Transgenic Expression of Helios in B Lineage Cells Alters B Cell Properties and Promotes Lymphomagenesis[1]

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Helios, a member of the Ikaros family of DNA-binding proteins, is expressed in multipotential lymphoid progenitors and throughout the T lineage. However, in most B lineage cells, Helios is not expressed, suggesting that its absence may be critical for B cell development and function. To test this possibility, transgenic mice were generated that express Helios under the control of an Ig μ enhancer. Commitment to the B cell lineage was unaltered in Helios transgenic mice, and numbers of surface IgM$^+$ B cells were normal in the bone marrow and spleen. However, both bone marrow and splenic B cells exhibited prolonged survival and enhanced proliferation. B cells in Helios transgenic mice were also hyperresponsive to Ag stimulation. These alterations were observed even though the concentration of ectopic Helios in B lineage cells, like that of endogenous Helios in thymocytes, was well below the concentration of Ikaros. Further evidence that ectopic Helios expression contributes to B cell abnormalities was provided by the observation that Helios transgenic mice developed metastatic lymphoma as they aged. Taken together, these results demonstrate that silencing of Helios is critical for normal B cell function. *The Journal of Immunology,* **2005, 175: 3508–3515.**

The commitment of hemopoietic stem cells to B and T lymphocyte lineages is regulated by transcription factors whose critical functions have been demonstrated in gene disruption experiments (1–5). Transcription factors that regulate lymphocyte commitment have also been found to block the development of alternative hemopoietic lineages when expressed ectopically in uncommitted progenitors. For example, T cell development is blocked by the B cell commitment factors EBF1 and Pax5, whereas B cell development is blocked by the T cell commitment factor Notch1 (6–9).

Ikaros is the founding member of a small family of DNA-binding proteins that plays a prominent role in lymphocyte development (3, 10, 11). The mouse and human genomes encode five members of the Ikaros family, which are characterized by a highly conserved C2H2 zinc finger DNA-binding domain near the N terminus and a second C2H2 zinc finger protein-protein interaction domain near the C terminus (10, 12–16). The C-terminal fingers support the formation of dimers and multimers between Ikaros family members (17–19). Numerous isoforms of Ikaros proteins are generated by alternative pre-mRNA splicing, although their functional significance in normal cells remains unknown (20, 21).

Two members of the Ikaros family, Pegasus and Eos, are broadly expressed. However, the other three, Ikaros, Aiolos, and Helios, are expressed primarily in hemopoietic cells, where they bind similar or identical DNA sequences (11–13, 16). Ikaros and Aiolos are expressed in most hemopoietic cells (12), but Helios expression is observed primarily in T lineage cells and some multipotential progenitors (13, 14). In thymocytes, Helios is 5- to 10-fold less abundant than Ikaros (13). Furthermore, coimmunoprecipitation experiments revealed that Helios is efficiently associated with Ikaros, whereas only a modest fraction of Ikaros associates with Helios (13). These findings led to speculation that Helios may be a limiting regulatory subunit of Ikaros complexes.

Because Ikaros family members are DNA-binding proteins, they are thought to regulate transcription and/or chromatin structure. However, their mechanisms of action remain poorly understood. Several findings, including predominant localization to pericentromeric heterochromatin and association with histone deacetylase complexes, suggest that Ikaros proteins contribute to gene silencing (3, 22, 23). Consistent with this hypothesis, Ikaros binding sites in the promoters of the *λ5* and *terminal transferase* (*Dntt*) genes were found to be critical for gene silencing during B and T cell development, respectively, in transgenic mice and stable transfection experiments (24, 25). However, Ikaros also contributes to the activation of *Cd8α* during thymocyte development, and Mi-2*β*, a major Ikaros-associated protein, was recently implicated in transcriptional activation (26, 27). A dual role in activation and silencing would be consistent with dual roles in both processes of Hunchback, the *Drosophila* orthologue of the Ikaros family (23).

Gene disruption experiments have provided important insights into the specific functions of Ikaros and Aiolos (3, 11). Four different strains of Ikaros mutant mice and one Aiolos mutant strain have been generated, all of which exhibit severe defects in lymphocyte development and function (28–36). The most severe defects were observed with two Ikaros mutant proteins that can associate with other Ikaros family members and block their functions (28, 36). Among the many interesting phenotypes that have been described, two of the most intriguing and consistent within the various Ikaros and Aiolos mutant strains are the development of lymphoma and reduced activation thresholds of lymphocyte populations.

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The T cell-restricted expression pattern of Helios is particularly interesting and suggests that this protein may be a critical regulator of T cell development. In addition, the extinction of Helios expression in B lineage cells may be critical for their normal development and function. To test this latter hypothesis, we expressed Helios under the control of the Ig μ enhancer. Normal numbers of B cells were observed in Helios transgenic mice, indicating that Helios expression from the Ig μ enhancer does not prevent lineage commitment or development. Surprisingly, however, defects in B cell function as well as B cell lymphoma were observed, even though the abundance of ectopic Helios in B lineage cells was well below that of Ikaros. Taken together, the results reveal that Helios and Ikaros contribute distinct functions during hemopoiesis and that Helios down-regulation is essential for normal B cell function.

Materials and Methods

Transgenic mice

A full-length murine Helios cDNA was amplified by RT-PCR, modified by addition of *Cla*I linkers and sequences encoding an N-terminal hemagglutinin (HA)[3] epitope tag, and inserted into the *Cla*I site of the pJT1 transgene expression plasmid (37). After DNA sequencing, the 5.9-kb expression cassette was excised (using *Not*I and *Kpn*I restriction enzymes), gel purified, and dialyzed. Oocytes from C57BL/6 mice were microinjected with the purified fragment at the University of California (Irvine, CA) transgenic mouse facility. Four transgenic founders were obtained. Heterozygous transgenic mice were used for the experiments (see Figs. 1–4), and homozygous mice for the experiments (Fig. 5). Both homozygous and heterozygous mice were used to characterize lymphomas (Fig. 6). The experiments reported in this article were approved by the University of California, Animal Research Committee.

