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Partial restoration of B cell development in Jak-3$^{-/-}$ mice achieved by co-expression of IgH and E$_\mu$-*myc* transgenes

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Abstract

Jak-3 is a non-receptor tyrosine kinase that plays an important role in coordinating signals received through a wide range of cytokine receptors, including the IL-7 receptor (IL-7R). Jak-3-deficient mice have a profound block in B cell development at the pro-to-pre-B cell transition and have very few peripheral B cells. This block has been postulated to reflect the inability of Jak-3$^{-/-}$ pro-B cells to respond to IL-7. Here we demonstrate that B cell development can be partially restored in Jak-3-deficient mice when they are bred to mice carrying both a rearranged Ig heavy chain (IgH/Igμ) transgene and a c-*myc* transgene expressed in the B cell lineage. Jak-3$^{-/-}$ mice expressing both of these transgenes exhibit significant increases in the number of B cells in the bone marrow and, to a lesser extent, in the spleen. However, very few rescued B cells were detectable in mice greater than 4 months of age. To determine whether resident hyperactivated Jak-3$^{-/-}$ peripheral T cells are responsible for the elimination of the rescued B cells in older mice, we bred IgH transgenic (Igμ Tg)/*myc* Tg/Jak-3$^{-/-}$ mice to T cell-deficient (TCR$\alpha^{-/-}$) mice. Data from these experiments suggest that the paucity of B cells in older Jak-3$^{-/-}$ mice is largely attributable to the lack of Jak-3 in the B cells themselves. Thus, Jak-3 seems to play several important roles in B cells: during development, to enable cell division, Ig gene rearrangement and cell differentiation, and in mature cells, to promote B cell survival in the periphery.

Introduction

Regulation of hematopoiesis is largely achieved by cytokines that bind to members of the hematopoietic receptor superfamily [reviewed in (1,2)]. This family of receptors lacks intrinsic enzymatic activity, but instead employs members of the Janus kinase (Jak) family of tyrosine kinases to activate and transmit relevant biological signals upon ligand binding. The cytoplasmic domains of many of these receptors are constitutively associated with individual members of the Jak family of cytoplasmic tyrosine kinases (Jak-1, Jak-2, Jak-3 and Tyk-2). Upon ligand binding, the associated Jaks are brought into close proximity, leading to their transphosphorylation and activation. Once activated, the Jaks phosphorylate tyrosine residues in the cytoplasmic domain of the receptors, leading to the recruitment to the receptor of Src homology 2 (SH2)-containing proteins, including members of the signal transducers and activators of transcription (STAT) family. Activation of STAT proteins by the Jaks can lead to downstream signals, including direct transcriptional activation by the STAT proteins themselves (3). Abnormal signaling through the Jak–STAT pathway results in hematopoietic disorders, including severe combined immunodeficiency and leukemia (4). The recent discovery of the CIS/SOCS/JAB/SSI family of inhibitors has contributed to understanding how this pathway is negatively regulated (5).

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Jak-3 mediates signaling through cytokine receptors that contain the IL-2R common γ chain (γ_c), including the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 (1,2). Jak-3 has also been shown to associate constitutively with CD40, a B cell co-receptor and member of the tumor necrosis factor receptor superfamily of surface molecules (6). Signaling through CD40 has a wide range of effects on B cells, including cell growth, survival and differentiation, isotype switching, rescue from apoptosis induced by Fas (CD95) or by surface Ig cross-linking, and up-regulation of expression of B7 (CD80), Fas, ICAM-1, CD23 and lymphotoxin (LT)-α (7).

Jak-3 (and γ_c)-deficient mice exhibit a severe combined immunodeficiency phenotype, similar to that observed in humans with Jak-3 mutations (8–14). Jak-3$^{-/-}$ and $\gamma_c^{-/-}$ mice have small thymuses and functionally unresponsive peripheral T cells with an activated/memory phenotype (10–14). The thymic defect in these mutant mice strongly resembles that seen in IL-7 and IL-7 receptor (IL-7R) knockout mice, suggesting that the block in Jak-3 mutant thymocyte development is largely due to the lack of the IL-7 signal (15,16). The dysregulation of the Jak-3-deficient T cells is also thought to contribute to the abnormal expansion of myeloid cells in Jak-3$^{-/-}$ mice, leading to splenomegaly by 4 months of age (17).

Jak-3 and γ_c-deficient mice have a severe block in early B cell development, leading to greatly reduced numbers of bone marrow (BM) and splenic B cells. Most of the B cells in adult Jak-3 mutant BM exhibit a pro/early pre-B cell phenotype (B220$^+$CD43$^+$), resembling that of mice lacking V(D)J recombinase (RAG-1 or RAG-2) genes (18,19). Based on these initial findings, it was postulated that the block in B cell development in Jak-3 mutant mice was entirely due to the lack of the IL-7 signal needed to induce B cell proliferation (10–12). IL-7 is required for both the proliferation and differentiation of early pre-B cells, but not for the proliferation of pre-pro-B cells to pro-B cells or for IgH gene rearrangement (20).

Jak-3 is also required for IL-2-mediated lymphocyte stimulation and differentiation. Of the three main target proto-oncogenes of IL-2 signaling—*bcl-2*, c-*fos* and c-*myc*—the latter two are critically dependent upon the protein tyrosine kinase (PTK) function of Jak-3. Cells expressing a mutant form of Jak-3 that can associate with IL-2 receptor γ, but has no PTK activity, display impaired c-*myc* and c-*fos*, but not *bcl-2* expression in response to IL-2 (21). Jak-3 mediates up-regulation of c-*myc* in response to IL-2 through its interactions with STAM1 and 2 (22,23). The c-*myc* gene encodes the transcription factor c-Myc, which heterodimerizes with a partner protein, termed Max, to regulate gene expression. Max also heterodimerizes with the Mad family of proteins to repress transcription, antagonize c-Myc and promote cellular differentiation (24). Burkitt's lymphoma is a highly malignant B cell tumor characterized by chromosomal translocations that constitutively activate the c-*myc* oncogene (25).

Transgenic (Tg) mice bearing a c-*myc* oncogene under control of the lymphoid-specific Ig heavy chain enhancer (E_μ) eventually develop clonal B lymphoid malignancies, but most young E_μ-*myc* Tg mice lack malignant clones (26,27). Young E_μ-*myc* Tg mice contain an abnormally expanded but non-malignant population of large B cell precursors. These cells, which represent several maturational stages, occupy the BM and develop in the spleen very early in life. Enforced c-*myc* expression in these mice favors proliferation over maturation, as the pre-B cell compartment is over-represented even in the peripheral lymphoid organs and mature B cell numbers are reduced. Thus, it is believed that c-Myc may normally function to regulate differentiation as well as to promote cell cycling, particularly since differentiation in several cell types is associated with down-regulation of c-*myc* (28,29).

At the initiation of our studies of B cell development in Jak-3$^{-/-}$ mice, we postulated that Jak-3 might play a role in conveying positive signals important for Ig gene rearrangement events or RAG expression. Thus, we reasoned that the developmental block in Jak-3 mutant B cells might be alleviated, at least partially, if the cells were provided a rearranged Igμ transgene. We show here that although an Igμ transgene does markedly increase the amount of rearrangement at the Igκ L chain locus in Igμ Tg/Jak-3$^{-/-}$ mice, it is not sufficient to restore normal numbers of either BM or peripheral B cells. Our studies went on to address whether enforced c-*myc* expression would compensate for the proliferative deficiency in Jak-3$^{-/-}$ B cells and also whether the presence of hyperactivated Jak-3$^{-/-}$ T cells directly affects the survival of Jak-3 mutant B cells.

Methods

Mice

Jak-3$^{-/-}$ mice (10) were the generous gift of Dr Leslie Berg (University of Massachussetts). Tg mice bearing a rearranged human IgH chain (Igμ Tg) (30) were bred in our facility from mice originally obtained from Drs Michel Nussenzweig (The Rockefeller University) and Phil Leder (Harvard). E_μ-*myc* transgenic mice (26) were obtained from Dr Charles Sidman (University of Cincinnati). TCRα$^{-/-}$ mice (31) were the gift of Dr Mark Soloski (Johns Hopkins University). All mice were maintained in microisolator cages under modified specific pathogen-free conditions in our facility.

