparable to that of $p65^{PI3K}$Tg mice (3), with a significantly larger $CD4^+$ T cell population and a slightly larger $CD11b^+$ population compared with controls (Fig. 1*C*).

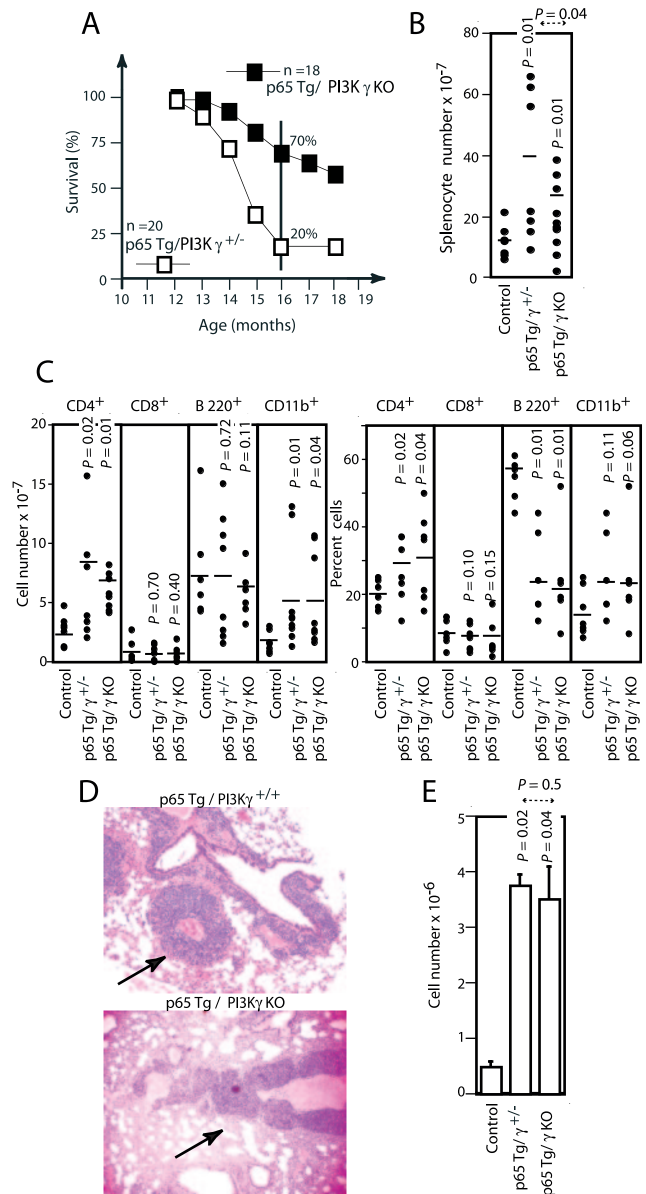

FIGURE 1. Prolonged life span and moderate reduction in lymphoproliferation in $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ mice. *A*, Percentage of survival of $p65^{PI3K}$Tg/PI3K$\gamma^{+/-}$ and $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ (knockout) littermates. χ^2 test, $p = 0.01$. *B*, Absolute number of splenocytes in $p65^{PI3K}$Tg/PI3K$\gamma^{+/-}$, $p65^{PI3K}$Tg/PI3Kγ knockout, and littermate control mice. Each dot represents a single mouse. *C*, Absolute number and percentage of $CD4^+$, $CD8^+$, $B220^+$, and $CD11b^+$ cells in splenocyte suspensions from control, $p65^{PI3K}$Tg/PI3K$\gamma^{+/-}$, and $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ mice. *D*, H&E staining of lung sections from $p65^{PI3K}$Tg/PI3K$\gamma^{+/+}$ and $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ mice. Lymphocyte infiltrate (arrows) is seen in both sections. *E*, Cytofluorometry analysis of lung suspensions from $p65^{PI3K}$Tg/PI3K$\gamma^{+/-}$ and $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ mice. Student's *t* test *p* values are indicated.