Southern and Western blot analyses

Tail tips from mice were digested overnight in 50 mM Tris-HCl (pH 8), 100 mM EDTA, 100 mM NaCl, 1% SDS, 0.5 mg/ml proteinase K at 55°C. DNA was purified by phenol-chloroform extraction and ethanol precipitation. DNA (10 μg) was then digested with *Bam*HI, electrophoresed through a 1% agarose gel, and transferred to nylon membranes (Amersham Pharmacia Biotech). Prehybridization and hybridization of a ^{32}P-labeled Helios cDNA probe were performed according to the manufacturer's protocol.

Western blots were performed using the SuperSignal West Femto kit (Pierce) with nuclear extracts or whole cell lysates from mouse tissues or transfected HEK 293T cells, and Abs directed against an HA epitope, Helios, or Ikaros (13). Abs directed against antiapoptotic proteins were obtained from Santa Cruz Biotechnology (Bcl-2, clone N-19; Bcl-x_L, clone P-19; Bax, clone H-62).

Flow cytometry

Flow cytometry of bone marrow cells and splenocytes was performed on a FACScan (BD Biosciences) using R-PE or PerCP rat anti-mouse CD45R/B220 (clone RA3-6B2), FITC rat anti-mouse CD8 (clone 53-6.7), R-PE rat anti-mouse CD4 (clone RM4-5; all from BD Pharmingen), and FITC rat anti-mouse IgM (clone 1B4B1; Southern Biotechnology Associates).

Cell proliferation assay

Splenocytes (1–2 $\times$ 10^5) or bone marrow cells (5 $\times$ 10^5) were cultured in triplicate in wells of 96-well plates for each condition: growth medium alone or medium supplemented with Con A (5 μg/ml), anti-IgM F(ab′)$_2$ (Jackson ImmunoResearch Laboratories), or with *Salmonella typhosa* LPS (50 μg/ml; Sigma-Aldrich). Cells were incubated for 3 days at 37°C, 5% CO_2. Sixteen hours before the end of the incubation, 1 μCi of [^{3}H]thymidine was added per well. Following harvest of cells, [^{3}H]thymidine incorporation was measured with a beta counter following dissolution in scintillation fluid.

Isotype-specific ELISA

Determination of Ig isotypes in mouse serum by ELISA was conducted using rabbit anti-mouse IgG+A+M (H + L chain specific) Abs (Zymed Laboratories) as the capture agent to which serum samples were added. Captured Ig isotypes were developed with biotinylated rabbit anti-mouse IgG1, IgG2a, IgG2b, IgG3, and IgM Abs. The Ig isotype concentrations were calculated by comparing the mean OD at 405 nm of duplicate wells of the tested sera to the curve obtained with purified standards.

T cell-dependent and -independent immune responses

Ten-week-old Helios transgenic and C57BL/6 control mice were immunized with either alum-precipitated NP_{12}-CG ((4-hydroxy-3-nitrophenyl)acetyl chicken gammaglobulin; 100 μg/mouse) or soluble NP-Ficoll ((4-hydroxy-3-nitrophenyl)-acetyl; 5 μg/mouse). Sera were collected 14 days later and the anti-NP Ab response was measured as previously described (38).

Immunohistochemistry and microscopy

Dissected organs were placed in 10% buffered formalin or fresh-frozen in OCT embedding compound. Routine histological sections were prepared from formalin-fixed, paraffin-embedded tissues and stained with H&E by standard procedures. Immunohistochemistry was performed on frozen tissues. Sections were fixed in cold absolute methanol or 95% ethyl alcohol for 5 min and washed in 1$\times$ PBS. Slides were then incubated separately with 1/100 or 1/200 rat anti-mouse CD45R (clone RA3-6B2; BD Pharmingen) or rat anti-mouse CD3 (clone 17A2; BD Pharmingen) primary Abs for 40 min in 1$\times$ PBS supplemented with 1/100 normal horse serum as a blocking agent (1/20 dilution; Invitrogen Life Technologies). This was followed by incubation with mouse-absorbed rabbit anti-rat Ig (1/50 dilution in normal horse serum; DAKO) for 30 min and then mouse-absorbed HRP-conjugated swine anti-rabbit Ig in normal mouse serum (1/50 dilution in normal horse serum; DAKO) for 30 min. Ab was localized with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide and sections were counterstained with hematoxylin and methyl green. Negative control slides received diluent only instead of primary Abs.

Results

Generation of transgenic mice expressing Helios in B cells

To study the consequences of Helios expression in progenitor and mature B cells, transgenic mice were generated that express the murine Helios cDNA under the control of the mouse Ig μ intronic enhancer, HSV thymidine kinase promoter, and human growth hormone polyadenylation signal (Fig. 1*A*). The Ig μ enhancer is thought to be active in all B lineage cells, as well as in common lymphoid progenitors, which have been proposed in recent models to be an early B lineage specified population (37, 39, 40). Sequences encoding an HA tag were placed at the 5′ end of the Helios cDNA. Fig. 1*B* shows a representative Southern blot of the three transgenic founders selected for further analysis. All three founders contained the integrated Helios cDNA. Expression of HA-Helios in bone marrow (Fig. 1*C*), spleen, and lymph nodes (data not shown) was confirmed by Western blot by using Abs against the HA epitope.

