Genetic Ablation of Protein Tyrosine Phosphatase 1B Accelerates Lymphomagenesis of p53-Null Mice through the Regulation of B-Cell Development

Nadia Dubé, 1,2 Annie Bourdeau, 1 Krista M. Heinonen, 1,3 Alan Cheng, 1,2 Ailsa Lee Loy, 1,2 and Michel L. Tremblay 1,2

1McGill Cancer Centre, 2Department of Biochemistry, and 3Division of Experimental Medicine, McGill University, Montreal, Quebec, Canada

Abstract

Protein tyrosine phosphatase 1B (PTP1B) is involved in multiple signaling pathways by down-regulating several tyrosine kinases. For example, gene-targeting studies in mice have established PTP1B as a critical physiologic regulator of metabolism by attenuating insulin signaling. PTP1B is an important target for the treatment of diabetes, because the PTP1B null mice are resistant to diet-induced diabetes and obesity. On the other hand, despite the potential for enhanced oncogenic signaling in the absence of PTP1B, PTP1B null mice do not develop spontaneous tumors. Because the majority of human cancers harbor mutations in p53, we generated p53/PTP1B double null mice to elucidate the role of PTP1B in tumorigenesis. We show that genetic ablation of PTP1B in p53 null mice decreases survival rate and increases susceptibility towards the development of B lymphomas. This suggested a role for PTP1B in lymphopoiesis, and we report that PTP1B null mice have an accumulation of B cells in bone marrow and lymph nodes, which contributed to the increased incidence of B lymphomas. The mean time of tumor development and tumor spectrum are unchanged in p53−/−/PTP1B−/− mice. We conclude that PTP1B is an important determinant of the latency and type of tumors in a p53-deficient background through its role in the regulation of B-cell development.

(Research Article) Cancer Res 2005; 65(21): 10088-95

Introduction

The protein tyrosine phosphatases (PTP) form a superfamily of at least 100 members (1, 2). Together with the protein tyrosine kinases (PTK), they modulate the cellular levels of tyrosine phosphorylation and regulate many cellular events such as differentiation, cell growth, motility, and proliferation (3). Therefore, deregulation of PTP activity can lead to aberrant signaling that can contribute to the development of various diseases in human, such as cancer, diabetes, inflammation, and autoimmunity (4). Numerous biochemical and genetic studies suggest a role for PTPs in oncogenic transformation (5, 6).

PTP1B is the prototype for the superfamily of PTPs and has been the most extensively studied within this group. This enzyme is widely expressed and localizes predominantly to the endoplasmic reticulum (ER; ref. 7). Biochemical and substrate-trapping studies have implicated PTP1B in the attenuation of signaling mediated by various receptor tyrosine kinase signaling pathways (reviewed in refs. 8–10), including the epidermal growth factor, platelet-derived growth factor, insulin, and insulin-like growth factor-1 (IGF-I) receptors. Additionally, PTP1B regulates kinases such as Src, p210Bcr-Abl, Janus kinase 2 (JAK2), and TYK2, as well as the transcription factor signal transducers and activators of transcription 5 (STAT5); thus, PTP1B is associated with oncogenic, metabolic, and cytokine signaling (9, 10).

The studies of PTP1B-deficient mice have shown that although this enzyme is dispensable for embryonic development, it is important for metabolic control (11, 12). Despite the potential role of PTP1B in enhanced oncogenic signaling through growth factor receptors, PTP1B-deficient mice do not spontaneously develop tumors. Both increased and decreased levels of PTP1B have been observed in different human tumors (reviewed in ref. 9), but strong genetic evidence for a role for PTP1B in cancer is still lacking. Therefore, we introduced the PTP1B gene deletion into p53 null mice to study the role of PTP1B in tumorigenesis and to determine if the genetic ablation of PTP1B would modify the rate of appearance and/or the type of tumors in this cancer-prone genetic model.

The P53 gene product, p53, encodes a transcription factor that regulates cell proliferation and apoptosis (13). Approximately 50% of human cancers, including tumors of the colon, breast, lung, and brain, display a p53 mutation (14, 15). In addition, germ line mutations in the p53 gene have been observed in Li-Fraumeni syndrome, which predisposes people to a number of tumors, including soft tissue sarcomas, osteosarcomas, brain tumors, breast cancers, and leukemias (16, 17). Mice deficient in p53 are developmentally viable but susceptible to the development of early spontaneous tumors (18–20), especially thymic lymphoma (21, 22).