Genotyping of mice

DNA was isolated from a small tail biopsy, then tested by PCR for the presence of an intact Jak-3 or TCRα gene, and for the human Igμ transgene, the E_μ-*myc* transgene and the neomycin gene (present in each of the Jak-3 and TCRα knockout constructs). Jak-3$^{-/-}$, Jak-3$^{+/-}$ and Jak-3$^{+/+}$ mice were distinguished by performing two PCR assays: first, for the neomycin gene, using *neo*-L (5′-CAG CTG TGC TCG ACG TTG TC-3′) and *neo*-R (5′-ACG CTA TGT CCT GAT AGC GG-3′) (22 cycles of 94°C 1 min, 58°C 30 s, 72°C 1 min, with 2mM $MgCl_2$; ~500-bp product); and next, for Jak-3 itself, using JAK3-1 (5′-TTA CGA GCT CCT CTC AGA C-3′) and JAK3-2 (5′-ACG ATG AAG TCG CTG TGC A-3′) (29 cycles of the same conditions; ~400-bp product). The Igμ transgene was detected in a PCR (30 cycles of 94°C 1 min, 66°C 2.5 min; 4 mM MgCl2) containing the forward primer HCH2 (5′-TCC AAG CTC ATC TGC CAG GCC ACG G-3′) and the reverse primer HCH3b (5′-ACC CAC GGC GCT GAA AGT GGC ATT G-3′), yielding a product of ~750 bp. Under the same PCR conditions, the E_μ-*myc* transgene was detected as a ~900-bp band (in addition to the 300-bp band amplified from the endogenous *myc* gene) using *myc*E3 (5′-CGG ACA CAC AAC

GTC TTG GA-3′) and *myc*5′UTR (5′-CTC TCA CGA GAG ATT CCA GC-3′). The TCRα gene was also amplified under these same conditions, using TCRCα-L (5′-CCA GAA CCC AGA ACC TGC TGT G-3′) and TCRCα-R (5′-CTG AAC TGG GGT AGG TGG CGT-3′), yielding a 270-bp product. Once we had crossed the Jak-3$^{-/-}$ and TCR$^{-/-}$ mice, we distinguished between the Jak-3/*neo* allele and the TCRα/*neo* allele by performing two different PCR reactions. First, we used a forward primer in the-3′ end of the neomycin gene PGK-P (5′-CAA AGC TGC TAT TGG CCG C-3′) and a reverse primer JAK3-3 (5′-GCA GAT CTG CCA AGC GAA CAG-3′) in the adjacent Jak-3 gene, to amplify a 485-bp band (also 30 cycles of 94°C 1 min, 66°C 2.5 min; 4 mM $MgCl_2$). For the TCRα/*neo* allele, we used PGK-P in combination with TCRCα-L under the same PCR conditions to amplify an ~500-bp band.

Cell preparation and purification

BM cells were isolated from femurs and tibias by careful disruption in PBS using a mortar and pestle. Cells were resuspended in PBS, depleted of bone fragments by passage over nylon mesh and pelleted at 1000 *g*. Splenocytes were obtained by crushing spleens between glass slides, then resuspending and pelleting the cells, as for BM. To retain as many cells as possible for an accurate B cell count, BM and spleen samples were not depleted of red blood cells by Ficoll treatment or hypotonic lysis; instead, red blood cells and dead cells were ignored during counts of cells resuspended in Trypan blue and were also gated out electronically after flow cytometric analysis. All cells were resuspended in FACS wash buffer (WB) (HBSS + 1% BSA + 10 mM HEPES buffer, pH 7.4) for staining.

Cell staining and flow cytometry

To stain cells for flow cytometric analysis, 1–1.5 × 10^6 cells were incubated on ice for 20 min in the presence of saturating amounts of the appropriate FITC-, phycoerythrin (PE)- and/or biotin-conjugated mAb, in a total volume of 100 μl of FACS WB. Cells were washed with 1.5 ml of FACS WB, pelleted and then resuspended in 100 μl FACS WB containing a saturating amount of Quantum Red–streptavidin. After another 20 min incubation on ice, the cells were washed and pelleted as before, and then resuspended in 0.5 ml of FACS WB and analyzed on a FACScan using CellQuest software (Becton Dickinson, Mountain View, CA). Detectors for forward (FSC) and side (SSC) light scatter were set on a linear scale, whereas logarithmic detectors were used for all three fluorescence channels (FL-1, FL-2 and FL-3). Compensation for spectral overlap between FL channels was performed for each experiment using single-color-stained cell populations. Wherever possible, instrument settings were saved to disk and used again with slight modifications if necessary in related experiments. All cells were collected ungated to disk and data were analyzed using CellQuest software. Unless otherwise noted, red blood cells and dead cells were excluded by electronically gating data on the basis of FSC versus SSC profiles; a minimum of 5 × 10^4 spleen cells or 1–2 × 10^5 BM cells were analyzed further.

Reagents for flow cytometry

Quantum Red–streptavidin was purchased from Sigma (St Louis, MO). Anti-B220–FITC, –PE or –biotin (clone RA3-6B2), anti-CD19–biotin (clone 1D3), anti-human IgM–FITC (clone G20-127) and anti-CD43–PE or –biotin (clone S7) mAb were obtained from PharMingen (San Diego, CA). Goat anti-mouse IgM–FITC, –PE or –biotin and goat anti-mouse IgD–biotin were purchased from Southern Biotechnology Associates (Birmingham, AL). Rat anti-mouse IgM (clone 331.12) mAb was purified from tissue culture supernatants and conjugated to FITC in our laboratory, according to established protocols (32).

Cell lines

103-bcl2 cells (33) were obtained from Dr Naomi Rosenberg (Tufts University) and were grown in RP10 (RPMI 1640 supplemented with 10% FBS, 4 mM glutamine, 10 mM HEPES, 50 μM β-mercaptoethanol and antibiotics) at 33°C in a 5% CO_2 incubator. For induction of IgL gene rearrangement, the cells were shifted to 39°C for 12–16 h before harvesting. DNA isolated from 3T3, a mouse fibroblast cell line, was provided by T. Morrow (Johns Hopkins University).

Ligation-mediated (LM)-PCR

LM-PCR assays to detect signal broken ends at the Igκ locus were performed essentially as described (34). To summarize, DNA was ligated to the BW linker (consisting of BW-1, 5′-GCG GTG ACCCGG GAG ATC TGA ATT C-3′ and BW-2, 5′-GAA TTC AGA TC-3′) overnight at 16°C (35). The reaction was then mixed with 100 μl PCR-L buffer (10 mM Tris pH 8.8, 50 mM KCl, 0.25% Tween 20 and 0.25% NP-40) and heated to 95°C for 15 min prior to PCR. The primary amplification consisted of 12 cycles of 94°C for 1 min and 66°C for 2.5 min each, using the linker-specific primer BW-H (5′-CCG GGA GAT CTG AAT TCC AC-3′) and a primer upstream of $J_κ1$ (κo3, 5′-AGT GCC ACT AAC TGC TGA GCC ACC-3′). One microliter of the primary reaction was used for a second amplification with BW-H and a nested primer, κo5 (5′-GCC CAA GCC TTC CAC GCA TGC TTG GAG-3′), consisting of 27 cycles of the same conditions. A 27-cycle control reaction (same conditions) was also performed, using the CD14-L (5′-GCT CAA ACT TTC AGA ATC TAC CGA C-3′) and CD14-R (5′-AGT CAG TTC GTG GAG GCC GGA AAT C-3′) primers and ~20 ng of linker-ligated template DNA (data not shown). One half of the final PCR product was analyzed by electrophoresis on a 0.7% agarose + 1.3% NuSieve (FMC Bioproducts, Rockland, ME) gel and blotted under alkaline conditions to a nylon membrane (Zetabind; Cuno, Meriden, CT); CD14 control reactions were analyzed by ethidium bromide staining. Blots were hybridized to ^{32}P-end-labeled locus-specific internal primer, κo6 (5′-AGC CAG ACA GTG GAG TAC TAC CAC-3′), and analyzed with a Phosphorimager using ImageQuant software (Molecular Dynamics, Eugene, OR).