A Western blot performed with Helios Abs revealed that HA-Helios in bone marrow cells from transgenic line D was less abundant than endogenous Helios in thymocytes from control mice or from the transgenic line (Fig. 1*D*). Because B lineage cells represent up to 30% of nucleated bone marrow cells, the overall abundance of HA-Helios in B cells appears comparable to that of endogenous Helios in thymocytes.

In thymocytes, Helios is 5- to 10-fold less abundant than Ikaros (13). Therefore, the HA-Helios in bone marrow cells may be less abundant than endogenous Ikaros. The relative concentrations of Ikaros and HA-Helios are relevant because of possible redundancy between these two proteins (see start of this report). If ectopic HA-Helios greatly increases the total concentration of Ikaros family members, any phenotypes observed could result from overexpression of this family. In contrast, if HA-Helios is expressed at limiting concentrations relative to endogenous Ikaros, the phenotypes would likely be due to specific effects of ectopic HA-Helios.

To determine the relative concentrations of endogenous Ikaros and HA-Helios, it was necessary to compare the sensitivities of the Ikaros and HA Abs. HA-Ikaros was overexpressed in HEK 293T cells. Three different amounts of nuclear extract from these cells

[3] Abbreviations used in this paper: HA, hemagglutinin; sIgM, surface IgM.

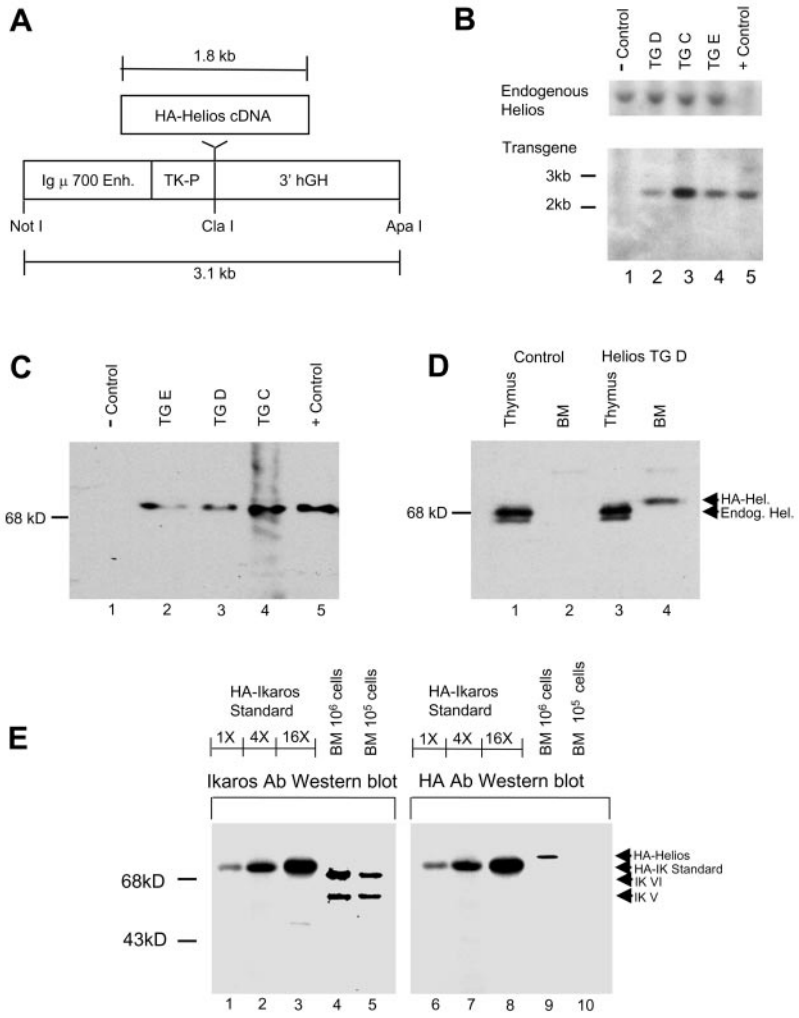

FIGURE 1. Expression of the Helios transgene. *A*, The Helios transgene construct contains the 700-bp IgH intronic enhancer (Ig μ 700 Enh.), HSV thymidine kinase promoter (TK-P), and human growth hormone mRNA splice site and polyadenylation signal (3′hGH). The full-length mouse Helios cDNA contains an HA epitope tag at the 5′ end. *B*, Presence of the microinjected DNA in transgenic founders was confirmed by Southern blot analysis using a radiolabeled probe spanning the thymidine kinase promoter and 5′ end of the HA-Helios cDNA. The expected size of the hybridization product is 2.4 kb. Results (*bottom*) are shown for a negative control (wild-type mouse DNA, *lane 1*), transgenic lines C–E (*lanes 2–4*), and a positive control (two genome equivalents of plasmid DNA, *lane 5*). The same blot was then probed with a radiolabeled Helios cDNA fragment to detect the endogenous Helios gene (*top*). *C*, Expression of HA-Helios protein in the three transgenic founders C–E (*lanes 2–4*) was monitored by Western blot, using nuclear extracts from bone marrow cells and an anti-HA mAb. Nuclear extracts from wild-type bone marrow cells (*lane 1*) and from HEK 293T cells (*lane 5*) containing overexpressed HA-Helios were also analyzed. *D*, Expression of endogenous Helios and HA-Helios in 10^6 thymocytes or bone marrow cells was monitored by Western blot, using anti-Helios polyclonal Abs. Whole cell extracts from wild-type mice (*lanes 1* and *2*) and transgenic line D (*lanes 3* and *4*) were analyzed. *E*, Relative quantities of endogenous Ikaros and HA-Helios in the bone marrow of transgenic line D were determined by Western blot, following normalization of signals obtained with overexpressed HA-Ikaros. Parallel blots were generated and were incubated with either Ikaros Abs (*lanes 1–5*) or HA Abs (*lanes 6–10*). For the purpose of normalization, exposures of the two blots were obtained that showed similar signals with the HA-Ikaros standards (*lanes 1–4* and *lanes 6–8*). The HA-Helios signal in 10^6 bone marrow cells (*lane 9*) was comparable to the Ikaros signal in 10^5 bone marrow cells (*lane 5*), suggesting that, on average, Ikaros is 10 times more abundant than HA-Helios.