Here, we report that in the absence of p53, PTP1B null mice display increased survival rates and earlier tumor development compared with p53−/−/PTP1B+/+ and p53−/−/PTP1B−/− mice. Mice lacking PTP1B in a p53 null background are more susceptible to develop lymphomas. Moreover, compared with p53−/−/PTP1B−/− and p53−/−/PTP1B+/− littermates, p53−/−/PTP1B−/− mice develop a higher percentage of B-cell lymphomas compared with T-cell lymphomas, suggesting that PTP1B regulates hematopoietic function. In this aspect, PTP1B null mice show an increased number of B cells in bone marrow and lymph nodes and an increased percentage of B cells in blood. Indeed, colony-forming assays in the presence of interleukin-7 (IL-7) confirm the increase in B-cell progenitors in the bone marrow of PTP1B null mice.

Note: N. Dubé is currently at the Department of Physiological Chemistry, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG Utrecht, the Netherlands.

A. Cheng is currently at the Life Sciences Institute, University of Michigan, 210 Washtenaw, Ann Arbor, MI 48109.

Requests for reprints: Michel L. Tremblay, McGill Cancer Centre, McGill University, 3655 Promenade Sir William Osler, Room 701, Montreal, Quebec, Canada H3G 1Y6. Phone: 514-398-7290; Fax: 514-398-6769; E-mail: michel.tremblay@mcgill.ca.

Cancer Res 2005; 65: (21). November 1, 2005 10088 www.aacrjournals.org

doi:10.1158/0008-5472.CAN-05-1353

© 2005 American Association for Cancer Research. Downloaded from cancerres.aacrjournals.org on April 14, 2017. © 2005 American Association for Cancer Research.
Additionally, B cells exhibit decreased apoptosis in the lymph nodes of PTP1B null mice. Taken together, these results indicate that PTP1B is an important determinant of the latency and type of tumors in a p53-deficient background. Our findings also show that PTP1B regulates B-cell development. To our knowledge, this is the first study to report the role of PTP1B in hematopoiesis and cancer in vivo.

Materials and Methods

Reagents and antibodies. Antibodies for flow cytometry were purchased from BD Biosciences (Mississauga, Ontario, Canada), Groovy Blue Genes (Vineland, Ontario, Canada), and Biosource (Camarillo, CA; anti–Annexin V). Antibodies for Western blotting were obtained from Upstate Biotechnology (Lake Placid, NY) and reagents for colony-forming assay were from StemCell Technologies (Vancouver, British Columbia, Canada; 1% methylcellulose and recombinant human IL-2).

Mice. Generation of PTP1B mutant mice and genotyping was described previously (11). Mice with a heterozygous-targeted p53 allele were obtained from Taconic (Albany, NY). All animal procedures were approved by the McGill University Research and Ethic Animal Committee, and experiments were carried out under the Canadian Council on Animal Care ethical regulations.

Tumorigenesis studies. p53 null mice either PTP1B+/-, PTP1B+/-, or PTP1B−/− (on a BALB/c × C57BL/6 background) were monitored biweekly for spontaneous tumor formation, were sacrificed when showing signs of illness, tumors, or possible signs of lymphomas (weight loss, enlarged lymph nodes or abdomen, wasting, and ruffled fur in the absence of overt tumors), and were subjected to necropsy. The thymus, spleen, and peripheral lymph nodes of each animal were examined for signs of enlargement. The tissues were placed in OCT Tissue Tek compound (Naperville, IL) for sectioning. Sections were prepared and stained with H&E or B220 and TCRβ and examined microscopically. Alternatively, cell suspensions of hematopoietic organs were prepared and stained with T- or B-cell markers and analyzed using a FACS calibur (Becton Dickinson, Franklin Lakes, NJ).

