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Genetics

Analysis of homozygous mutant chimeric mice: Deletion of the immunoglobulin heavy-chain joining region blocks B-cell development and antibody production

(B-cell deficiency/immunoglobulin gene rearrangement/gene targeting/embryonic stem cells)

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ABSTRACT Using a recently described method for efficiently deriving homozygous targeted alleles in embryonic stem cells, we produced chimeric mice whose tissues were derived partially from embryonic stem cells bearing homozygous deletion of the mouse immunoglobulin heavy-chain joining (J_H) region. Characterization of these chimeric mice indicated that homozygous J_H deletion leads to arrest of B-cell development at an early stage, resulting in a total lack of peripheral B cells and serum IgM. These results were confirmed in mice containing the homozygous J_H deletion in their germ line. This novel B-cell-deficient mouse strain provides a tool for studying the recombination and expression of exogenous immunoglobulin genes introduced into the mouse germ line.

Generation of mice with specific mutations in the immunoglobulin (Ig) loci, via gene targeting in mouse embryonic stem (ES) cells, can be a powerful approach for elucidating the processes of Ig gene assembly and expression and their role in B-cell development. Such mice could obviate the limitations associated with studies of transformed cell lines (1) or mice carrying Ig transgenes in a background of functional endogenous Ig genes (2).

One valuable model system would be a mouse in which the process of Ig gene assembly is completely arrested. The failure of SCID (3) and RAG-deficient (4, 5) mice to properly assemble Ig genes and to produce antibodies is due to mutant genes that act in trans to impair rearrangement of the Ig and T-cell receptor genes. Thus, reconstitution of B-cell development with unrearranged Ig transgenes cannot be studied in these mutant hosts.

Our strategy to impair Ig gene assembly was to mutate a crucial cis-acting sequence involved in this process. As joining of the diversity (D) and heavy-chain joining (J_H) gene segments is thought to be a prerequisite for heavy-chain variable region (V_H)–D rearrangement, light-chain gene rearrangement, and B-cell differentiation (1, 6, 7), disruption of this event by gene targeting might be sufficient to prevent Ig heavy-chain gene assembly and therefore block B-cell development and antibody production, thus providing a suitable host for introduction of unrearranged Ig transgenes.

To this end we deleted the J_H region from one allele by gene targeting in ES cells. Furthermore, from single allele-targeted ES cells, we selected a homozygous mutant ES cell clone which gave rise to chimeric mice with a homozygous mutant B lineage, for evaluating the effect of the J_H deletion on B-cell development. In addition, chimeric mice were bred to generate mice heterozygous and homozygous in their germ line for the J_H deletion (ΔJ_H).

MATERIALS AND METHODS

Targeting-Vector Construction. Plasmid pUC18-J_H, containing a 6.1-kb *Eco*RI genomic fragment of the mouse Ig heavy-chain (Igh) locus (8), was digested with *Xho* I and *Nae* I to delete the 2296-bp fragment containing the entire J_H region and the D_{Q52} gene segment, which was replaced by a 1150-bp *Xho* I–*Bam*HI fragment of pMC1NeopolA (9, 10) to form the targeting vector pmHΔJ. The unique pUC-derived *Nde* I site was used for linearization.

Transfection and Selection of Heterozygous and Homozygous ΔJ_H-Targeted ES Cell Lines. About 2×10^7 E14TG2a ES cells (11) were electroporated (240 V, 500 μF, Bio-Rad Gene Pulser) with the linearized targeting vector (50 μg/ml). G418 (200 μg/ml) was added after 24 hr. G418-resistant ES colonies were picked and analyzed in pools of four by PCR analysis (45 cycles of 1 min, 94°C; 2 min, 55°C; 3 min, 72°C) using two primers, 5′-ACGGTATCGCCGCTCCCGAT-3′ and 5′-AGTCACTGTAAAGACTTCGGGTA-3′, located 120 bp 5′ of the *Bam*HI site in the neomycin-resistance gene and 161 bp 3′ of the insertion site within the Igh gene, respectively. PCR products were analyzed by electrophoresis. Clones from PCR-positive pools were analyzed individually. To generate a ΔJ_H-homozygous mutant ES cell line, ES 110-1 cells (5×10^3 per 100-mm plate) were selected in G418 (1400 μg/ml) for 10 days (12); 260 colonies survived (20% of the expected survival in G418 at 200 μg/ml).