PCR-fragment length polymorphism

RNA was isolated by the guanidinium isothiocyanate method as described previously (36). cDNA was prepared by mixing 3 μg of RNA with 1 × Life Technologies (Grand Island, NY) reverse transcription buffer, 1 mM dNTPs, 10 mM DTT, 20 U

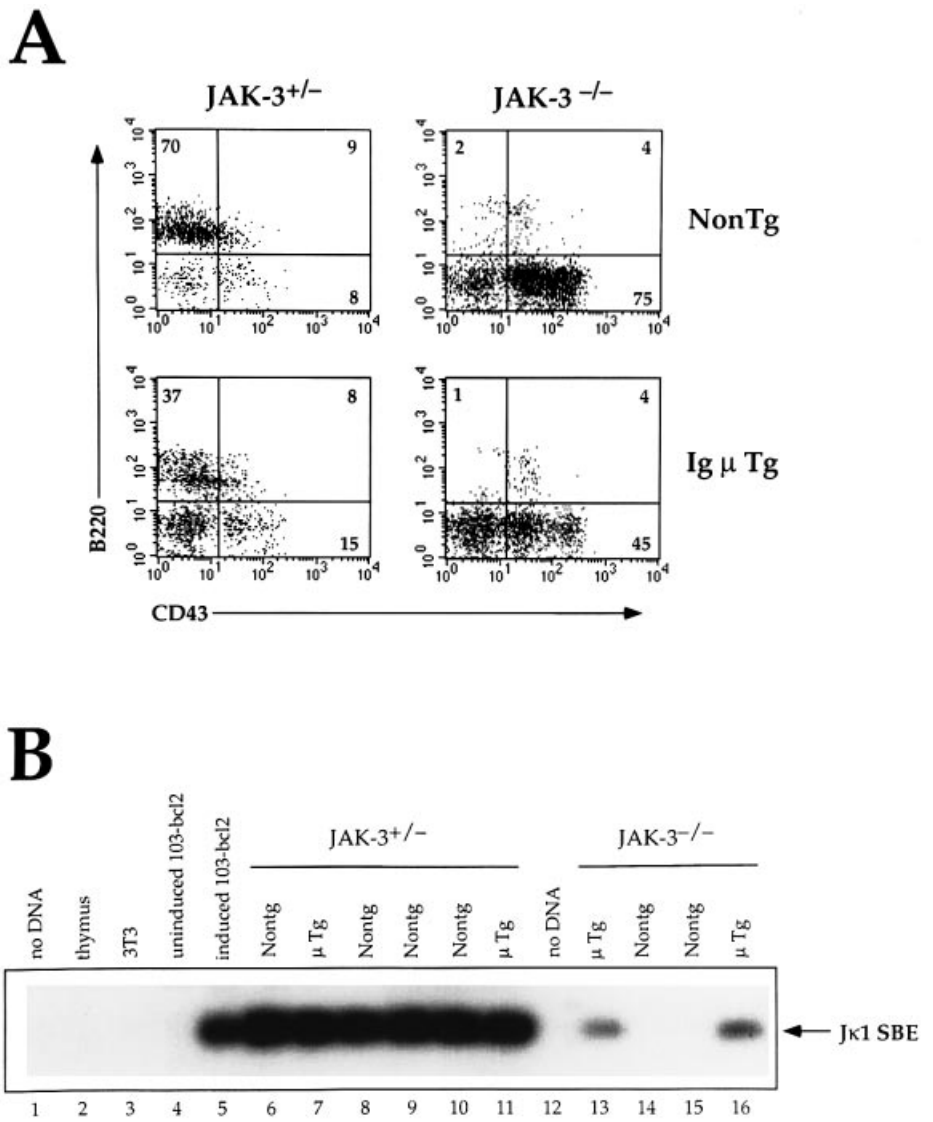

Fig. 1. A rearranged Igμ transgene does not restore B cell numbers in Jak-3-deficient mice, but does provoke increased Igκ light chain gene rearrangements. (A) BM cells from Jak-3$^{+/-}$ (left-hand panels) and Jak-3$^{-/-}$ (right-hand panels) mice that were either non-Tg (upper panels) or Igμ Tg (lower panels) were stained with FITC–anti-B220 and anti-CD43–biotin followed by Quantum Red–streptavidin, and analyzed on a FACScan. The data were gated on viable lymphocytes (large and small) based on their light scatter characteristics; the percentages of cells in each quadrant are indicated. (B) LM-PCR to detect SBE at $J_\kappa 1$ was performed as described in Methods using template DNA isolated from: BALB/c thymus (lane 2), 3T3 fibroblasts (lane 3), uninduced 103-bcl2 cells (lane 4), 103-bcl2 cells induced to undergo rearrangement upon shifting them from 37 to 39°C for 15 h (lane 5), non-Tg Jak-3$^{+/-}$ BM (lanes 6 and 8–10), Igμ Tg Jak-3$^{+/-}$ BM (lanes 7 and 11), non-Tg Jak-3$^{-/-}$ BM (lanes 14 and 15) and Igμ Tg Jak-3$^{-/-}$ BM (lanes 13 and 16); reactions performed without DNA template were also included as controls (lanes 1 and 12).

RNasin, 100 pM random hexamers and 400 U MMLV reverse transcriptase, and incubating for 2 h at 42°C, followed by 5 min at 95°C to inactivate the reverse transcriptase. One-tenth of this reaction was then added to the PCR. DNA was isolated by lysing cells in the presence of SDS and EDTA, incubating overnight with proteinase K, and performing two phenol:$CHCl_3$ extractions and one $CHCl_3$ extraction, followed by precipitation in ethanol/0.3 M ammonium acetate. DNA from 1–4 × 10^4 cells, or one-tenth of a cDNA reaction, was first amplified (20 cycles of 94°C for 1 min, 66°C for 2.5 min, followed by 1 cycle of 72°C for 10 min) with a primer downstream of J_H3 (JHA: 5′-TGC CTC AGA CTT CAA GCT TCA GTT CTG G-3′) and a degenerate V_HJ558 gene family-specific primer (V_H558-FR1: 5′-ARG CCT GGG RCT TCA GTG AAG-3′). A portion of this reaction (1 μl of 25 μl total) was used in a second round of amplification (20–25 cycles under the same conditions as used in the first round) using $V_H186.2$-FR3 (5′-AGC AGC CTG ACA TCT GAG GAC TC-3′) and an end-labeled primer located within J_H1 (JHB2: 5′-GAA TGG AAT GTG CAG AAA GAA AAA AGC C-3′). Results similar to those shown in Fig. 6 were obtained from DNA templates using primers downstream of J_H2 or J_H3. For ^{32}P-end-labeling of the oligonucleotide

primers, T4 polynucleotide kinase (New England Biolabs, Beverly, MA) was used according to the manufacturer's instructions. The labeled oligonucleotides were purified on a QiaQuick spin column (Qiagen, Valencia, CA) according to the manufacturer's instructions. Three microliters of the second PCR were run on a 6% acrylamide sequencing gel. The sequencing gels were subsequently analyzed using a PhosphorImager and ImageQuant software (Molecular Dynamics) or were visualized by autoradiography.

Results

The B cell compartments in the BM of Jak-3 mutant and RAG mutant mice are similar, and consist primarily of arrested pro-B (B220$^+$CD43$^+$) cells (10–12,18,19). A small number of IgM$^+$ B cells can be detected in young (<40-day-old) Jak-3$^{-/-}$ mice, but adult mice are virtually devoid of mature peripheral B cells (D. Thomis and L. Berg, pers. commun.). It has been shown previously that crossing RAG-1-deficient mice to mice carrying a rearranged human IgH (μ) transgene is sufficient to drive the mutant B cells across the pro-to-pre-B cell transition (37,38). Thus, while RAG mutant B cells are arrested at the B220$^+$CD43$^+$ pro-B cell stage, B cells in RAG × μ BM are largely B220$^+$CD43$^-$ (pre-B cells). With this in mind, we asked whether the block at the pro-B cell stage of development in Jak-3$^{-/-}$ mice might be rescued similarly by expression of the Igμ transgene, presuming that a functional Igμ protein might mediate induction of IgL gene rearrangement and progression to the next stage of development. When we crossed Jak-3$^{-/-}$ mice to Igμ Tg mice, we found that the surface phenotype of BM B cells from Igμ Tg/Jak-3$^{-/-}$ mice is virtually indistinguishable from that of their non-Tg Jak-3$^{-/-}$ counterparts (Fig. 1A). Furthermore, very few B220$^+$CD43$^-$ B cells are present in the peripheral lymphoid organs of adult Igμ Tg/Jak-3$^{-/-}$ mice (data not shown and see below).