Genomic multiplex PCR for p53 alleles. Technical advice was provided by Taconic. Forward PCR primers used were 5'-GTTGGAGGAGGA-CAAAAGTCTGAGGAGC-3' [intron 4; wild type (WT) and null/knockout (KO)]. Reverse primers used were 5'-ATGGAGCTGTTACCTGTCAAC-ACT (WT), 5'-TTTACGCAGCTTCGTGCATGCT (null/KO), and 5'-CTAGTGTACACACAGTCTGATG-3' (control). PCR was done in buffer containing 1.5 mmol/L MgCl2, 100 pmol of each primer, 0.2 mmol/L deoxynucleotide triphosphates, and 0.4 unit of AmpliTaq Gold DNA polymerase (Roche Molecular Biochemicals, Laval, Quebec, Canada). Reaction conditions were as follows: denaturation at 94°C for 50 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute for 35 cycles. Products were analyzed on a 1.5% agarose gel and visualized by ethidium bromide staining. A 200-bp band from the 5’ primer in intron 4 plus a 3’ primer in the polymerase II promoter in the targeting construct is diagnostic for the presence of the null allele, and the WT p53 allele gives a 600-bp band with the same 5’ primer in intron 4 but with a 3’ primer that corresponds to the exon5/intron 6 junction that is present in the normal p53 gene. PTP1B mice were genotyped as previously described (11).

Methylcellulose colony-forming assay. Bone marrow cells (5 × 103) isolated from 6- to 7-week-old PTP1B WT and KO mice were plated in duplicate in 1% methylcellulose and Iacro's modified Dulbecco's medium with 30% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 10−4 mol/L β-mercaptoethanol, and 10 ng/ml recombinant human IL-2. Colonies were counted 7 to 8 days later using an inverted microscope at ×40 magnification.

Flow cytometry. Fresh cell suspensions from total spleen, thymus, bone marrow, lymph nodes, and lymphocytes isolated from blood were prepared from 6- to 7- or 14- to 15-week-old male mice, in PBS containing 2% FBS, and filtered through a 70- to 100-μm cell strainer. An equal volume of cell suspension was used and incubated with purified anti-CD16/CD32 antibody (BD Biosciences) to block nonspecific binding to Fcγ receptors followed by a combination of the indicated antibodies. Data acquisition and analysis were done on FACS scan or FACScalibur flow cytometer (Becton Dickinson) using Cell Quest software.

Lymphocyte isolation from whole blood. Lymphocytes were isolated using “Lympholyte” (Cedarlane, Hornby, Ontario, Canada) according to the manufacturer’s instructions and resuspended in PBS containing 2% FBS.

B-cell isolation from spleen. B cells from PTP1B WT and KO mice were obtained by isolating CD19+ cells using EASY Sep magnetic beads system (StemCell Technologies) according to the manufacturer’s protocol.

Annexin V staining. Single-cell suspensions of lymph nodes and spleen were prepared and apoptosis was determined by Annexin V staining following the manufacturer’s instructions (Biosource). Determining the status of cells (live, dead, or apoptotic) was based on gating of cells by their size (side scatter and forward scatter), plasma membrane integrity (propidium iodide staining), and redistribution of plasma membrane phosphatidylserine (Annexin V binding). Cells were analyzed using a FACScan or FACScalibur flow cytometer (Becton Dickinson).

Statistical analysis. Values are reported as the average of at least three independent experiments ± SE. Statistical analysis was done using Kaplan-Meier analysis (Fig. 1) and two-tailed, unpaired Student’s t test.

Results

The absence of PTP1B decreases survival of p53 null mice. To explore the role of PTP1B in tumorigenesis, we generated p53 and PTP1B double KO mice by breeding the two lines, and we monitored tumor formation and survival of these mice (Fig. 1). By 20 weeks of age, 60% of the p53−/−/PTP1B+/+ mice had tumors compared with 71% of the p53−/−/PTP1B−/− and 89% of the p53−/−/PTP1B−/−, clearly showing that the absence of PTP1B potentiates tumor development of p53 null mice (Fig. 1). In accordance with previously reported data (18), of the 20 p53−/−/PTP1B−/− mice studied, 75% developed obvious neoplasms by 25 weeks of age. Some tumors appeared as early as 12 weeks, the mean time of tumor appearance being 20.7 weeks. Similarly, the 40 p53−/−/PTP1B−/− mice analyzed in our study presented a
mean time of tumor development of 20 weeks, suggesting that the time of tumor appearance does not differ significantly between p53·/−/PTP1B·/− and p53·/−/PTP1B·/− mice (P = 0.09). All 55 p53·/−/PTP1B·/− mice developed tumors by 25 weeks of age. The mean time of tumor appearance was 16.4 weeks, and tumor incidence increased rapidly between 14 and 18 weeks of age. Hence, the rate of tumor development was clearly and significantly accelerated in p53 null mice lacking PTP1B compared with p53·/−/PTP1B·/− and p53·−/−/PTP1B·/− mice as determined by the Kaplan-Meier analysis. Thus, PTP1B is an important determinant of tumor onset in a p53 null background.