Chimeric Mice. Generation of chimeric mice and their breeding were carried out as described (13).

Flow Cytometry of Peripheral Blood and Bone Marrow Cells. Peripheral blood mononuclear cells (PBMCs) and bone marrow cells were purified on Ficoll/Hypaque (Accurate), stained with monoclonal antibody (mAb), and analyzed on a FACScan (Becton Dickinson). mAbs were phycoerythrin (PE)-anti-B220 (Pharmingen), PE-anti-Thy-1.2 (5a8, Caltag), and fluorescein isothiocyanate (FITC)-anti-Ly-9.1, -IgM^a, or -IgM^b (Pharmingen). The anti-Ly-9.1 mAb stained >99% of lymphocytes from the 129/J mouse strain and 0–1% of lymphocytes from the B6 strain. The anti-IgM allotype reagents stained 90–97% of B cells in the appropriate strain and <1% of B cells in the inappropriate strain.

To identify an Ly-9.1$^-$ IgM^{b-} CD43$^+$ B220$^+$ B6-derived subpopulation (see Fig. 5*A*), the DK207 chimeric bone marrow cells were treated with anti-CD43 (mAb S7, provided by J. D. Kemp, University of Iowa), followed by FITC-F(ab′)$_2$ goat anti-rat IgG (FGAR) (Caltag, South San Francisco, CA). Purified rat IgG (Cappel) was then added to saturate the free FGAR binding sites. Cells were then incubated with PE-anti-B220, biotinylated anti-Ly-9.1, and biotinylated anti-IgM^b in

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Abbreviations: ΔJ_H, J_H deletion; ES cell, embryonic stem cell; Igh, immunoglobulin heavy chain; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell.

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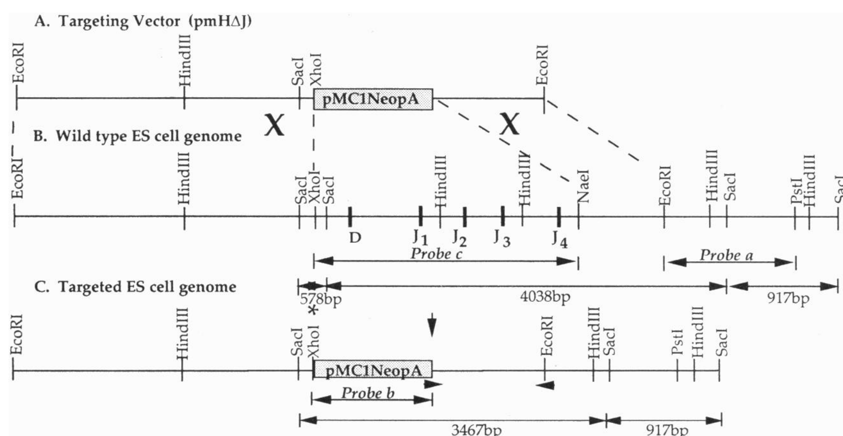

FIG. 1. Scheme for deletion of the mouse J_H region by gene targeting in ES cells. (*A*) pmHΔJ targeting vector. (*B*) Genomic structure of the mouse J_H region and flanking sequences. The four J_H genes and the D_{Q52} segment are indicated. (*C*) Predicted structure of the targeted locus. Arrowheads indicate the approximate positions of the primers used for the PCR screening. Hybridization probes used: probe *a*, *Eco*RI–*Pst* I fragment, 3′ of the insertion site; probe *b*, *Xho* I–*Bam*HI fragment of the *neo* gene; probe *c*, *Xho* I–*Nae* I fragment spanning the J_H region. Expected fragment sizes detected by probes *a*, *b*, and *c* are shown. The 578-bp fragment (asterisk) is undetected due to short overlap with probe *c*.