To determine whether the lack of Jak-3 alters the production or function of the V(D)J recombinase, we next assayed for RAG-1 and -2 gene expression in Jak-3$^{-/-}$ versus Jak-3$^{+/-}$ BM by RT-PCR. Despite the greatly reduced numbers of B cells in Jak-3$^{-/-}$ BM, we were able to detect both RAG-1 and -2 transcripts in both non-Tg and Igμ Tg/Jak-3$^{-/-}$ whole BM (data not shown). We went on to test the functional competence of the RAG proteins expressed in the Jak-3 mutant B cells by performing LM-PCR (34), an assay for signal broken ends (SBE), which are DNA intermediates of Ig gene rearrangement. A positive signal generated in this assay indicates the presence of double-strand DNA breaks at the IgL gene fragment of interest, reflecting recombinase activity at that site (39). As shown in Fig. 1(B), there are strong signals in DNA from Jak-3$^{+/-}$ BM cells (either non-Tg or Igμ Tg; Fig. 1B, lanes 6–11), but no measurable breaks at the Jκ1 gene segment in DNA isolated from non-Tg Jak-3$^{-/-}$ BM (Fig. 1B, lanes 14 and 15). However, Jak-3 mutant B cells carrying the Igμ transgene do exhibit RAG-mediated breaks in the IgL locus (Fig. 1B, lanes 13 and 16). This difference is not a reflection of an increased number of B cells in the Igμ Tg mice, since both non-Tg and Igμ Tg mice on the Jak-3$^{-/-}$ background have very similar numbers of BM B cells (Fig. 1A and data not shown). Instead, these LM-PCR results indicate that in the presence of a functionally rearranged IgH chain, IgL chain gene rearrangement can occur in cells lacking Jak-3, implying that at least one aspect of the pre-BCR signal is intact.

The inability of Jak-3 mutant B cells expressing the Igμ transgene to reconstitute the peripheral B cell compartment despite being capable of activating IgL gene rearrangement suggests that Jak-3 might play a role in B cell expansion. We tested whether the proliferative block in Jak-3 mutant B cells, presumably the result of a defective IL-7 signaling pathway, might be overcome by the B cell-specific overexpression of the proto-oncogene c-*myc* (26,27). Our rationale for these experiments was that if Jak-3-deficient B cells were provided both a rearranged IgH chain gene and the capacity for essentially unlimited growth, they might expand to nearly normal levels. We bred the E_μ-*myc* Tg mice onto the (Jak-3$^{-/-}$ × Igμ Tg) background and intercrossed the F_1 progeny, generating F_2 mice with eight different genotypes (Fig. 2). We then isolated and counted BM (Fig. 2A) and splenocytes (Fig. 2B) from mice of various ages, stained them with mAb specific for various B cell surface markers, and analyzed them by flow cytometry to determine the percentage and absolute number of B cells in each of these tissues. As shown in Fig. 2(A), the E_μ-*myc* transgene alone caused a significant expansion of B cells in the BM of many of the Jak-3$^{-/-}$ mice (Fig. 2A, second column, third row), and BM B cell numbers (listed in parentheses in Fig. 2, under each percentage) were further elevated (sometimes to 'normal' levels) in mice carrying both the *myc* and Igμ transgenes (Fig. 2A, fourth row). Note that the rescued B cells in the Jak-3$^{-/-}$ *myc* Tg mice are larger than normal B cells (i.e. FSC^{high} 'blasts'), as is typically seen in Jak-3-intact mice expressing the *myc* transgene (26,27). The same general trend held true for splenic B cells in these mice (Fig. 2B). However, the fraction of B cells in the spleens of the double Tg (Igμ/Eμ-*myc*) mice on the Jak-3-deficient background never reached normal (non-Tg Jak-3$^{+/-}$) levels and certainly never approached the numbers achieved in Jak-3$^{+/-}$ *myc* Tg mice (Fig. 2B, rows 3 and 4).

We further analyzed the surface phenotype of the B cells in the BM and spleen from mice of each genotype (Fig. 3). In the BM, gated B220$^+$ cells in a non-Tg Jak-3$^{-/-}$ mouse are nearly all IgM$^-$ and nearly half of the mutant B cells express CD43 (Fig. 3A, top row). Introduction of the Igμ transgene into the mutant background increases the percentage of IgM^{low} cells, but a large fraction of the Jak-3$^{-/-}$ B cells are still CD43$^+$ (Fig. 3A, second row). The introduction of the *myc* transgene drastically reduces the fraction of IgM$^+$ cells in both Jak-3$^{-/-}$ and Jak-3$^{+/-}$ mice (Fig. 3A, bottom rows), as expected from previous studies of Eμ-*myc* Tg mice (27). In Jak-3$^{-/-}$ mice with or without either of the transgenes, 25–50% of the cells remain CD43$^+$.

Interestingly, the partial restorative effect of the E_μ-*myc* and Igμ transgenes on the B cell compartment in Jak-3$^{-/-}$ mice only occurred in a fraction of the mice analyzed and waned significantly as the mice aged (Fig. 4). We followed cohorts of mice from 4 to 29 weeks of age, and counted B cells in the BM and spleen at various time points. As shown in Fig. 4, both non-Tg Jak-3$^{-/-}$ and Igμ Tg Jak-3$^{-/-}$ mice at all ages tested have only negligible numbers of BM and splenic B cells compared to their Jak-3$^{+/-}$ counterparts (Fig. 4, top row). Among the *myc* Tg/Jak-3$^{-/-}$ mice, a small fraction (two of 14) had numbers of BM B cells that overlapped with their *myc* Tg/Jak-3$^{+/-}$

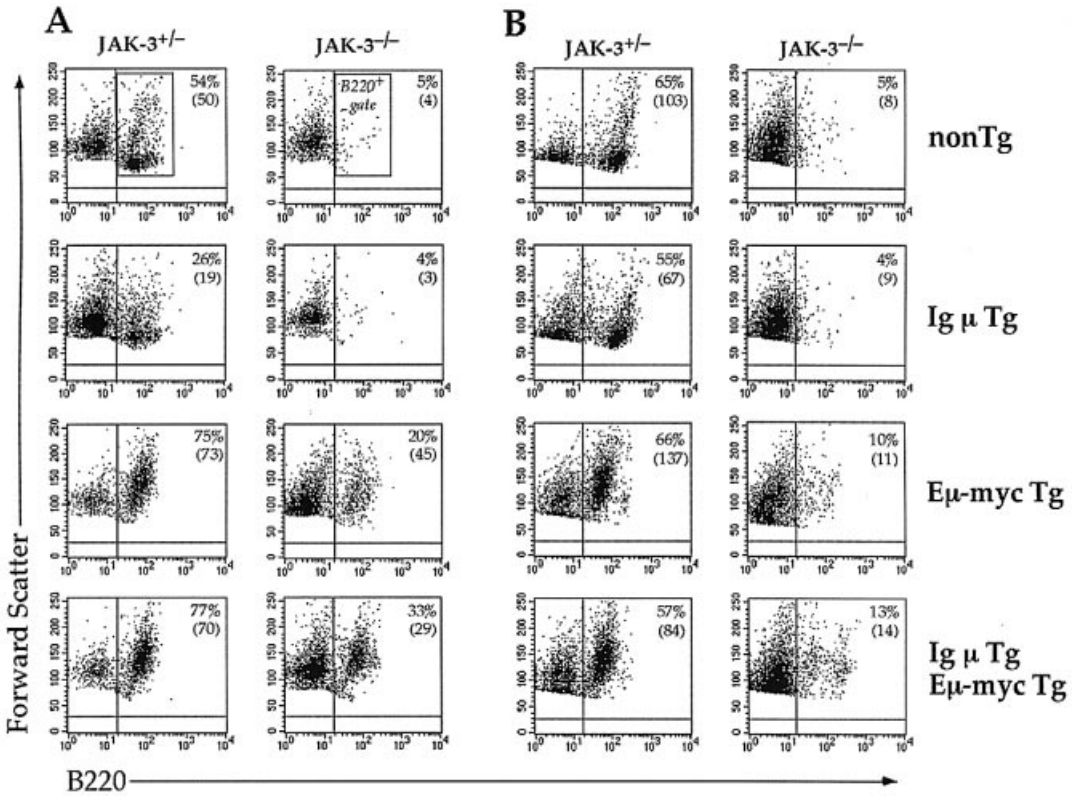

Fig. 2. Partial restoration of B cell numbers is achieved in Jak-3$^{-/-}$ mice provided an E_μ-*myc* transgene. BM cells (A) and splenocytes (B) isolated from either Jak-3$^{+/-}$ or Jak-3$^{-/-}$ mice that were non-Tg (top row), Igμ Tg (second row), E_μ-*myc* Tg (third row) and Igμ/E_μ-*myc* double Tg (bottom row) were stained with FITC–anti-B220 and PE–anti-CD43, and analyzed by flow cytometry. Data were gated to exclude red blood cells and dead cells (FSC^{low}); the percentage of viable B220$^+$ cells in each sample is indicated in the upper right-hand quadrants and the actual number ($\times 10^6$) of viable BM B cells is listed below each percentage in parentheses. The B220$^+$ gate used for the data in Fig. 3 is also indicated (A, top row). Note the increased size (FSC^{high}) of the B cells in the E_μ-*myc* Tg mice. Data shown are from a cohort of 8-week-old mice and are representative of results obtained with young mice (<14 weeks of age), except for the E_μ-*myc* Tg/Jak3$^{-/-}$ plot, which derives from the mouse with the highest number of B cells we observed among this strain (see Fig. 4).