Analysis of tumors in p53 null mice lacking PTP1B. We characterized the type of tumors encountered in p53·/−/PTP1B·/− and p53·−/−/PTP1B·/− mice. Hematopoietic tissues including thymus, lymph nodes, and bone marrow, as well as lymphocytes isolated from blood were analyzed by flow cytometry using antibodies to T cell–specific (TCRβ, CD4, and CD8) and B cell–specific (CD19 or B220, CD24, and IgD antibodies) markers. At the time of necropsy, two major types of lymphomas were observed. The first type, thymic lymphoma, was invariably of T-cell origin. Most of the thymic lymphomas were CD4·CD8− (Fig. 2A, middle), indicating that they arose from the transformation of relatively immature thymocytes. We also observed some cases where the cells were CD4·CD8+ (Fig. 2A, right), suggesting a more differentiated stage. The second type of lymphoma, a peripheral type, was of B-cell origin and often arose from all of the major peripheral lymph node groups and the spleen. Splenomegaly frequently accompanied the peripheral lymphoma type, and in some cases, the enlarged spleen filled much of the abdominal cavity. The peripheral lymphomas of B-cell origin were all positive for the B-cell markers CD19 or B220, and B-cell accumulation was observed in the lymph nodes (mesenteric and axillary; Fig. 2B, middle and right), as well as in the bone marrow and blood (Fig. 2D). In addition, the B lymphomas analyzed were from pre-B (Fig. 2C) or immature origin. In the latter case, the majority of B cells in the lymph nodes stained negative for IgD (data not shown).
suggesting an accumulation of immature B cells in the periphery. The mice that exhibited soft or solid tumors without the presence of lymphomas were classified as "other" (see Fig. 3A).

**Increased incidence of lymphomas and higher proportion of B lymphomas in p53/PTP1B double null mice.** It was previously reported that 70% of the p53 null mice developed lymphomas, 70% to 75% of which were of T-cell origin and contained primarily immature (CD4⁺CD8⁺) T cells, whereas the remaining, which originated in the spleen and peripheral lymph nodes, were characterized as B-cell lymphomas (18–20, 23, 24). Similarly, we found that 70% of p53⁻/⁻ PTP1B⁺/⁺ mice developed lymphomas, and the 30% remaining exhibited solid tumors (Fig. 3A). Lymphomas arose between 18 to 19 weeks on average and 71% were of thymic origin, and 29% were B lymphomas (Fig. 3B). Thus, p53⁻/⁻ PTP1B⁻/⁻ mice develop lymphomas to the same extent as p53 null mice. Interestingly, 91% of the double null p53/PTP1B mice developed lymphomas (Fig. 3A), where 46% originated from T cells and 54% from B cells (Fig. 3B). The T lymphomas occurred on average at 17.4 weeks, whereas the B lymphomas occurred at 15.5 weeks. These results indicate that the absence of the PTP1B gene in a p53-deficient background results in an increased proportion and earlier appearance of B-cell lymphomas.

**PTP1B is expressed in hematopoietic tissues.** To confirm the role of PTP1B in lymphoma incidence, we examined B-cell development in PTP1B null mice, because hematopoietic abnormalities could contribute to the accelerated rate of tumor development and to the shift from T to B lymphomas. Given that Donehower et al. reported that p53 null mice have no obvious immune defects (18), we focused our analysis on the hematopoietic function of PTP1B null mice.

**PTP1B Regulates Lymphomagenesis of p53 Null Mice**

Figure 3. Modification of the spectrum of tumors from p53 null mice lacking PTP1B. Classification of tumors from p53⁻/⁻ PTP1B⁺/⁺ (n = 23) and p53⁻/⁻ PTP1B⁻/⁻ mice (n = 32). A, lymphomas were characterized by flow cytometry or immunohistochemistry. The remaining tumors were classified as others and correspond to all nonlymphoma tumors (soft and solid tumors independently of the site of origin). n = number of mice studied. B, proportion of T versus B lymphomas in p53⁻/⁻ PTP1B⁺/⁺ and p53⁻/⁻ PTP1B⁻/⁻ mice.