the presence of excess rat IgG. This was followed by tricolor streptavidin (Caltag). CD43$^+$ B220$^+$ Ly-9.1$^+$ cells (ES-DK207-derived) (Fig. 5*B*) were treated similarly except that anti-IgM^b was omitted. To identify a Ly-9.1$^-$ IgM^{b-} Thy-1.2$^+$ B220$^+$ B6 subpopulation (Fig. 5*C*), we used FITC-anti-Ly-9.1, FITC-anti-IgM^b, PE-anti-B220, and biotinylated anti-Thy-1.2 followed by tricolor streptavidin. Thy-1.2$^+$ B220$^+$ Ly-9.1$^+$ cells (ES-DK207-derived) (Fig. 5*D*) were treated similarly except that FITC-anti-IgM^b was omitted. Purified anti-Fc$_\gamma$ receptor II (Pharmingen) was added to reduce nonspecific staining.

Detection of Serum IgM. Serum IgM^a was captured by using plastic-immobilized anti-IgM^a mAb and detected with biotinylated-anti-mouse IgM mAb followed by streptavidin-alkaline phosphatase (Pharmingen) and *p*-nitrophenyl phosphate. A_{405} was measured with a UVmax spectrophotometer (Molecular Dynamics).

RESULTS

Generation and Characterization of Heterozygous and Homozygous ΔJ$_H$-Mutant ES Cells. E14TG2a ES cells were electroporated with the linearized targeting vector pmHΔJ. Of 650 G418-resistant colonies screened by PCR, 5 produced the expected 1407-bp PCR signal (data not shown). J_H targeting was confirmed by Southern blot analysis. *Sac* I-digested DNA from the parent line showed the two expected fragments (4038 and 917 bp) hybridizing to probe *a* (Figs. 1 and 2*A*). The 5 targeted ES cell clones showed the pattern expected for one native and one targeted allele: a 4038-bp fragment of decreased intensity compared with the parent line, a 917-bp fragment, and an additional 3467-bp fragment (Figs. 1 and 2*A*). Rehybridization with the *neo* probe (*b*) showed that only the 3467-bp fragment contained *neo* sequences and demonstrated the absence of additional random integration events. Deletion of the J_H region from the targeted allele was confirmed with probe *c*, which detected only the 4038-bp fragment. These conclusions were confirmed by analysis of *Hin*dIII-digested DNA (data not shown).

The effect of a recessive genetic mutation such as ΔJ_H can be evaluated in mice homozygous for the mutation. However, evaluation of the effect of ΔJ_H on Ig expression and B-cell development can be accelerated by ΔJ_H-homozygous ES cells, which can be used to produce chimeric mice containing a population of ES-derived lymphoid cells homozygous for the mutant allele. We generated ΔJ_H-homozygous ES cells by subjecting the ΔJ_H-heterozygous ES 110-1 cell line to elevated levels of G418 (1400 μg/ml), using a previously described approach (12). Southern blot analysis of seven of the surviving colonies indicated that one, ES DK207, had lost the native heavy-chain allele and acquired a second, ΔJ_H-targeted allele, as evidenced by the inability of probes *a* and *c* to detect the wild-type 4038-bp fragment (Fig. 2*B*). Karyotypic analysis of ES DK207 (14) indicated that, like the parent line ES 110-1, about 80% of the cells analyzed had 40 chromosomes (data not shown), suggesting that two targeted Igh alleles were present. The heterozygous and homozygous mutant ES cell lines were microinjected into C57BL/6J blastocysts and chimeric mice were generated.

Analysis of Heterozygous and Homozygous ΔJ$_H$-Mutant B Cells in Chimeric Mice. B cells were evaluated in chimeras