Lymphoproliferation was also observed in lymph nodes (data not shown). T cells from $p65^{PI3K}$Tg mice infiltrate many nonlymphoid tissues (3). Histological analysis of lung (Fig. 1*D*) and kidney (data not shown) indicated that PI3Kγ deletion does not reduce the magnitude of infiltrates in $p65^{PI3K}$Tg mice. These infiltrates are enriched in T cells (3), and flow cytometry analysis of lung suspensions showed no significant differences between T cell infiltrates in $p65^{PI3K}$Tg/PI3K$\gamma^{-/-}$ and $p65^{PI3K}$Tg/PI3K$\gamma^{+/-}$ mice (Fig. 1*E*). I_B-PI3Kγ deletion thus prolonged $p65^{PI3K}$Tg mouse survival, but

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Table I. *SLE-like disease in p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ and p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice*

Analysis	Mice: p65^{PI3K} Tg/PI3K$\gamma^{+/-}$ (n = 20)	Mice: p65^{PI3K} Tg/PI3Kγ KO (n = 18)
Disease incidence at 16 mo (%)[a]	80	33
Healthy at 20 mo (%)[b]	0	33
Advanced GN (score 3–4)[c] (%)	85	16
Moderate GN (score 2–1) (%)	15	33
Normal kidney (%)	0	50

[a] Mice were analyzed at appearance of disease symptoms.
[b] Mice with no external signs of disease.
[c] Glomerulonephritis was determined at necropsy performed when animals showed first disease signs; <15 mo for p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ and >17 mo for p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice.

did not abrogate T cell lymphoproliferation or invasion in these mice.

Reduced renal disease in p65^{PI3K}Tg/PI3Kγ-deficient mice

SLE-like disease development is accompanied by polyclonal hypergammaglobulinemia and proteinuria (3, 7). p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice developed proteinuria with age, which was low or absent in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ littermates (data not shown). Total anti-DNA Ab levels, as well as IgM, IgG1, IgG2a, and IgG2b levels, were reduced in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mouse serum compared with p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ littermates (Fig. 2*A*).

Histological examination of kidney sections showed renal lesions in most p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice, including frequent hyaline casts in tubules, increased mesangial cells, inflammatory infiltration, hypercellular glomeruli, thickening of capillary walls and vascular obliteration, at an intensity similar to that in p65^{PI3K}Tg/PI3K$\gamma^{+/+}$ mice (Fig. 2*B*). These signs indicate severe mesangioproliferative GN (Berden score grades 2–4) (19) (Fig. 2*B*). These features were reduced or absent in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice, whose GN scores ranged from 0 to 1 (Fig. 2*B*; Table I). Granular immune complexes were abundant in p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice, and were minimal or absent in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice (Fig. 2*C*). Proteinuria, renal lesions, and immune complexes suggest renal failure as the cause of early death of p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mouse. We detected no gender differences. Renal disease was thus less severe in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice, despite the presence of invasive lymphoproliferation, indicating that PI3Kγ deletion specifically hinders lupus development.

Reduction in CD4$^+$ memory T cell numbers in p65^{PI3K}Tg/PI3Kγ-deficient mice

CD4$^+$ memory cell accumulation, a characteristic of systemic lupus, is found in p65^{PI3K}Tg mice (3). These cells are implicated in disease development (6, 10–12). As PI3Kγ regulates T cell activation (14, 15), we examined whether PI3Kγ deletion could reduce CD4$^+$ memory cell numbers. Despite the fact that PI3Kγ deletion did not significantly affect the memory T cell pool in non-Tg mice (Fig. 3*A*), both the proportion and absolute numbers of CD4$^+$CD44^{high} and CD4$^+$CD62L^{low} memory cells were significantly reduced in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ compared with p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice (Fig. 3, *A* and *B*). As p65^{PI3K}-induced CD4$^+$ memory T cell expansion involves reduction of cell death rates within this population (3), we examined whether PI3Kγ deletion restores normal CD4$^+$ memory T cell death rates in p65^{PI3K}Tg mice. CD4$^+$CD44^{high} memory cell death in vivo was