Figure 4. Accumulation of immature B cells in bone marrow of PTP1B null mice. A, B, and C, flow cytometry analysis of antibody-stained bone marrow cells isolated from PTP1B⁺/⁺ and PTP1B⁻/⁻ mice. Representative of five independent experiments with a total of n = 12 to 14 mice per group. *, P < 0.02; **, P < 0.0004 (t test).

PTP1B is a ubiquitously expressed enzyme, but its pattern of expression in hematopoietic tissues has not yet been confirmed. Therefore, tissues and cells were isolated from adult PTP1B WT and null (KO) mice, and expression was verified by immunoblotting using an antibody against PTP1B. PTP1B is expressed in stromal cells derived from the bone marrow of 7-day-old WT pups, as well as in adult bone marrow, lymph nodes, spleen, thymus, and splenic B cells (data not shown). These results show that PTP1B is expressed in all hematopoietic tissues.

**Accumulation of immature B cells in bone marrow of PTP1B null mice.** The higher proportion of B lymphomas observed in p53/PTP1B double null mice prompted us to examine the number and developmental stages of B cells in the bone marrow of PTP1B null mice. Bone marrow cells were isolated and stained with anti-CD19 or anti-B220 antibodies and analyzed by flow cytometry. As shown in Fig. 4A, PTP1B null mice displayed a significant 20% increase in the proportion of B lineage cells (P < 0.02). To examine whether the absence of PTP1B altered B-cell differentiation, we stained bone marrow cells with antibodies against B220, IgM, and IgD, the latter being developmental markers of B cells. A significant 15% increase was observed in the immature B-cell fraction (IgM⁻IgD⁺; P < 0.004; Fig. 4B). Thus, the absence of...
PTP1B causes an accumulation of IgM<sup>-</sup>IgD<sup>-</sup> B cells in the bone marrow.

As depicted on Fig. 4B, the mature population of B cells (IgM<sub>low</sub>IgD<sub>high</sub>) is decreased in PTP1B null bone marrow. To confirm this observation, we stained the cells with anti-CD19 and anti-IgD antibodies. Two distinct IgD<sup>+</sup> B-cell populations are observed in WT mice (Fig. 4C), IgD<sub>low</sub> and IgD<sub>high</sub>. In contrast, the second population is decreased by 51% (P < 0.004) in the bone marrow of PTP1B null mice.

**Increased pre-B colony formation in bone marrow of PTP1B null mice.** To confirm the increase in progenitor B cells in PTP1B null bone marrow, colony-forming assays were done using bone marrow cells harvested from WT and PTP1B null 6- to 7-week-old mice. The cells were grown in 1% methylcellulose supplemented with 10 ng/mL of IL-7, which induces the growth of pre-B colonies (StemCell Technologies). Cells isolated from PTP1B null bone marrow formed thrice more colonies than WT cells (P < 0.003; Fig. 5A), showing that the defect is in part cell autonomous. Flow cytometry analysis of B220, CD43, and CD25 antibody-stained colonies confirmed their identity as pre-B cells, CD43<sup>+</sup>CD25<sup>−</sup> and CD43<sup>+</sup>CD25<sup>−</sup> (Fig. 5B). In all cases, over 99% live cells were B220 positive (data not shown).

**Increased proportion of B cells in peripheral lymph nodes of PTP1B null mice.** We next determined if B cells accumulated in peripheral lymphoid organs such as lymph nodes, blood, and spleen of PTP1B null mice. To quantify B/T cell ratio, cell suspensions of lymph nodes and spleen, or peripheral blood lymphocytes from adult mice (7- to 14-week-old) were stained with anti-CD19 and anti-TCR<sup>β</sup> antibodies and analyzed by flow cytometry. In PTP1B null mice, an increase of 20% in the proportion of B lineage cells was observed in the lymph nodes (P < 0.02), as well as a 40% increase in the percentage of B cells in blood (P < 0.007; Fig. 6A, top and middle, respectively). However, PTP1B null mice displayed a normal B/T cell ratio in the spleen (Fig. 6A, bottom). Additionally, we determined whether the maturity of B cells was modified in these organs by staining the cell suspensions with anti-CD19 and anti-IgD antibodies. These cells express IgD in equivalent proportions in the lymph nodes, blood, and spleen (Fig. 6B).