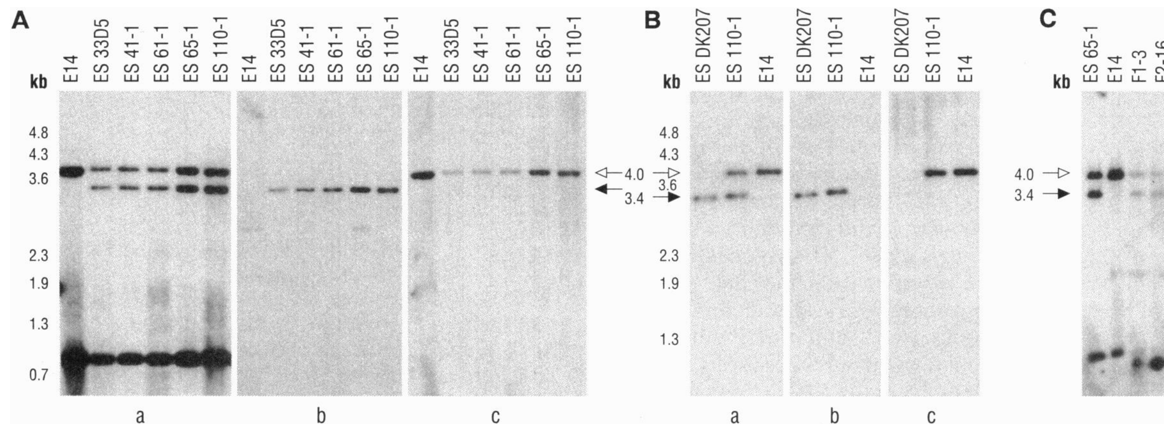

FIG. 2. Southern blot analysis of ΔJ_H-heterozygous and -homozygous mutant ES cell lines and of ΔJ_H-heterozygous mice. Genomic DNAs were digested with *Sac* I. Blots were hybridized successively with probes *a*, *b*, and *c*, as indicated below each autoradiograph. (*A*) Untransfected E14TG2a ("E14") and the five PCR-positive ES clones: ES 33D5, ES 41-1, ES 61-1, ES 65-1, and ES 110-1. (*B*) ΔJ_H-homozygous ES DK207, ΔJ_H-heterozygous ES 110-1, and E14TG2a. (*C*) Tail DNA from offspring derived from mating of the ES 65-1-derived chimeric female and a C57BL/6J male; probe a was used. The faint 3-kb band detected with probe *b* in E14TG2a and ES 65-1 in *A* corresponds to a *neo* gene band in residual feeder cells in these DNA preparations.

that had been generated from wild-type E14TG2a ES cells or from ES cell lines that are heterozygous (ES 110-1 or ES 65-1) or homozygous (ES DK207) at the targeted J_H region. Mice with similar degrees of chimerism, as judged by coat color, were compared. ES cell (129 strain)- and blastocyst (C57BL/6-B6)-derived B and T lymphocytes could be distinguished with a mAb to the 129-specific Ly-9.1 cell surface marker (15). In addition B cells of ES cell (IgM^a) and blastocyst (IgM^b) origin could be distinguished with IgM allotype-specific reagents. PBMCs were stained for two-color analysis with antibodies to the B-cell-specific marker B220 (16) or anti-Thy-1.2 (T cells) and with antibodies to either Ly-9.1 or IgM allotypes. As shown in Fig. 3*A a–d*, ES-derived B cells ($B220^+$ IgM^{a+} or $B220^+$ Ly-9.1^+) and T cells (Ly-9.1^+ Thy-1.2^+) were detected in the peripheral blood of chimeric mice generated from the wild-type ES cells, confirming the ability of this cell line to give rise to lymphoid cells *in vivo*. Analysis of chimeras generated from ΔJ_H-heterozygous ES 65-1 (Fig. 3*A e–h*) or ES 110-1 (Fig. 4) cells demonstrated the presence of $B220^+$ IgM^{a+} Ly9.1^+ B cells containing a single, intact, ES-derived Igh locus. A comparison of the Ly-9.1^+ B-cell/T-cell ratios in the wild-type (Fig. 3*A a* and *b*) and ΔJ_H-heterozygous (Fig. 3*A e* and *f*) chimeras indicated that deletion of the J_H region from one Igh allele was not detrimental to B-cell development. Similar results were obtained from chimeric spleen (Fig. 4).