were then analyzed on parallel Western blots, using Ikaros Abs to probe one blot and HA Abs to probe the other (Fig. 1*E*, *lanes 1–3* and *6–8*). Each Western blot also contained two different amounts of total bone marrow nuclear extract derived from transgenic line D (Fig. 1*E*, *lanes 4*, *5*, *9*, and *10*). By varying the time of exposure to film, exposures of the two blots that yielded comparable signals with the HA-Ikaros standards were obtained (Fig. 1*E*). A comparison of the Ikaros (Fig. 1*E* , *lanes 4* and *5*) and HA-Helios (Fig. 1*E*, *lanes 9* and *10*) signals observed in these normalized gel images suggests that endogenous Ikaros is ~10-fold more abundant than the ectopic HA-Helios (i.e., the Ikaros signal in Fig. 1*E*, *lane 5* is comparable to the HA-Helios signal in *lane 9*, which was derived from 10 times more cells). Because transgenic HA-Helios increases to only a small extent the total concentration of Ikaros family members, any effects of HA-Helios are likely to represent specific consequences of Helios misexpression, rather than an increase in the overall concentration of Ikaros proteins. It is important to note, however, that these results represent average expression levels within bone marrow populations. We cannot rule out the possibility that, at some stages of B cell development, HA-Helios may be more abundant than endogenous Ikaros.

B cells are present in Helios transgenic mice

To determine whether B cell development occurs in Helios transgenic mice, B cells were first monitored in the bone marrow. Despite a reduction in the total number of $CD45R^+$ cells in Helios

transgenic mice, transgenic and control animals had comparable frequencies of surface IgM-positive ($sIgM^+$) cells (Fig. 2*A*). Total numbers of $sIgM^+$ cells were also comparable, as total bone marrow cellularity was the same in control and Helios transgenic animals (data not shown).

An analysis of spleen cellularity revealed no difference in cell numbers in Helios transgenic and wild-type mice (data not shown). The percentages of splenic $CD45R^+$ $sIgM^+$ B cells were also comparable in transgenic and wild-type mice (Fig. 2*B*). Further histopathological analysis of the spleen revealed normal frequencies of $CD4^+$ and $CD8^+$ T cells (Fig. 2*C*) and normal tissue architecture (data not shown). All three Helios transgenic lines yielded similar results, although results are shown only for transgenic line D.

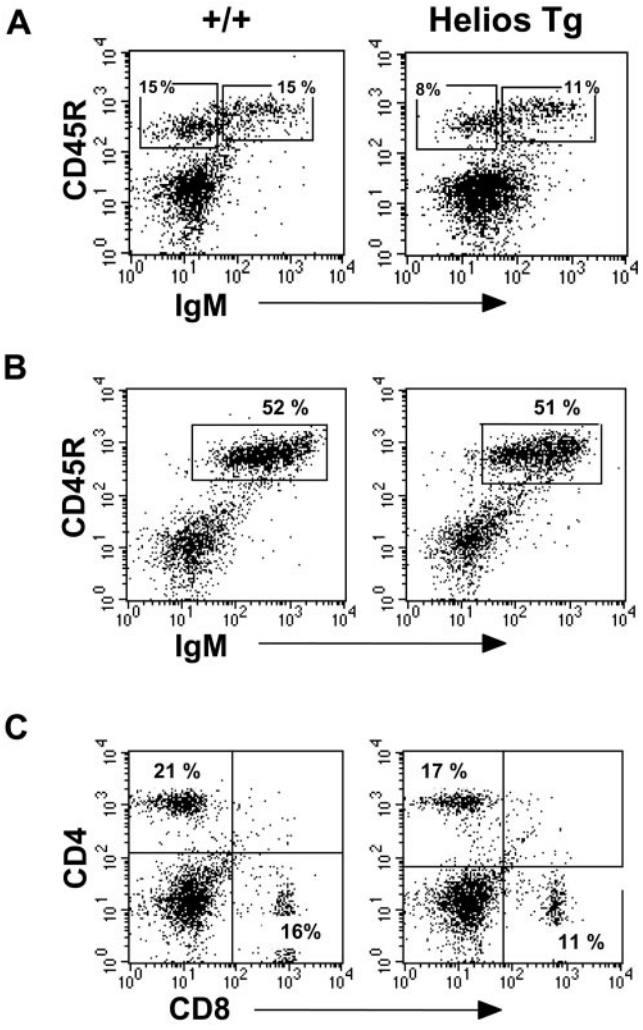

FIGURE 2. Analysis of lymphocytes in bone marrow and spleen from Helios transgenic mice. *A*, Bone marrow cells from wild-type and Helios transgenic mice were analyzed using CD45R and IgM Abs. The frequencies of $CD45R^+$ IgM^+ and $CD45R^+$ IgM^- cells are indicated. The profile shown is representative of the analysis of 10 wild-type mice and 25 transgenic mice (see below). *B*, The frequencies of splenic B cells in wild-type and transgenic mice were analyzed using CD45R and IgM Abs. *C*, The frequencies of splenic T cells in wild-type and transgenic mice were analyzed by flow cytometry using CD4 and CD8 Abs. The profile shown is representative of the results obtained with eight wild-type and 24 Helios transgenic mice.