To determine if a decrease in apoptosis caused the accumulation of B cells in the lymph nodes of PTP1B null mice, we stained cells harvested from lymph nodes with Annexin V and propidium iodide and found that B cells lacking PTP1B exhibited a 50% reduction in apoptosis (WT, 20 ± 5% versus KO, 10 ± 5%; P < 0.002; Fig. 6C, top). Therefore, B cells accumulate in lymph nodes of PTP1B null mice and display decreased apoptosis. Importantly, apoptosis of B220-negative cells (all non-B cells) was unchanged in the lymph nodes. In contrast, the apoptotic index of B cells was unchanged in the spleen of PTP1B null mice (21 ± 10%) compared with WT controls (19 ± 8%; Fig. 6C, bottom). The apoptotic index observed for the WT hematopoietic organs is similar to previously reported values (25–27).

PTP1B null mice present lymphadenopathy and increased B-cell size. We observed that adult PTP1B null mice presented enlarged lymph nodes (lymphadenopathy). Cell counts from lymph nodes of 6- to 7-week-old male mice revealed an increase of 50% in the number of cells in PTP1B null mice (WT, 3.28 × 10<sup>6</sup> ± 7.45 × 10<sup>6</sup> versus KO, 6.35 × 10<sup>6</sup> ± 3.78 × 10<sup>6</sup>; P < 0.03; data not shown). The lymph nodes from 7- to 15-week-old PTP1B<sup>−/−</sup> mice had larger B cells (CD19<sup>+</sup>) than WT littermates (33% increase; P < 0.002), as determined by forward scatter characteristics (data not shown). In addition, we observed a higher number of large B cells in bone marrow (13%; P < 0.004) and spleen (40%; P < 0.009) in 15-week-old PTP1B null mice compared with WT mice (data not shown). Importantly, the increase in cell size was specific to B cells in these hematopoietic organs. These findings suggest that genetic ablation of PTP1B results in increased B-cell size, which correlates with increased activation status of B lymphocytes (28). Thus, these changes correspond with impaired B-cell development and predisposition to B-cell lymphomas (29).

**Discussion**

Previous studies suggest that PTP1B acts a positive regulator as well as a negative regulator of oncogenesis, and both increased and decreased levels of PTP1B have been observed in variety of human cancers (reviewed in ref. 9). Intuitively, it is often speculated that inhibition of PTP1B may lead to increased PTK signaling and ultimately oncogenesis; however, despite potentially enhanced oncogenic signaling, PTP1B null mice do not overly undergo tumorigenesis, and strong genetic evidence for a role of PTP1B in cancer is still lacking. Using p53 deficiency as a general model of tumorigenesis, we now report that in the absence of p53, PTP1B<sup>−/−</sup> and KO mice display decreased survival rates (Fig. 1) showing that genetic ablation of PTP1B potentiates tumor development of p53 null mice. The meantime of tumor development, however, is similar in both p53<sup>−/−</sup>PTP1B<sup>−/−</sup> and p53<sup>−/−</sup>PTP<sup>−/−</sup> mice (20.7 and 20.0 weeks, respectively), suggesting that the absence of one allele of the PTP1B gene does not significantly alter the time of tumor appearance. In addition, p53/PTP1B double null mice are more susceptible to the development of lymphomas compared with p53<sup>−/−</sup>PTP1B<sup>−/−</sup> and p53<sup>−/−</sup>PTP<sup>−/−</sup> mice (Fig. 3A) and develop twice as many B-cell lymphomas (Fig. 3B). This prompted us to look at the role of PTP1B in B-cell development, because hematopoietic defects could contribute to the accelerated tumorigenesis and to the shift in the development of T to B lymphoma. This is indeed the case. The bone marrow of PTP1B null mice contains an increased proportion of immature IgM<sup>-</sup>IgD<sup>-</sup> B cells (Fig. 4B) but a reduced number of mature...
IgM<sup>low</sup>IgD<sup>high</sup> B cells (Fig. 4C). Accordingly, we observed a higher number of pre-B colonies in colony-forming assays of bone marrow cells lacking PTP1B (Fig. 5A). Moreover, B cells accumulate in lymph nodes (Fig. 6A, top), lymphocytes isolated from blood (middle), and spleen (right) cell suspensions isolated from WT and PTP1B null mice. The fluorescence-activated cell sorting plots are representative of at least three independent experiments. Columns, average obtained for all experiments, with a total of \( n = 8 \) to 11 mice (6- to 8-week-old) per group. B, flow cytometry analysis of antibody-stained lymph nodes (left), lymphocytes isolated from blood (middle), and spleen (right) cell suspensions isolated from WT and PTP1B null mice. The fluorescence-activated cell sorting plots are representative of at least three independent experiments. Columns, average obtained for all experiments, with a total of \( n = 8 \) to 11 mice (6- to 8-week-old) per group. C, Annexin V staining of lymph nodes (top) and spleen (bottom) cell suspensions. The apoptotic index was determined by counting the number of Annexin V–positive cells. The fluorescence-activated cell sorting plots are representative of at least three independent experiments. Columns, average obtained for all experiments, with a total of \( n = 9 \) to 11 mice (6- to 8-week-old) per group. *, \( P < 0.02 \); **, \( P < 0.002 \); ***, \( P < 0.007 \) (t test).