In marked contrast to the wild-type and ΔJ_H-heterozygous 65-1 or 110-1 chimeras, mice generated from the ΔJ_H-homozygous ES DK207 cell line lacked Ly-9.1^+ $B220^+$ or IgM^{a+} $B220^+$ B cells in peripheral blood (Fig. 3*A j* and *k*), and virtually all $B220^+$ cells expressed the IgM^b allotype (Fig. 3*A l*). The lack of ES-derived peripheral B cells was not due to a block in lymphopoiesis, since ES-derived, Ly-9.1^+ $B220^-$ cells represented 12% of total PBMCs (Fig. 3*A j*). Of these, half were Thy-1.2^+ T cells (Fig. 3*A i*). Thus, the ES DK207 cell line failed to produce IgM^{a+} peripheral B cells in chimeric mice but gave rise to both Thy-1.2^+ T cells and to an uncharacterized population of Thy-1.2^- $B220^-$ PBMCs (Fig. 3*A i* and *j*). Similar observations were made in ES DK207 chimeric spleens (Fig. 4). Our data indicate, therefore, that deletion of the J_H region from both alleles, but not from one allele, blocks development of mature IgM^a-producing B cells.

The lack of ES-derived peripheral B cells in the DK207 chimeras was corroborated by the absence of serum IgM^a. By ELISA, the IgM^a serum levels were high (detectable at serum dilutions $>2 \times 10^4$) in chimeras derived from the wild-type or the ΔJ_H-heterozygous ES cell lines (Fig. 3*B*), although ES-derived B cells represented as few as 4% of the peripheral B cells (Fig. 4). However, IgM^a was undetectable even at the highest concentration of DK207 serum tested (1:20 dilution), indicating a reduction in IgM^a by at least a factor of 1000 in DK207 chimeras (Fig. 3*B*). Similar levels of IgM^b were detected in the sera of all chimeras tested (data not shown). This represents additional evidence that homozygous deletion of the J_H region completely blocks B-cell maturation and IgM production.

Analysis of Homozygous ΔJ_H-Mutant B-Cell Precursors. To elucidate the stage at which the development of ES DK207-derived B cells is blocked, we analyzed cells from the bone marrow, the primary site of B lymphopoiesis in the adult mouse (17). Like peripheral blood and spleen, the bone marrow of DK207 chimeras lacked ES-derived IgM^{a+} B cells but did contain B6-derived IgM^{b+} B cells (data not shown). However, in contrast to peripheral blood and spleen (Fig. 4 *A* and *E*), DK207 chimeric bone marrow contained a small number (2.6–2.9%) of Ly-9.1^+ $B220^+$ cells (Fig. 4*I*), which was 5- to 10-fold greater than the nonspecific staining observed in B6-derived bone marrow cells (0.2–0.4%). Com-