Augmented proliferative response of transgenic $sIgM^+$ B cells

Taken together, the above data indicate that overexpression of Helios does not block B cell development. Subsequent studies examined B cell function in Helios transgenic mice. We first assessed the proliferative potential of splenocytes and bone marrow cells from transgenic and control mice in response to LPS. Both populations in Helios transgenic mice incorporated approximately three times more [^{3}H]thymidine than control cells (Fig. 3*A*). Hyperproliferation of transgenic B cells was also observed upon stimulation through the BCR with anti-IgM $F(ab')_2$ (Fig. 3*B*). This hyperproliferative response was observed at a very low concentration of anti-IgM $F(ab')_2$ (0.625 μg/ml), which failed to activate wild-type

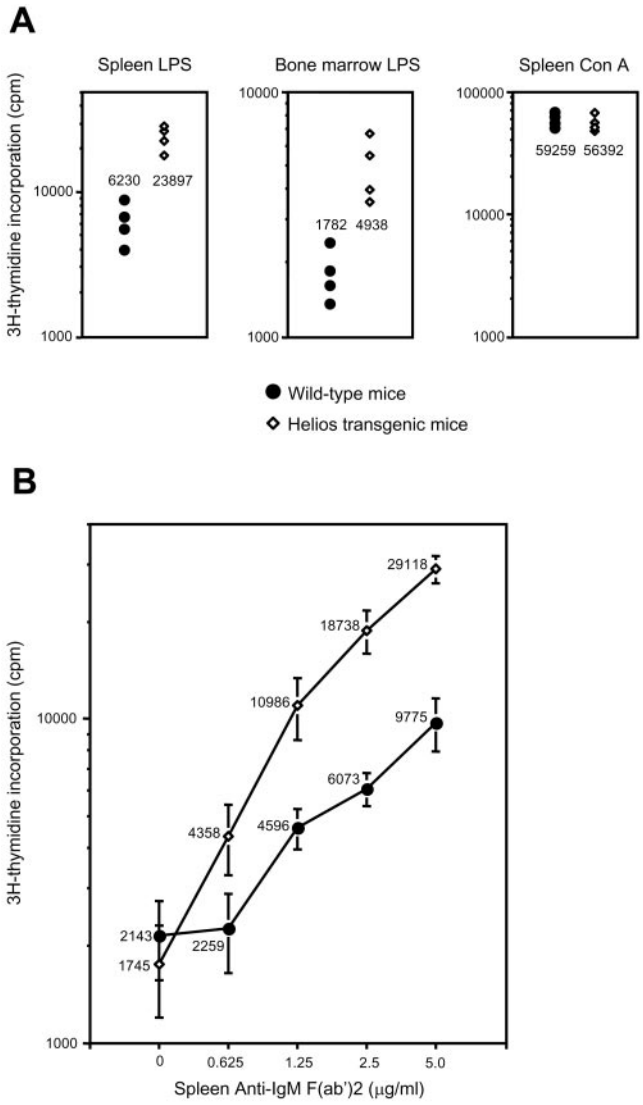

FIGURE 3. In vitro proliferation of bone marrow cells and splenocytes from Helios transgenic mice. *A*, Splenocytes (*left* and *right panels*) or bone marrow cells (*middle panel*) from wild-type mice (●) or Helios transgenic mice (◇) were incubated for 48 h with LPS (*left* and *middle panels*), Con A (*right panel*), or medium alone (data not shown). Cells were pulsed with [^{3}H]thymidine for 15 h and were then harvested, washed, and the incorporated [^{3}H]thymidine counted. Each experiment was performed in triplicate. Results are shown as cpm of incorporated [^{3}H]thymidine. *B*, Splenocytes from wild-type mice (●) or Helios transgenic mice (◇) were incubated with different concentrations of anti-IgM $F(ab')_2$ for 48 h, pulsed with [^{3}H]thymidine for an additional 15 h, harvested, washed, and analyzed for [^{3}H]thymidine incorporation.

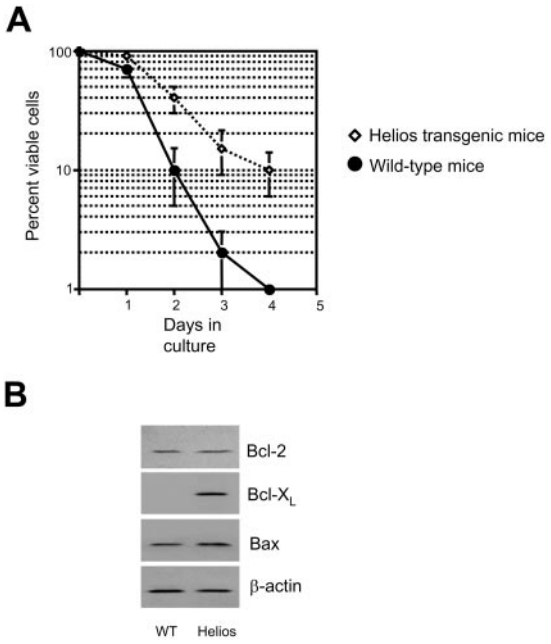

FIGURE 4. Survival of transgenic splenocytes and expression of anti-apoptosis proteins. *A*, Splenocytes from wild-type mice (●) and Helios transgenic mice (◇) were cultured in RPMI 1640 medium plus 10% FCS. Percentages of viable cells after 0–4 days in culture were determined by trypan blue staining of an aliquot of cells. *B*, The expression of the anti-apoptotic proteins Bcl-2, Bcl-x_L, and Bax in splenocytes from wild-type and Helios transgenic mice was monitored by Western blot. β-actin protein was monitored as a control.