littermates (i.e. $>30 \times 10^6$). Two other young *myc* Tg/Jak-3$^{-/-}$ mice had at least 20×10^6 BM B cells (comparable to non-Tg Jak-3$^{+/-}$ mice), but only one mouse in this group had substantial numbers (50×10^6) of splenic B cells (Fig. 4, third row). We suspect that the large degree of variability within each group may reflect the mixed genetic background (including C57BL/6 and 129/SvJ) of the mice.

B cells in mice bearing both Igμ and *myc* transgenes fared better. Within this group (Fig. 4, bottom row), six of 13 mice <4 months of age had numbers of BM B cells comparable to those of their Jak-3$^{+/-}$ counterparts and three of 15 in this age range had relatively high numbers ($>50 \times 10^6$) of splenic B cells. Interestingly, none of the 10 Igμ Tg/*myc* Tg Jak-3$^{-/-}$ mice >4 months of age had significant numbers of BM or splenic B cells. Although the combination of both Igμ and E_μ-*myc* transgenes did lead to the highest B cells numbers in the Jak-3-deficient mice, it is not clear how much of an effect was contributed by both transgenes versus the E_μ-*myc* transgene alone. The combined effect of the two transgenes together seemed to mediate a mild enhancement of B cells numbers over those in mice with just the E_μ-*myc* transgene, but only in a subset of the younger mice (Fig. 4 and see below). Thus, it is not clear whether the Igμ transgene plays a crucial role in this B cell restoration or not.

By plotting all of the data from each of the eight genotypes side by side, we could more clearly visualize a trend, particularly in the BM (Fig. 5A and B). Introduction of the Igμ transgene onto the Jak-3$^{-/-}$ background led to a very mild, but not statistically significant, increase in the number of BM (but not splenic) B cells in young mice (means $\pm$ SD for BM were $3.5 \pm 1.3 \times 10^{-6}$ for non-Tg Jak-3$^{-/-}$ mice versus $5.1 \pm 2.2 \times 10^{-6}$ for their Igμ Tg counterparts, $P = 0.078$; for spleen, $6.8 \pm 3.2 \times 10^{-6}$ versus $8.9 \pm 3.8 \times 10^{-6}$, $P = 0.256$) (Fig. 5A and C). B cell numbers increased further in the young *myc* Tg mice ($13.7 \pm 13.0 \times 10^{-6}$ in BM, $P = 0.014$ versus non-Tg Jak-3$^{-/-}$ BM and $14.4 \pm 6.3 \times 10^{-6}$ in spleen, $P = 0.002$), but the largest increase was found in the young double Tg (Igμ/E_μ-*myc*) Jak-3 mutant mice ($23.85 \pm 15.06 \times 10^{-6}$ in BM, $P = 0.0001$; and $30.45 \pm 32.12 \times 10^{-6}$ in spleen, $P = 0.019$), resulting in an almost 5-fold increase in BM B cell numbers compared to non-Tg Jak-3 mutant mice (Fig. 5A). It is also clear from these graphs that the effects of the transgenes on B cells wane in the older mice (no P values between the groups of mice >14-week-old mice were <0.05) (Fig. 5, filled triangles).