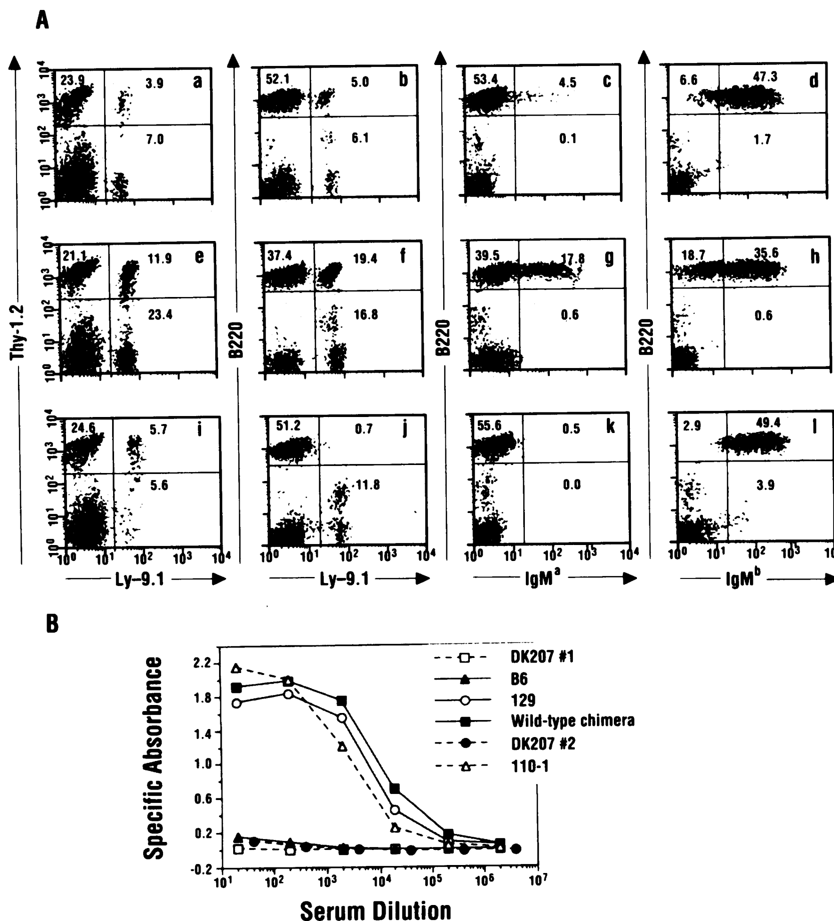

FIG. 3. Lack of ES-derived B cells and serum IgM^a in DK207 chimeric mice. (*A*) PBMCs from chimeric mice stained for expression of B220, Thy-1.2, Ly-9.1, IgM^a, and IgM^b and analyzed by two-color flow cytometry. PBMCs derived from a wild-type chimera (*a–d*), a 65-1 chimera (*e–h*), and a DK207 chimera (*i–l*) were stained for Ly-9.1 and Thy-1.2 (*a*, *e*, and *i*), Ly-9.1 and B220 (*b*, *f*, and *j*), IgM^a and B220 (*c*, *g*, and *k*), and IgM^b and B220 (*d*, *h*, and *l*). Numbers in each quadrant refer to the percent of total cells in that quadrant. In the same experiment, PBMCs from another DK207 chimera were analyzed with similar results. With PBMCs from a control B6 mouse (Ly-9.1^- IgM^{a-}), staining background was determined to be 0.7% $B220^+$ Ly-9.1^+ and 0.7% $B220^+$ IgM^{a+}. (*B*) Serum IgM^a allotype measured by ELISA. Shown are titrations of serum samples from individual mice.

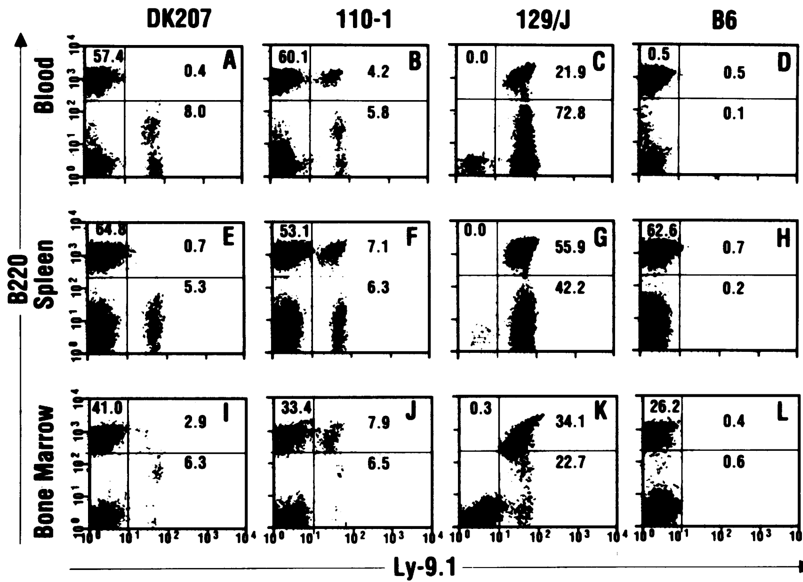

FIG. 4. ES-derived Ly-9.1$^+$ B220$^+$ B-cell precursors found in the bone marrow of chimeric mice derived from the DK207 ES cell line. Ficoll/Hypaque-purified leukocytes from peripheral blood (*A–D*), spleen (*E–H*), and bone marrow (*I–L*) of individual mice [DK207 chimera (*A*, *E*, and *I*), 110-1 chimera (*B*, *F*, and *J*), 129/J control mouse (*C*, *G*, and *K*), and B6 control mouse (*D*, *H*, and *L*)] were stained with mAb reactive with Ly-9.1 and B220 and analyzed by two-color flow cytometry. Numbers in each quadrant indicate percent of total events in that quadrant. Quantitatively similar results were obtained in a separate experiment performed on another set of mice (data not shown).

pared to the equivalent populations in 110-1 or 65-1 chimeric mice (Fig. 4*J*), in DK207 chimeric mice there was a clear deficit in the brightest Ly-9.1$^+$ B220$^+$ cells, which are mostly IgM$^+$ (ref. 7 and data not shown). This suggested that DK207-derived B220$^+$ bone marrow cells mainly consisted of Ly-9.1$^+$ B220$^+$ IgM^{a-} B-cell precursors, whose maturation to IgM^{a+} B cells was blocked by homozygous deletion of J_H.