B cells. These data support the notion that quiescent transgenic cells require a lower threshold of BCR engagement to be stimulated (see *Discussion*). In contrast, the proliferative response of splenic T cells to Con A was found to be comparable in transgenic and wild-type mice (Fig. 3*A*).

Prolonged survival of sIgM$^+$ B cells from Helios transgenic mice

Cultured splenic B cells from Helios transgenic mice showed significantly prolonged survival (Fig. 4*A*). Although the majority of normal splenocytes placed in RPMI 1640 growth medium supplemented with 20% FCS died within 4 days following initiation of cultures, transgenic splenocytes died at a slower rate, with 10 times more cells present after 4 days in culture. Because no proliferation was observed in these experiments (data not shown), the survival differences must have resulted from increased cellular life span. We have also tested peripheral B cells for the presence of proteins involved in apoptosis and found that the Bcl-x_L gene is strongly up-regulated in B cells from Helios transgenic mice, with the Bax gene up-regulated to a lesser extent (Fig. 4*B*).

Abnormal Ig isotype distribution in Helios transgenic mice

Despite the presence of normal numbers of mature B cells, sera from Helios transgenic mice contained increased concentration of IgG2b, with no significant differences in other Ig isotypes (Fig. 5*A*). To examine Ig production during an immune response, 10-wk-old transgenic and wild-type mice were immunized with NP-CG or NP-Ficoll to test their immune responses to stimulation via T cell-dependent (NP-CG) and T cell-independent (NP-Ficoll)

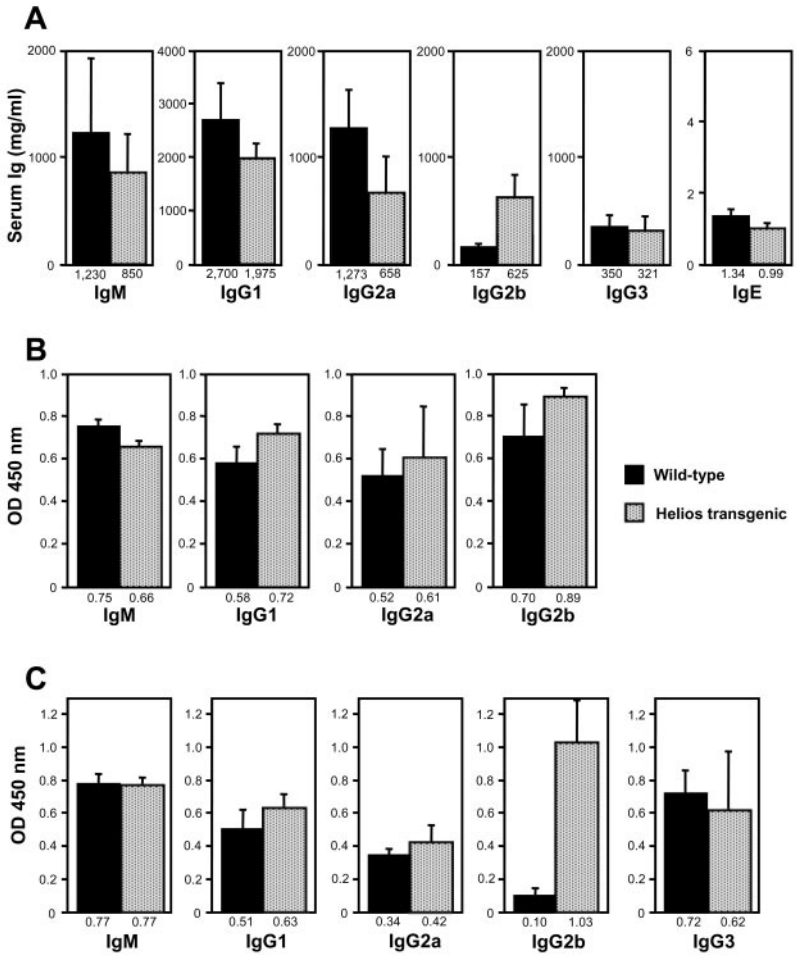

FIGURE 5. Ig isotype expression in Helios transgenic mice. *A*, Serum Ig levels were monitored by ELISA in six transgenic and six wild-type mice at 6 mo of age. Mean Ig concentrations and SD are shown. The statistical significance of the increased IgG2b concentration was $p < 0.001$. *B* and *C*, Serum anti-NP Ig levels in response to NP-CG (*B*) and NP-Ficoll (*C*) were estimated by ELISA and are shown as units of absorbance at 405 nm. In each experiment, four transgenic and four control mice were analyzed at 10 wk of age.

pathways, respectively. As shown in Fig. 5*B*, 2 wk after immunization with NP-CG, the concentrations of Ig isotypes, including IgG2b, were comparable in transgenic mice and controls. In contrast, 2 wk after immunization with NP-Ficoll, the concentration of IgG2b was significantly higher in transgenic mice in comparison to wild-type mice (Fig. 5*B*).

Ectopic expression of Helios induces B cell lymphoma in transgenic mice

Sixteen percent of transgenic animals older than 1 year, and one mouse at 9 mo of age, developed a rapidly progressing wasting disease, characterized by lethargy and weight loss. Some of these animals had neck or axillary adenopathy and the majority had a distended abdomen with easily palpable masses. Mice with these physical features were found in all three HA-Helios transgenic lines, but not in old wild-type mice. Necropsies showed that, in most cases, animals had an enlarged spleen and markedly enlarged axillary, cervical, and abdominal lymph nodes (Fig. 6*A*). Additional histological examination of grossly involved and uninvolved organs demonstrated multifocal, monomorphous lymphocyte expansions with moderate to high mitotic rates indicative of rapidly progressing lymphoma. Lymphoid architecture was disrupted by massive tumor cell infiltration of lymph nodes (Fig. 6*B*, *panel 1*) and spleen (data not shown). In some animals, heavy infiltration of solid organs, such as lungs, liver, and kidneys was also observed (Fig. 6*B*, *panels 2–4*). Overall, these observations were consistent with the presence of a rapidly progressing lymphoma.