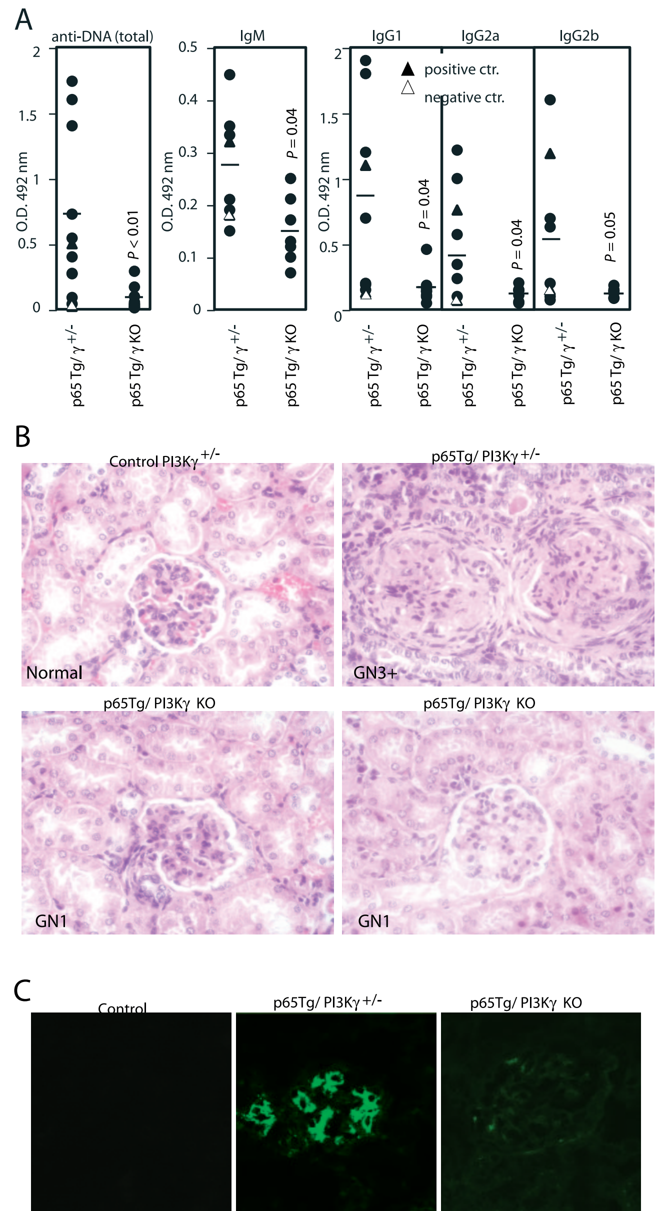

FIGURE 2. Reduced renal disease in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice. *A*, Anti-dsDNA total Ab, IgM, IgG1, IgG2a, and IgG2b in p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ and p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mouse sera. Ab titers are represented as absorbance at $OD_{492\ nm}$ at a 1/1600 serum dilution. △, Titer of a serum pool from littermate wild-type mice; ▲, mean Ab titer from groups of p65^{PI3K}Tg and MRL/*lpr* mice, which yielded similar results. Student's *t* test *p* values are indicated. *B*, H&E-stained kidney sections from representative PI3K$\gamma^{+/-}$ (normal) and p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice (GN score 3), and two p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice (GN score 1). *C*, FITC anti-IgG Ab-stained IgG deposits in representative mice. IgG deposits are seen in p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice and are notably reduced or absent in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ kidney. Control non-Tg kidney is shown for comparison.

higher in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ compared with p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice (Fig. 3*C*). Accordingly, spontaneous death of cultured CD4$^+$ T cells from p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice was also significantly higher than those of p65^{PI3K}Tg/PI3K$\gamma^{+/-}$ mice (Fig. 3*D*). In addition, we observed reduction of CD4$^+$ T cell survival after PI3Kγ deletion in non-Tg mice (Fig. 3*D*). Together, the findings show that amelioration of lupus disease in p65^{PI3K}Tg/PI3K$\gamma^{-/-}$ mice correlates with reduced CD4$^+$ memory cell survival.