To identify these Ly-9.1$^+$ B220$^+$ bone marrow cells as B-cell precursors and determine at what stage of development these precursors were arrested, three-color flow cytometry was performed with antibodies to cell surface markers that define early B-cell precursors (17, 18): CD43 (18) and Thy-1.2 (19) in conjunction with the Ly-9.1 marker. In addition, by excluding from analysis IgM^{b+} B cells, we analyzed only B6-derived B-cell precursors (*Materials and Methods*). As previously reported (7, 20), these precursor cells expressed lower levels of B220 (B220^{dull}) than mature peripheral B cells. Likewise, most Ly-9.1$^+$ B220$^+$ DK207-derived cells were B220^{dull} (Fig. 5). However, we did observe a small number of Ly-9.1$^+$ B220^{bright} cells (Fig. 5), but these were not studied further.

Consistent with previous reports (18), B220^{dull} IgM$^-$ bone marrow in control B6 and 129 mice contained both CD43^{bright} and CD43^{dull} cells (data not shown). Both in control mice (B6, 129) and in the B6-derived pool from chimeric bone marrow, 70 ± 17% (mean ± SD, n = 7; range, 53–87%) of B220^{dull} bone marrow cells were CD43^{dull} (Fig. 5). However, in the two DK207 chimeric mice analyzed, only 7–18% of B220^{dull} Ly-9.1$^+$ bone marrow cells were CD43^{dull} (Fig. 5*B*). The paucity of CD43^{dull} cells in DK207 bone marrow is consistent with an early block in maturation (18) due to deletion of J_H from both alleles.

Like CD43, Thy-1.2 is expressed by B220$^+$ early pro-B cells but is lost as these cells mature, so that pre-B cells (cytoplasmic IgM$^+$, surface IgM$^-$) are Thy-1.2$^-$ (19). From the results obtained for CD43 expression we expected that the majority of DK207-derived B-cell precursors would display Thy-1.2 antigen and few cells would be Thy-1.2$^-$. Indeed, only 8–18% of the Ly-9.1$^+$ B220^{dull}, DK207-derived bone marrow cells were Thy-1.2$^-$ (Fig. 5*D*), consistent with an early block in maturation. We also noted heterogeneous expression of Thy-1.2 by DK207-derived Ly-9.1$^+$ B220^{dull} cells, with the brightest Thy-1.2$^+$ cells expressing the lowest levels of B220. It remains a possibility that the Thy-1.2^{bright} cells were mature T lymphocytes whereas the Thy-1.2^{dull} population contained the developmentally arrested B-cell precursors. Finally, another indication of the immaturity of ES DK207-derived B220^{dull} B-cell progenitors was their increased average size compared with B6-derived B220^{dull} IgM^{b-} cells (not shown).

In summary, in ES DK207 chimeric mice, it appears that due to homozygous deletion of J_H, development of the ES-derived B lineage is arrested at a stage characterized by relatively large cell size and a B220^{dull} IgM$^-$ CD43$^+$ Thy-1.2$^+$ cell surface phenotype, similar to that described for mutant mice (3–5) and for early precursor populations in normal mice (18).