Further analyses demonstrated that over 95% of cells from all tumors examined were positive for CD45R and CD5 (Fig. 6*C*, *panels 1* and *2*) and negative for CD43 (Fig. 6*C*, *panel 3*), and CD3 (data not shown), which are indicative of a mature, $CD5^+$ B lineage-derived malignancy. Finally, these tumor cells could be propagated in vitro, which confirmed their transformed phenotype (data not shown). Therefore, the fact that Helios transgenic mice from three independent lines developed B cell lymphomas with similar histological appearances late in life strongly suggests that formation of these tumors resulted from ectopic expression of Helios in B lineage cells and the accumulation of occult secondary events.

Discussion

Of the three Ikaros family members that are primarily expressed in hemopoietic cells, Helios is unique in that it is largely excluded from the B cell lineage. Our results provide evidence that extinction of Helios expression is necessary for normal B cell function. Ectopic expression of Helios under the control of the Ig μ enhancer resulted in prolonged survival and enhanced proliferation of mature B cells, hyperresponsiveness to Ag stimulation, and the development of B cell lymphoma. These abnormalities were observed in mice that expressed Helios at concentrations that were below the concentration of endogenous Ikaros. Because the ectopic Helios appears to raise the overall concentration of Ikaros family members in B lineage cells by only a small amount, the results suggest that Ikaros and ectopic Helios contribute distinct regulatory activities.

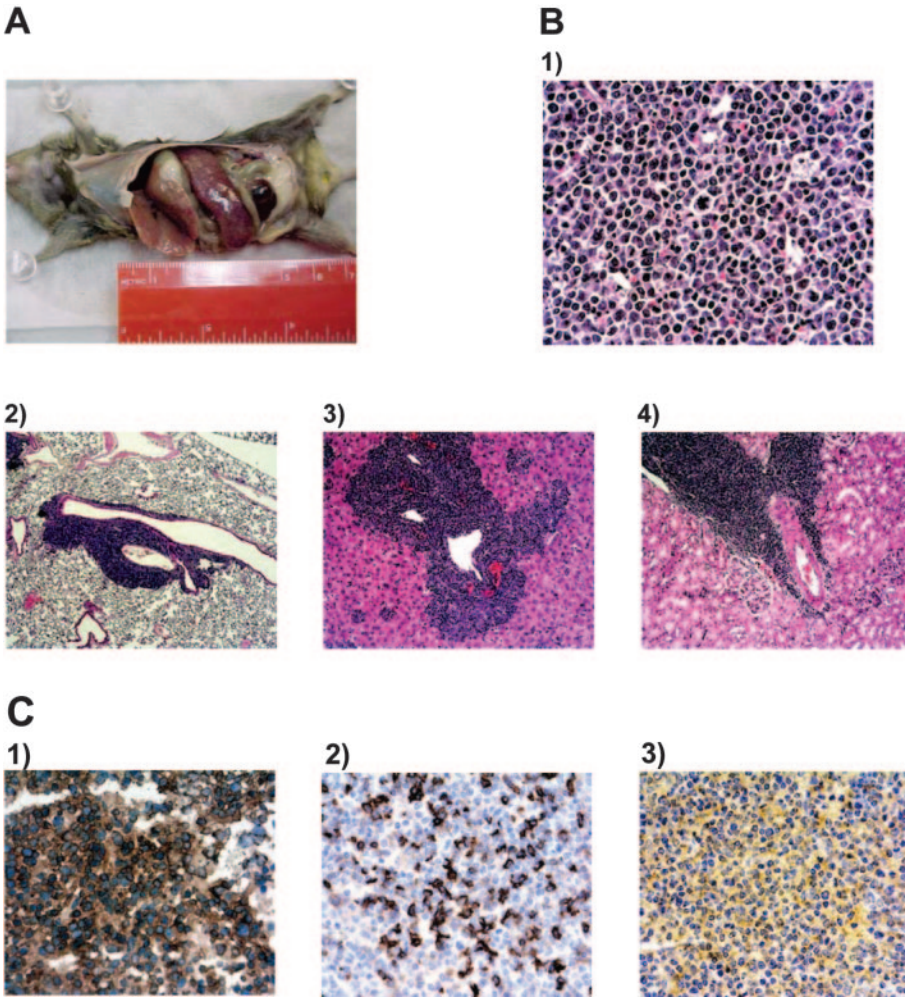

FIGURE 6. Development of lymphoma in Helios transgenic mice. Gross and microscopic examination of Helios transgenic mice >1 year of age demonstrated an aggressive lymphoproliferative disorder with multiorgan involvement. *A*, Exposed abdominal cavity demonstrating massively enlarged spleen and marked mesenteric lymphadenopathy. *B*, H&E stained sections of lymph node (*panel 1*, magnification ×400), lungs (*panel 2*, magnification ×40), liver (*panel 3*, magnification ×100), and kidney (*panel 4*, magnification ×100) demonstrate an aggressive lymphoproliferative disorder. The lymph node architecture is completely effaced by monomorphic, $CD45R^+/CD3^-$ lymphoid cells. Mitotic activity is brisk and apoptotic cells are abundant, indicating a rapidly proliferating lesion. Neoplastic B cells surround larger lung airways and vascular channels (*panel 2*), form mainly periportal and central venous aggregates in the liver (*panel 3*), and form interstitial space clusters near glomeruli and proximal tubules in the kidney (*panel 4*). *C*, Immunohistochemistry of a representative tumor mass is shown. Tumor mass was stained with CD45R (*panel 1*), CD5 (*panel 2*), and CD43 (*panel 3*) Abs. The results reveal that tumor cells are $CD45R^+$, $CD5^+$, and $CD43^-$.