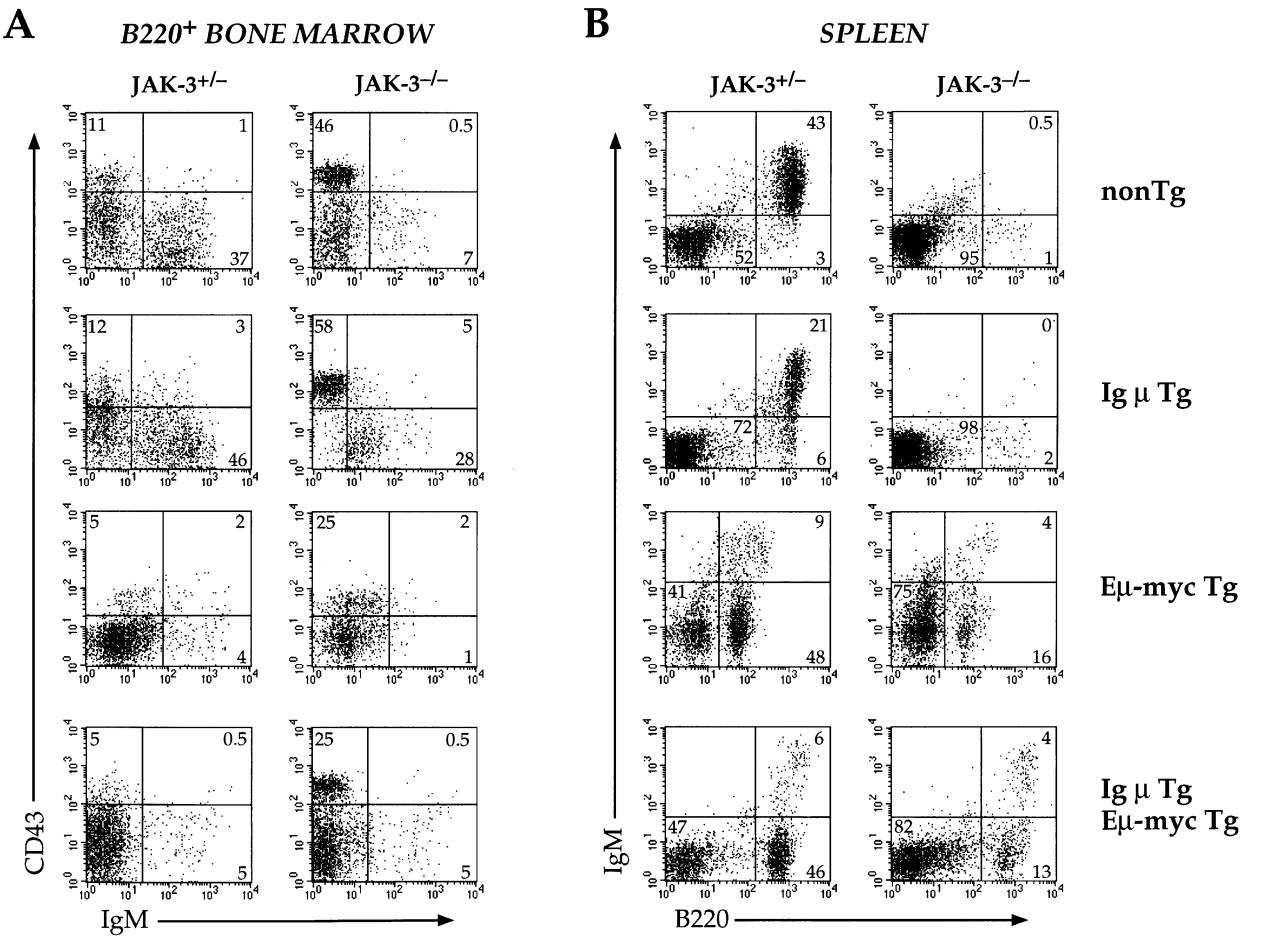

Fig. 3. The surface phenotype of Jak-3$^{-/-}$ B cells in the BM and spleen is similar, but not identical, to that of their Jak-3$^{+/-}$ counterparts. (A) BM cells were stained for three-color flow cytometry with anti-IgM (mouse or human)–FITC, anti-B220–PE and anti-CD43–biotin followed by Quantum Red–streptavidin (note that the E_μ-*myc* Tg cells in the third row were stained with anti-B220–FITC, anti-CD43–PE and anti-IgM–biotin followed by Quantum Red–streptavidin). Viable cells were further gated on B220$^+$ cells, using the gate shown in Fig. 2A (top row), and the resulting IgM versus CD43 profiles are shown for Jak-3$^{+/-}$ or Jak-3$^{-/-}$ mice that were non-Tg (top row), Igμ Tg (second row), E_μ-*myc* Tg (third row) and Igμ/E_μ-*myc* double Tg (bottom row). Note that (for logistical reasons) most of the data do not derive from the same mice shown in Fig. 2. (B) Total splenocytes from mice of each genotype stained with anti-IgM (mouse or human)–FITC and anti-B220–PE (non-Tg and double Tg), anti-B220–PE and goat anti-human IgM–biotin followed by Quantum Red–streptavidin (Igμ Tg) or anti-B220–FITC and anti-IgM (mouse or human)–biotin followed by Quantum Red–streptavidin were analyzed by flow cytometry; data from all live-gated splenocytes is shown. The percentages of cells in each quadrant are indicated. Note that although all of the mice were not analyzed on the same day, each Jak-3$^{+/-}$ versus Jak-3$^{-/-}$ pair was age-matched (<14 weeks old) and analyzed together using the same antibody reagents, and therefore can be directly compared.

The surface phenotype of the rescued splenic B cells in the young Igμ Tg/*myc* Tg Jak-3$^{-/-}$ mice varied somewhat between individual mice, but we generally detected both mature IgM$^+$CD43-B220^{high} cells and some IgM$^-$CD43$^+$B220^{low} cells, resembling those in Jak-3$^{+/-}$ *myc* Tg mice (Fig. 3 and data not shown). At this point, we questioned whether the *myc* transgene had enabled the expansion and survival of just a few clones of B cells in the Jak-3-deficient mice or if the B cell repertoire in these mice was reasonably diverse. To undertake a molecular analysis of the IgH repertoire of Jak-3$^{+/-}$ and Jak-3$^{-/-}$ mice, we looked for differences in bulk populations of spleen cells by using PCR-fragment length polymorphism analysis (40). This assay consists of a PCR step using primers that amplify across the VDJ junction, followed by separation of the amplified products on a polyacrylamide gel, allowing an assessment of both CDR3 length heterogeneity and the distribution of in-frame and out-of-frame rearrangements within a population of cells. We used a degenerate upstream primer that recognizes most members of the large V_H186.2 family and a downstream primer hybridizing to sequences in J_H1 to amplify either cDNA or genomic DNA from whole adult spleen, or genomic DNA from splenocytes from 6-day-old pups.

As shown in Fig. 6, the IgH repertoire in non-Tg or *myc* Tg Jak-3$^{-/-}$ peripheral B cells is reasonably diverse. As expected, amplification from cDNA templates enriched for in-frame, presumably functional, IgH rearrangements in all mice tested. Interestingly, the Jak-3-deficient cells from 8-week-old adult mice seemed to have nearly as large a collection of IgH rearrangements as their Jak3$^{+/-}$ counterparts (Fig. 6, lanes 2–

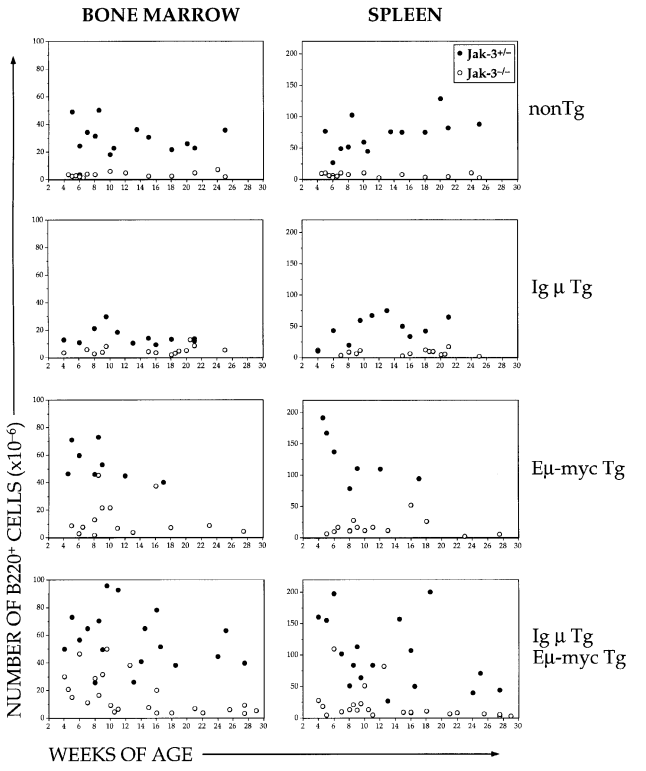

Fig. 4. The partial restorative effect of the E_μ-*myc* and Igμ transgenes on the B cell compartment in Jak-3$^{-/-}$ mice is lost as the mice age. The total number of B cells in the BM (left-hand panels) and spleen (right-hand panels) for Jak-3$^{+/-}$ (filled circles) and Jak-3$^{-/-}$ (open circles) mice of various ages carrying the transgenes indicated, was calculated by multiplying the number of Trypan blue-excluding cells obtained from each mouse by the percentage of live B220$^+$ cells determined by flow cytometric analysis (see Fig. 2). Mean values ± SD from the mean of the key groups are listed in the text. Statistical significance of the differences between the groups was determined by calculating *P* values using Student's unpaired *t*-test; data were considered significantly different when $P < 0.05$.

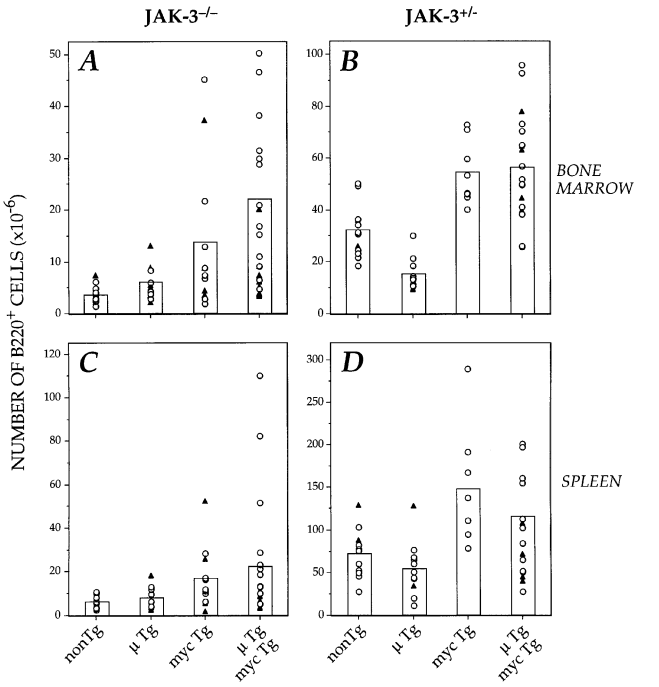

Fig. 5. IgM and E_μ-*myc* transgenes synergize to increase the number of B cells in the BM and, to a lesser extent, in the spleen of Jak-3$^{-/-}$ mice. Data from Fig. 4 are plotted to directly compare the effects of the IgM and/or E_μ-*myc* transgenes on the number of B cells present in either Jak-3$^{-/-}$ (left-hand panels) or Jak-3$^{+/-}$ (right-hand panels) BM (upper panels) and spleen (lower panels). Each open circle represents a single mouse aged 4–14 weeks; each closed triangle depicts a mouse >14 weeks of age. The bars represent the mean value for each group.