Figure 6. Abnormal B/T cell ratio in lymph nodes of PTP1B null mice. A, flow cytometry analysis of antibody-stained lymph nodes (top), lymphocytes isolated from blood (middle), and spleen cells (bottom) isolated from WT and PTP1B null mice. The fluorescence-activated cell sorting plots are representative of at least three independent experiments. Columns, average obtained for all experiments, with a total of \( n = 8 \) to 11 mice (6- to 8-week-old) per group. B, flow cytometry analysis of antibody-stained lymph nodes (left), lymphocytes isolated from blood (middle), and spleen (right) cell suspensions isolated from WT and PTP1B null mice. The fluorescence-activated cell sorting plots are representative of at least three independent experiments. Columns, average obtained for all experiments, with a total of \( n = 8 \) to 11 mice (6- to 8-week-old) per group. *, \( P < 0.02 \); **, \( P < 0.002 \); ***, \( P < 0.007 \) (t test).

Increasing evidence suggests that disruption of various components of the B-cell receptor (BCR) signaling pathways leads to a block in different stages of transitional B-cell development (32). Of interest, mice deficient in CD45, a receptor PTP, have been shown to accumulate IgM<sup>high</sup>IgD<sup>low</sup> late transitional B cells (33, 34). Similarly,
immature B cells accumulate in bone marrow of PTP1B null mice. This suggests that PTP1B could be involved in BCR signaling, or that BCR expression could be modified in the absence of PTP1B.

It is known that B cells having completed their maturation process in the periphery and returning to the bone marrow as mature recirculating B cells, bearing low levels of IgM and high levels of IgD, mediate an efficient humoral immune response (35, 36). Therefore, our next challenge will be to determine the immunologic consequences of the reduced number of mature recirculating IgDlowIgDhigh B cells in the bone marrow of the PTP1B null mice (Fig. 4B), which may include an impaired ability to mount an immune response to bacterial or viral infection.

IL-7 is another important player in B-cell differentiation, as shown by gene-targeted mice-deficient in IL-7 or IL-7R, where the loss of IL-7/IL-7R interaction led to a severe impairment of B-cell development beyond the pro–B-cell stage (37, 38). Because this cytokine receptor signals through the JAK1 and JAK3, it is unlikely that PTP1B regulates signaling downstream of this pathway, as suggested by the negative regulation exerted by PTP1B on JAK2 and TYK2 phosphorylation specifically (39–43). We stained bone marrow B cells using an anti-CD127 (IL-7Rα) antibody and found no difference in the IL-7R content in PTP1B WT and KO mice (data not shown). Alternatively, the levels of IL-7 could be modulated in the absence of PTP1B.

Dysregulation of JAK2, STAT3, and STAT5 is involved in malignant cellular transformation, and constitutive activation of these molecules is found in various types of leukemias (44–46). Because JAK2 and STAT5 are known substrates of PTP1B, we have looked at their activation status in hematopoietic organs of p53−/−PTP1B+/−, p53−/−PTP1B−/−, and p53−/−/PTP1B−/− mice. We observed heterogeneous phosphorylation of JAK2, STAT3, and STAT5 in the tissues analyzed that was independent of the PTP1B genotype and have not observed any difference in the expression level of these proteins (data not shown). Consequently, we have not identified a pathway regulated by PTP1B in these hematopoietic tissues and suggest that the p53−/−PTP1B−/− phenotype shows an effect on hematopoietic development rather than in a specific pathway.