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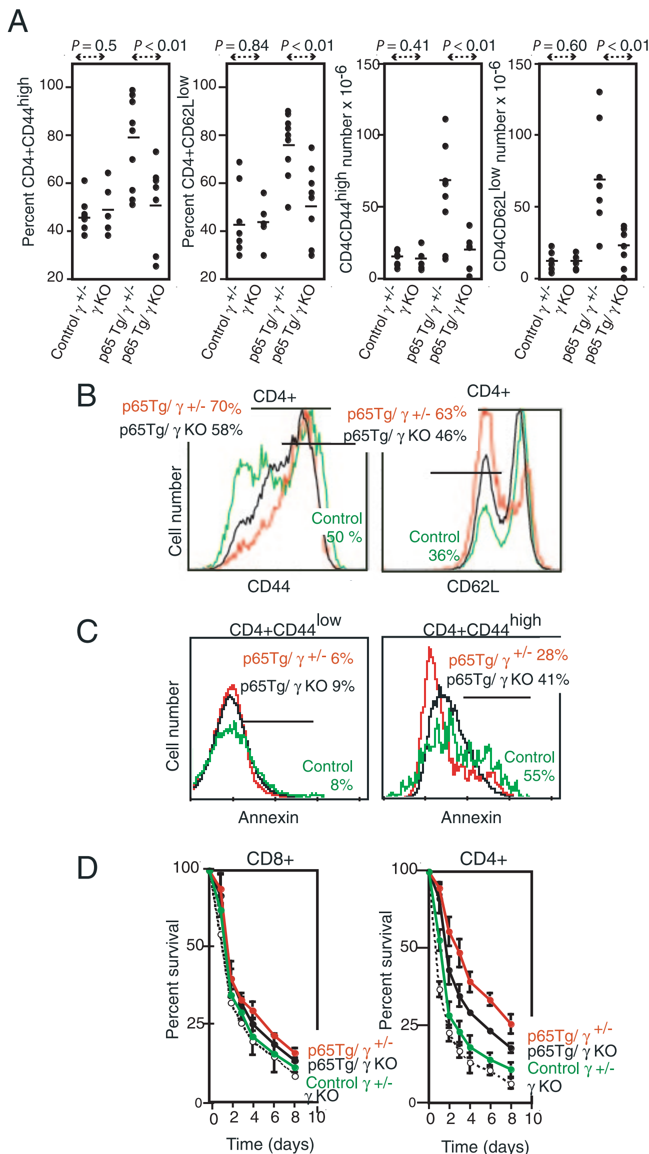

FIGURE 3. Reduction in $CD4^+$ memory cell numbers in $p65^{PI3K}Tg/PI3K\gamma^{-/-}$ mice. *A*, The proportion and absolute numbers of $CD4^+CD44^{high}$ and $CD4^+CD62L^{low}$ cells were examined in $PI3K\gamma^{+/-}$, $PI3K\gamma^{-/-}$, $p65^{PI3K}Tg/PI3K\gamma^{+/-}$, and $p65^{PI3K}Tg/PI3K\gamma^{-/-}$ littermate mice spleens. Student's *t* test *p* values are indicated. *B*, CD44 and CD62L expression was examined in representative control (green), $p65^{PI3K}Tg/PI3K\gamma^{+/-}$ (red), and $p65^{PI3K}Tg/PI3K\gamma^{-/-}$ (black) spleens. Percentages of $CD44^{high}$ and $CD62L^{low}$ cells are indicated. *C*, $CD4^+$ cell death rates in spleen as examined by log fluorescence intensity of annexin V binding in $CD4^+CD44^{high}$ and $CD4^+CD62L^{low}$ populations in control (green), $p65^{PI3K}Tg/PI3K\gamma^{+/-}$ (red), and $p65^{PI3K}Tg/PI3K\gamma^{-/-}$ (black) mice. The percentage of annexin V-positive cells is indicated. A representative mouse from one experiment of four with similar results. *D*, Proportions of viable $CD4^+$ and $CD8^+$ T cells at different time points of in vitro culture, considering initial $CD4^+$ and $CD8^+$ numbers as 100%. $PI3K\gamma^{+/-}$ (green), $PI3K\gamma^{-/-}$ (dashed black), $p65^{PI3K}Tg/PI3K\gamma^{+/-}$ (red), and $p65^{PI3K}Tg/PI3K\gamma^{-/-}$ (black). Comparison of surviving T cells from the different mutant mice with control $PI3K\gamma^{+/-}$ mice at day 8 in three assays yielded a significant difference ($p < 0.05$).