5). PCR products generated from genomic DNA taken from the same spleen donors yielded a much smaller population of IgH gene rearrangements (Fig. 6, lanes 8 and 10). This result may reflect inefficient amplification by Taq polymerase of the small fraction of genomic DNA contributed by B cells in the Jak-3-deficient spleens, particularly considering the degree of splenomegaly in these mice (17). We did not observe any significant alteration of the IgH repertoire when the *myc* transgene was present (Fig. 6, lanes 3 and 5). We obtained essentially the same results using DNA from neonatal spleen, with all four of the non-Tg Jak-3$^{-/-}$ samples exhibiting a reasonably large collection of distinct IgH rearrangements (Fig. 6, lanes 12–15), although still less diverse than their Jak-3$^{+/-}$ counterparts (Fig. 6, lanes 11 and 16). These data suggest that despite the severe effects on B cell generation imposed by Jak-3 deficiency, under certain conditions the V(D)J recombinase seems to be capable of functioning normally in Jak-3$^{-/-}$ mice. This finding is consistent with our earlier observations that Jak-3$^{-/-}$ BM contains detectable RAG-1 and -2 transcripts, and that Igμ Tg/Jak-3$^{-/-}$ BM exhibits functional recombinase activity (Fig. 1 and data not shown).

Although the *myc* transgene can partially restore B cell numbers in Jak-3 mutant mice, most of the rescued B cells disappear by the time the mice reach 4 months of age (Figs 2–5). Since this timeframe coincides with the appearance of a substantial population of hyperactivated Jak-3$^{-/-}$ peripheral T cells (41), we postulated that the Fas ligand (FasL)$^+$ T cells might be responsible for the elimination of the rescued B cells, perhaps by inducing apoptosis through a Fas–FasL pathway (42), or by secreting cytokines that directly or indirectly lead to B cell elimination (17). We addressed this question by breeding the Jak-3$^{-/-}$ mice, with both Igμ and *myc* transgenes, to T cell-deficient (TCRα$^{-/-}$) mice (31).

As before, we analyzed groups of mice representing each of the16 potential genotypes at various ages by counting the numbers of B cells in the BM and spleen (Fig. 7). In general, the numbers of B cells in Jak-3$^{+/-}$TCRα$^{-/-}$ mice were similar to those in their TCRα$^{+/+}$ and TCRα$^{+/-}$ counterparts (Figs 4 and 5, and data not shown). However, among the Jak-3$^{-/-}$ mice we noted that the number of B cells present in young Jak-3$^{-/-}$ TCRα$^{-/-}$ mice was generally higher than in their Jak-3$^{-/-}$ TCRα$^{+/-}$ littermates with an intact T cell compartment, although these differences were not statistically significant (Fig. 7A and C). For example, $P = 0.494$ for the number of BM cells in E_μ-*myc* Tg/Jak-3$^{-/-}$TCRα$^{-/-}$ versus E_μ-*myc* Tg/Jak-3$^{-/-}$TCRα$^{+/-}$ mice and $P = 0.065$ for the number of spleen cells in the same groups. In the double-Tg mice, $P = 0.468$ for the number

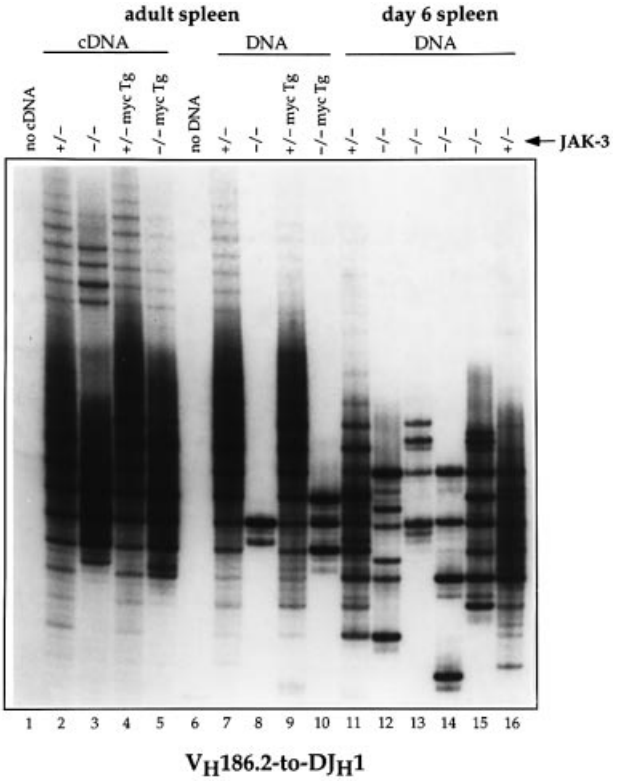

Fig. 6. The IgH repertoire in non-Tg or *myc* Tg Jak-3$^{-/-}$ peripheral B cells is reasonably diverse. PCR-fragment length polymorphism analysis of V_H186.2–DJ_H1 rearrangements was performed as described in Methods, using as template either DNA (lanes 7–16) or cDNA (lanes 2–5) isolated from splenocytes of adult (8-week-old) non-Tg Jak-3$^{+/-}$ (lanes 2 and 7) or Jak-3$^{-/-}$ (lanes 3 and 8) mice, of adult E_μ-*myc* Tg Jak-3$^{+/-}$ (lanes 4 and 9) or Jak-3$^{-/-}$ (lanes 5 and 10) mice, and of day 6 non-Tg Jak-3$^{+/-}$ (lanes 11 and 16) or Jak-3$^{-/-}$ (lanes 12–15) mice. Control reactions lacking template were also included (lanes 1 and 6). Results similar to these were obtained from DNA templates using primers downstream of J_H2 or J_H3 (data not shown).

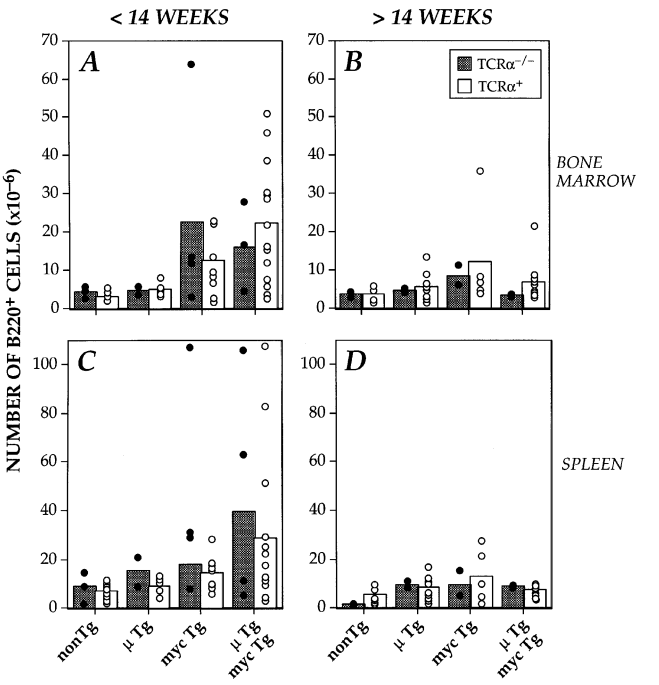

Fig. 7. Young Jak-3$^{-/-}$ mice lacking T cells have similar numbers of peripheral B cells as do their TCRα^+ counterparts and the absence of hyperactivated T cells does not restore the B cell compartment in older Jak-3$^{-/-}$ mice. The average numbers of BM (A and B) and splenic (C and D) B cells from either TCR$\alpha^{-/-}$ (shaded bars) or TCRα^+ (open bars) Jak-3$^{-/-}$ mice are plotted. Data from individual TCR$\alpha^{-/-}$ (filled circles) and TCRα^+ (open circles) are included to demonstrate the experimental variability. The TCRα^+ data include those from both TCR$\alpha^{+/-}$ and TCR$\alpha^{+/+}$ (Fig. 4) mice. T cell deficiency also did not significantly affect the numbers of B cells in Jak-3$^{+/-}$ mice (data not shown).

of BM cells in Igμ/E_μ-*myc* Tg/Jak-3$^{-/-}$TCR$\alpha^{-/-}$ versus Igμ/E_μ-*myc* Tg/Jak-3$^{-/-}$TCR$\alpha^{+/-}$ mice and $P = 0.603$ for the number of spleen cells. More importantly, the B cell population in older Jak-3$^{-/-}$ mice lacking T cells did not expand compared to Jak-3$^{-/-}$TCRα^+ mice, even in mice carrying the *myc* transgene (Fig. 7B and D). These data suggest that the paucity of B cells in older Jak-3$^{-/-}$ mice is largely attributable to the lack of Jak-3 in the B cells themselves, as their survival does not improve in the absence of hyperactivated FasL$^+$ Jak-3-deficient T cells. However, it remains formally possible that other cells, such as macrophages, could mediate the elimination of the rescued Jak-3$^{-/-}$ B cells.