Of interest, it was shown that IGF-I produced by bone marrow stromal cells potentiates the proliferative stimulus provided by IL-7 thereby regulating B-cell differentiation (47). It will be interesting to determine if increased signaling through the IGF-IR pathway provides a proliferative advantage to the bone marrow B cells in PTP1B null mice. In addition, the consequence of removal of PTP1B on IL-7-induced cell proliferation and apoptosis is under investigation. This should give further insight into the signaling pathways involved in this process, and how PTP1B regulates B-cell proliferation and apoptosis. For instance, Wnt5a heterozygous mice are susceptible to the development of B lymphomas with age, and Wnt5a has been reported as a negative regulator of B-cell proliferation by inhibiting the response of B cells to IL-7 (48).

It was previously reported that apoptosis is the only p53 function selected against during the development of murine lymphomas (49), and that the absence of p53 reduced pro–B-cell apoptosis (50). In this regard, it was observed that loss of PTP1B leads to resistance to serum withdrawal, etoposide killing, and ER stress-induced apoptosis, through the AKT/PKB and c-jun N-terminal kinase pathways, respectively (51, 52). It remains unclear, however, why and how PTP1B modulates B-cell apoptosis in specific cell types and compartments. Thus, inhibition of PTP1B could provide a prosurvival signal to transformed cells, suggesting that PTP1B contributes to the maintenance of apoptosis in specific cell types. It is specifically the case in the lymph nodes of PTP1B null mice, where B cells accumulate and exhibit decreased apoptosis (Fig. 6C, top). Therefore, it stands to reason that the removal of PTP1B in a p53−/− background would further decrease apoptosis, and together with the accumulation of B cells in the bone marrow of PTP1B null mice (Fig. 4A), these events could trigger the development of B-cell lymphomas in p53/PTP1B double null mice.

At this stage, we cannot exclude that the B-cell developmental phenotype of PTP1B−/− mice could lead to the increase in B-cell lymphomas observed in the p53−/−PTP1B−/− by augmenting the pool of susceptible B lineage target cells that sustain cooperating genetic lesions due to loss of p53. In addition, because PTP1B negatively regulates PTK signaling, it remains possible that increased PTK signaling in PTP1B−/− mice provides an accelerated B-cell expansion in preference to T-cell transformation or to other types of tumors in a p53 null background.

In summary, our studies reveal that PTP1B is an important determinant of the latency and type of tumors in a p53−/− background and suggest that PTP1B acts as a tumor suppressor in this specific context. We show that the absence of one allele of the PTP1B gene does not alter the mean time of tumor development in p53 null mice. Our results reveal a novel and important role of PTP1B in immune function. To our knowledge, this is the first demonstration of a role for PTP1B in B-cell development and hematopoiesis, as well as in cancer development in vivo. In this regard, the correlation of expression levels of PTP1B with neoplastic disease status in humans will be of invaluable help to clearly define its role in tumorigenesis. Further studies on the role of PTP1B in lymphoid cell development, proliferation, and differentiation are ongoing.

Acknowledgments

Received 4/18/2005; revised 8/5/2005; accepted 8/30/2005.

Grant support: Cancer Research Society (M.L. Tremblay), Canadian Institutes of Health Research grant MOP-62887 (M.L. Tremblay), Canadian Institutes of Health Research doctoral award (N. Dube), Cancer Research Institute of New York cancer research foundation scholarship (K.M. Heinonen), and Jeanne and Jean-Louis Levesque Chair in Cancer Research (M.L. Tremblay).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

We thank Drs. Nicole Beauchemin and Christophe Blanchetot for critical reading of the article; Eva Mignon, Michelle Read, and Courtney Trott for excellent technical help; and Eric Massicotte and Martine Dupuis for technical advice on flow cytometry.

References

7. Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG. The nontransmembrane tyrosine phosphatase PTP-1B.
Genetic Ablation of Protein Tyrosine Phosphatase 1B Accelerates Lymphomagenesis of p53-Null Mice through the Regulation of B-Cell Development

Nadia Dubé, Annie Bourdeau, Krista M. Heinonen, et al.