Discussion

Enhanced I_A-PI3K activity in T cells corrects some of the thymocyte differentiation defects observed in I_B-$PI3K\gamma^{-/-}$ mice (15), suggesting partial compensation between I_A- and I_B-PI3K isoforms. We thus examined whether I_B-PI3Kγ deletion could reduce the pathological consequences of increased I_A-PI3K activity in mature T cells (3). I_B-PI3Kγ deletion did not abolish I_A-PI3K-induced T cell invasion or lymphoproliferation, but reduced $CD4^+$ memory cell survival, as well as GN incidence and severity, prolonging $p65^{PI3K}Tg$ mouse life span. Therefore, although I_A is activated by Tyr kinases and I_B by GPCR (1, 2), I_B-PI3Kγ deletion was capable of selectively ameliorating I_A-PI3K-induced lupus.

Normal quiescent T cells have low levels of PI3K lipid products, which increase only after cell activation (1, 2). PI3Kγ-deficient mice show reduced T cell activation and impaired macrophage and neutrophil mobilization; however, they have a relatively normal hemopoietic cell composition in basal conditions (13, 14). Interference with PI3Kγ is thus predicted to induce minor side effects. Enhanced I_A-PI3K activation causes lupus (3–5). Moreover, endogenous PI3K activation was observed in a graft-vs-host-induced murine systemic lupus model (20) and in lupus-prone MRL/*lpr* mice (21). Our preliminary data suggest that PI3K activity is also increased in human SLE T cells (>75% of patients, $n = 17$, in progress). Increased basal PI3K activity may thus constitute a susceptibility factor for SLE, or be required for $CD4^+$ memory cell maintenance. Validation of PI3Kγ as a target in systemic lupus, suggested by genetic PI3Kγ interference in this study, is further supported by pharmacological studies using PI3Kγ inhibitors in lupus-prone mice (21).

Chronic inflammatory autoimmune diseases are triggered by distinct factors, including defects in immune response down-regulation as well as central or peripheral tolerance defects, resulting in maintenance of autoreactive $CD4^+$ memory cells (22). In the $p65^{PI3K}Tg$ model, lupus is a consequence of excessive $CD4^+$ T cell survival (3), resulting in $CD4^+$ memory cell accumulation. Recovery from SLE-like disease following PI3Kγ deletion correlates with a reduction in $CD4^+$ memory T cell numbers. As pathogenic $CD4^+$ memory cells contribute to SLE development (10–12), the decrease in memory cells is probably a basic mechanism by which PI3Kγ inhibition ameliorates lupus. The decrease in $CD4^+$ memory cells with helper activity is probably responsible for the diminished B cell activation, and may reduce macrophage and neutrophil activation. The studies presented nonetheless do not exclude a direct effect of PI3Kγ deletion on these populations.

No differences in disease development were observed between male and female $p65^{PI3K}Tg$ mice (3). As higher female SLE incidence in other models correlates with a higher proportion of pathogenic $CD4^+$ cells (10), the observation that the $p65^{PI3K}$ transgene enhances $CD4^+$ cell survival similarly in males and females (3) explains the lack of gender-susceptibility differences. Similarly, PI3Kγ deletion reduced survival of $CD4^+$ memory cells in a gender-independent fashion, explaining why both males and females recovered following PI3Kγ deletion.