Although ectopic expression of Helios resulted in multiple B cell abnormalities, we did not observe a defect in B cell commitment. Because other regulators of commitment to the B and T lymphocyte lineages, including Pax5, EBF, and Notch1, severely blocked development of alternative lineages when expressed in early progenitors (6–9), Helios may not be a critical regulator of T cell commitment. One caveat of this tentative conclusion is that Helios was expressed in the transgenic mice under the control of an Ig μ enhancer, whereas Pax5, EBF, and Notch1 were primarily studied by overexpression in multipotential progenitors via retroviral transduction and bone marrow reconstitution. Although the Ig μ enhancer is likely to drive expression of transgenes in common lymphoid progenitors, expression of the Helios transgene in common lymphoid progenitors was not confirmed and the enhancer may not support expression in earlier multipotential progenitor cells. Thus, an analysis of Helios-deficient mice and overexpression of Helios in multipotential progenitors by retroviral transduction will be needed to further explore its potential role in the B and T lymphocyte lineage decision.

Preliminary data indicate that pre-B cell numbers were reduced in the bone marrow of Helios transgenic mice. This conclusion was based on the finding that the frequency of $CD45R^+CD43^-$ sIgM cells was lower in Helios transgenic mice than in control animals (Fig. 2*A*, and data not shown). Further studies will be required to determine whether this finding reflects a specific reduction in pre-B cells or whether earlier progenitor stages are also reduced. Nevertheless, even if Helios overexpression results in a reduction in some stages of B cell development, the number of $sIgM^+$ B cells in the bone marrow was not reduced. The normal numbers of IgM^+ B cells can be explained by their increased survival and proliferation relative to wild-type cells, although homeostatic mechanisms must also be considered.

The hyperproliferation of mature B cells and the reduced activation threshold in response to anti-IgM are reminiscent of the phenotypes of B cells from Aiolos mutant mice and mice expressing abnormally low concentrations of Ikaros (32–35). These similarities raise the possibility that ectopic Helios antagonizes critical functions of Aiolos and Ikaros. Antagonism could be accomplished by formation of Helios-Aiolos or Helios-Ikaros heterodimers or multimers, thereby diminishing the formation of Aiolos-Aiolos or Aiolos-Ikaros complexes, or by competition for common coactivators or corepressors, or for common DNA recognition sites in target genes. Because ectopic Helios appears to be less abundant than endogenous Ikaros, the ectopic Helios may compete primarily with Aiolos (whose relative concentrations are not known).

The Helios transgenic mice showed a significant increase in basal IgG2b and a large increase in IgG2b levels following immunization with NP-Ficoll, with no difference in IgG2b concentrations relative to wild-type mice following immunization with NP-CG. NP-Ficoll is representative of thymus-independent type 2 Ags, which are characterized by the presence of large polysaccharide molecules with highly repetitive antigenic sites (41). Clinically important, thymus-independent type 2 Ags are those present in the capsules of *Haemophilus influenzae b*, *Streptococcus pneumoniae*, and *Neisseria meningitides* (42). Normal humoral responses to thymus-independent type 2 Ags yield selective Ig subclass production, predominantly of the IgG3 isotype, as early as 7 days postimmunization (43). Like wild-type mice, Helios transgenic mice responded to thymus-independent type 2 Ags with increased production of IgG3, but they also produced large amounts of IgG2b. Thus, ectopic expression of Helios appears to lead to altered isotype switching or aberrant regulation of IgG2b-expressing B cells. Interestingly, Aiolos-deficient mice and mice expressing reduced concentrations of Ikaros also exhibit altered Ig isotype profiles (32, 35). However, the alterations differ from those observed in the Helios transgenic mice, as Aiolos-deficient mice produce elevated levels of IgG1, IgG2a, and IgE and decreased levels of IgM, and Ikaros mice produce elevated levels of IgG2a and reduced levels of IgG3 (32, 35).

A fraction of the relatively aged Helios transgenic mice developed multifocal B cell lymphoma. The relatively old age and low frequency of mice that developed lymphoma indicates that aberrant Helios expression in B cells is not sufficient to cause lymphoma. Rather, Helios appears to promote tumor formation and requires other secondary events for malignant transformation. Again, the formation of B cell tumors is reminiscent of Aiolos-deficient mice (32). Interestingly, expression of human Helios has been documented in a substantial percentage of childhood pre-B and mature B cell acute lymphoblastic leukemias, raising the possibility that misexpression of Helios contributes to these malignancies (44).

It is important to emphasize that expression of Bcl-2 in transgenic mice under the control of an Ig μ enhancer yielded a phenotype that was similar to that of the Helios transgenic mice. In the Bcl-2 transgenic mice, alterations in B cell repertoire and the secretion of autoantibodies was observed (45, 46). Lymphomagenesis was also observed at a frequency and age similar to those observed with the Helios transgenic mice, although the lymphomas obtained in Bcl-2 transgenic mice were generally derived from cells at earlier stages of B cell development (45, 46). These results suggest that the enhanced survival and enhanced lymphomagenesis in Helios transgenic mice could be due primarily to the upregulation of Bcl-2 family members.

In summary, existing biological models indicate that Ikaros expression is broadly important for hemopoiesis, whereas Aiolos regulates the proliferative response, activation threshold, and maturation of mature B cells. Although the role of Helios in T cell development remains undefined, our results demonstrate that the extinction of Helios expression is required for normal B cell function.

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Disclosures

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