Discussion

We have attempted to restore B cell development and differentiation in lymphopenic Jak-3-deficient mice by introducing a pre-rearranged Igμ transgene, in the presence or absence of an additional transgene directing the overexpression of *myc* in the B cell lineage. Our intent was to overcome the requirement for Ig gene rearrangement in the mutant mice by providing their B cells with a functional Igμ protein that might mediate induction of IgL gene rearrangement and progression to the next stage of development. This approach has proven at least partially successful in at least one other system. Specifically, B cells in RAG-deficient mice expressing the same Igμ transgene are able to progress from the pro-B (CD43$^+$) to the pre-B (CD43$^-$) cell stage of development (where they are blocked due to an inability to rearrange IgL genes) (37,38).

Interestingly, however, we found that the BM B cell compartment in Jak-3-deficient mice expressing the Igμ transgene is similar to that of their non-Tg counterparts (Fig. 1A). However, one important difference we did find was that the Igμ transgene stimulates a substantial increase in rearrangement activity at the Igκ locus (Fig. 1B). We postulate that these Igμ Tg/Jak-3$^{-/-}$ B cells can achieve surface expression of both the pre-BCR and mature BCR, but that their further differentiation and expansion is blocked, due at least in part to an inability to respond to IL-7 and, possibly, to IL-2. Thus, Jak-3 deficiency dissociates the ability of the pre-BCR signal to activate Igκ locus rearrangement from its ability to promote the maturation and survival of pre-B cells.

Due to the similarities between the B cell compartment in Jak-3-, IL-7- and IL-7Rα-deficient mice, many have reasoned

that the block in B cell development in the Jak-3$^{-/-}$ mice is largely due to their inability to respond to IL-7 (15,16). Analogous to Jak-3$^{-/-}$ mice, there is an incomplete block in B cell development in the BM of IL-7$^{-/-}$ and IL-7R$^{-/-}$ mice at the transition from the pro-B to the pre-B cell stage, resulting in peripheral lymphoid organs that contain abnormally low numbers of mature surface Ig-expressing B cells. Tg expression of the anti-apoptotic protein Bcl-2 in IL-7R$^{-/-}$ mice rescues the defect in T cell development and in mature T cell function (43). However, Bcl-2 overexpression does not rescue B lymphopoiesis, although it can enhance survival of those mature B cells that escape the developmental arrest (44). Similarly, Bcl-2 overexpression can rescue T cell development, but not B cell development, in $\gamma_c^{-/-}$ mice (45) and in Jak-3$^{-/-}$ mice (46). Thus, the essential role of IL-7R signaling in B cells cannot be replaced by Bcl-2, indicating that during B lymphopoiesis IL-7R signaling is necessary for promoting cell division or differentiation, or for inhibiting a Bcl-2-insensitive pathway to apoptosis (44).

Subsequent analyses of the IL-7R$\alpha^{-/-}$ mice revealed that the number of pro-B cells undergoing IgH rearrangements in these mice is normal, but there is a severe reduction in pre-B cells with complete IgH rearrangements, and in surface IgM$^+$ B cells. This block stems not only from defective proliferation but also from impeded IgH rearrangements, where D–J_H recombination occurs normally, but rearrangement of V_H segments is progressively impaired as their distance increases upstream of DJ (47). This leads to infrequent rearrangement of most D-distal V segments, remarkably reducing diversity. Since our studies were not as directed at this particular issue as those of Corcoran *et al.*, we cannot conclusively state whether or not D-distal IgH rearrangement is similarly impeded in Jak-3$^{-/-}$ B cells. However, we did easily detect a wide variety of V_H186.2 (a large subfamily of J558, the most 5′-V_H locus) rearrangements in spleen cells from adult Jak-3$^{-/-}$ mice using a PCR-fragment length polymorphism assay (Fig. 6), suggesting that rearrangement to these D-distal V_H segments is not silenced in Jak-3-deficient B cells. However, because our rearrangement analysis was performed using peripheral B cells, it is unclear whether rearrangements using V_H186.2 is efficient in these mice or whether selection processes have actually changed the relative representation of V_Hs in the periphery.

After the productive rearrangement of Ig heavy chain genes, pre-B lymphocytes undergo a limited number of cell divisions in response to IL-7, constrained by an inhibitory signal initiated by antigen receptor assembly. This mechanism to limit IL-7-dependent proliferation may be essential for the proper regulation of peripheral B lymphocyte numbers (48). We reasoned that if Jak-3-deficient B cells were provided both a rearranged IgH chain gene and the capacity for 'unlimited' growth, they might expand to reasonably normal, if not increased, numbers in the Jak-3 mutant mice. It has been shown that B cell activation is associated with a marked transient rise in c-*myc* expression and that Jak-3 is required for c-*myc* gene induction signaled by IL-2 (21,22). Thus, enhancing *myc* expression in Jak-3-deficient B cells might restore the cell growth pathway and enable B cell expansion. We also knew that enforced *myc* expression is not sufficient to block the differentiation of all B cells, since B cells from E_μ-*myc* Tg mice are at least partially functional both *in vitro* and *in vivo* (49). E_μ-*myc* mice can mount specific antibody responses, if sometimes delayed, and the serum from non-immunized E_μ-*myc* mice contains normal levels of both IgM and IgG. Thus, constitutive expression of the c-*myc* gene appears to retard B cell differentiation, but does not grossly impair immunologic function in the intact animal (49).

Interestingly, expression of both the Igμ and the E_μ-*myc* transgenes in Jak-3$^{-/-}$ mice did partially restore the B cell compartment, but significant numbers of rescued B cells were generally not observed in mice >14 weeks of age (Figs 2–5). In accordance with previous reports (27), a small fraction of aged *myc* Tg mice in our colony did develop B lymphoid malignancies (data from tumor-bearing mice were not included in this report). Tumors were more common among Jak-3$^{+/-}$ than Jak-3$^{-/-}$ mice, which was to be expected based on the reduced frequency of B cells in the Jak-3-deficient background (Figs 2–5 and data not shown). Although we have shown that the presence of activated (FasL$^+$) Jak-3$^{-/-}$ T cells may contribute slightly to a decreased number of splenic B cells in mice aged 4–14 weeks, T cells do not seem to be responsible for the death of the B cells in older mice (Fig. 7). Instead, we postulate that Jak-3, perhaps based on its association with CD40 (6,50,51), may be a key player in maintaining peripheral B cell homeostasis.

Since the initiation of our studies, there has been one report of partial restoration of B cell development in Jak-3$^{-/-}$ mice, where the authors observed modest increases in peripheral B cell numbers after injecting newborn Jak-3-deficient mice with IL-3 for 14 days (52). In those studies, IL-3 was thought to be acting on an IL-3Rα^+/IL-7Rα^+/B220^{low} precursor population in the BM and inducing its expansion via a Jak-2-dependent pathway. Notably, the numbers of B cells found in most of the young Igμ/E_μ-*myc* Tg Jak-3$^{-/-}$ mice in our experiments (Figs 2–5) far exceed those rescued in the IL-3-treated Jak-3$^{-/-}$ mice (52). However, in agreement with Brown *et al.*, we would anticipate that the lack of Jak-3 in the rescued B cells in our system would preclude their functional reconstitution, because the cells would not be able to respond to any of the cytokines that require γ_c and Jak-3. Furthermore, the presence of the *myc* transgene would preclude us from fairly assessing the proliferative capacity of the rescued cells.

It is possible that Jak-3, in addition to its affiliation with CD40, associates with receptors for the recently identified tumor necrosis factor family member, B lymphocyte stimulator (BLyS) (53), which is thought to play a crucial role in B cell homeostasis (54–56). It remains to be determined whether Jak-3 mediates signaling through one or all of the BLyS receptors (including TACI, BCMA and BAFF-R) (56). However, given that our veritable 'sledgehammer' of enforced *myc* and Igμ expression only partially, and transiently rescues B cell numbers in Jak-3 mutant mice, a possible direct or indirect role for Jak-3 in BLyS-mediated B cell homeostasis merits investigation.

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Abbreviations

γ_c	common γ chain
BLyS	B lymphocyte stimulator
BM	bone marrow
FasL	Fas ligand
IL-7R	IL-7 receptor
Jak	Janus kinase
LM-PCR	ligation-mediated PCR
LT	lymphotoxin
PE	phycoerythrin
PTK	protein tyrosine kinase
SBE	signal broken ends
STAT	signal transducer and activator of transcription
Tg	transgenic
WB	wash buffer

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