Regarding specific and redundant I_A and I_B isoform functions, enhanced I_A-PI3K activity compensates the defective pre-TCR-triggered $CD4^-CD8^-$ differentiation and TCR-induced $CD4^+$ cell generation in I_B-$PI3K\gamma^{-/-}$ mice. This suggests that the two isoforms cooperate to trigger T cell differentiation (15). Concurring with this, the $PI3K\gamma\delta^{-/-}$ phenotype shows a striking T cell development blockade (23). In this study, we show that both I_A and I_B isoforms regulate $CD4^+$ memory cell survival in mature T cells. Comparison of in vitro $PI3K\gamma^{-/-}$ and $PI3K\gamma^{+/-}$ T cell survival supports an independent role for PI3Kγ in $CD4^+$ T cell survival. PI3K also regulates T cell activation in vitro and in vivo (14) (our data not shown).

Despite PI3Kγ involvement in T cell activation and survival, the proportion of memory T cells in non-Tg $PI3K\gamma^{+/-}$ and $PI3K\gamma^{-/-}$

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mice was similar, suggesting that a homeostatic mechanism compensates the potentially reduced memory cell survival and generation in PI3K$\gamma^{-/-}$ mice. Similar homeostatic correction is found in the PI3Kγ contribution to T cell differentiation. Although defective T cell differentiation is detected in newborn PI3K$\gamma^{-/-}$ mice, T cell populations are near normal in 15-day- to 1-mo-old animals, with only a modest reduction in peripheral CD4$^+$ T cell numbers (15).

Our data show that PI3Kγ deficiency reduces CD4$^+$ memory T cell survival in p65^{PI3K}Tg mice. Nonetheless, we cannot exclude that one effect of PI3Kγ deletion in this model is a reduction in memory cell generation, as suggested by experiments in MRL/*lpr* mice (21). In these mice, PI3Kγ inhibition also ameliorates lupus and reduces pathogenic CD4$^+$ memory cell numbers (21). In MRL/*lpr* mice, however, the reduction in CD4$^+$ memory T cells is not linked to variations in survival, because apoptosis is defective due to the *lpr*/Fas mutation (7). The reduction in CD4$^+$ memory cells in PI3Kγ inhibitor-treated MRL/*lpr* mice is thus probably the result from a reduction in memory cell generation.

Although the consequences of PI3Kγ interference are similar regarding lupus amelioration and CD4$^+$ memory cell reduction, inhibition of PI3Kγ in MRL/*lpr* mice reduces hypercellularity more effectively, with a selectively greater effect on the CD4$^+$ cell pool (21). Total CD4$^+$ T cell numbers are also reduced in PI3K$\gamma^{-/-}$ mice (15). This effect is not seen in PI3K$\gamma^{-/-}$/p65^{PI3K}Tg mice, however, suggesting that enhanced I_A activation has a unique function in inducing lymphoproliferation that is not counteracted by I_B deficiency.

PI3Kγ is involved in macrophage, neutrophil, and thymocyte migration (13–15). PI3Kγ deletion, however, did not reduce T cell invasion induced by enhanced I_A-PI3K activity. Accordingly, T cell homing to lymph nodes is only moderately affected by PI3Kγ deletion (24). T cell invasion thus probably involves I_A-PI3K activation through a Tyr kinase pathway, whereas thymus growth may require I_B-PI3K activation through a GPCR. As for I_A and I_B isoform control of T cell activation and survival, either receptors that trigger these responses activate both isoforms, or distinct receptors that activate I_A and I_B, respectively, cooperate in promoting CD4$^+$ cell survival and activation.

The results show that whereas I_A isoforms are dominant in the induction of lymphoproliferation and invasion, I_B-PI3Kγ deletion reduces CD4$^+$ memory cell survival, even in the presence of active I_A isoforms. As pathogenic CD4$^+$ memory cell increase contributes to multigenic murine and human lupus (6–12), the selective reduction of CD4$^+$ memory cells and subsequent amelioration of systemic lupus upon I_B-PI3Kγ deletion suggest that this isoform is a promising target for SLE treatment.

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Disclosures

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