Analysis of B Cells in Homozygous ΔJ_H-Mutant Mice. Chimeric mice produced from ΔJ_H-heterozygous mutant ES 65-1 cell line were mated with B6 mice to obtain mice heterozygous in their germ line for ΔJ_H, as demonstrated by Southern blot analysis (Fig. 2*C*) and by flow cytometry using allotype-specific mAb (data not shown). Heterozygotes were

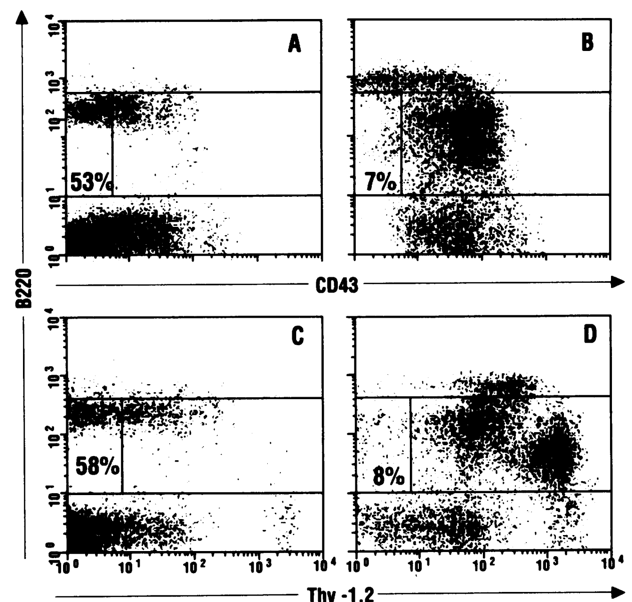

FIG. 5. Expression of CD43 and Thy-1.2 by the majority of ES-derived, B220^{dull} B-lineage cells in bone marrow of DK207 mice. CD43 (*A* and *B*) or Thy-1.2 (*C* and *D*) and B220 staining are shown for subpopulations of bone marrow cells: Ly-9.1$^-$ IgM^{b-} B6-derived cells (*A* and *C*) and Ly-9.1$^+$ ES DK207-derived cells (*B* and *D*). Upper and lower horizontal lines define B220^{dull} cells. The percentage of B220^{dull} cells that express low or negligible levels of CD43 or Thy-1.2 is indicated in each panel.

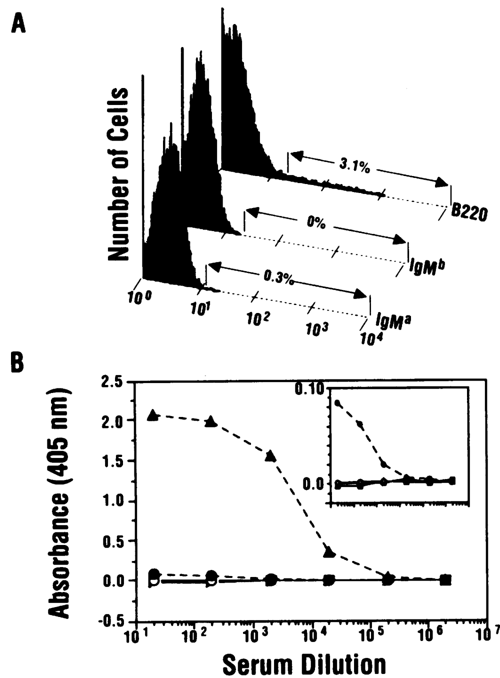

FIG. 6. Absence of peripheral blood B cells and IgM in ΔJ_H-homozygous mice. Peripheral blood was obtained from mice derived from intercrossing ΔJ_H-heterozygous mice. (*A*) PBMCs were isolated and stained with fluorochrome-conjugated antibodies to IgM^a, IgM^b, or B220 and analyzed by flow cytometry. Results are shown for one representative 4-week-old mouse, and the percent stained cells is indicated for each profile. For PBMCs from five additional 3- to 5-week-old ΔJ_H-homozygous mice, 1.8 ± 1.0% were $B220^+$. In control mice, IgM^{a+}, IgM^{b+}, and $B220^+$ cells represented 32%, 46%, and 53% of PBMCs, respectively. (*B*) Serum obtained from B-cell-deficient mice was assayed by ELISA for IgM^a. △, □, and ○, Serum from three individual B-cell-deficient ΔJ_H-homozygous mice; ●, IgM^b (B6) negative control serum; ▲, IgM^a (BALB/c) positive control serum. (*Inset*) Expansion of the vertical scale between 0.00 and 0.10 absorbance unit, showing low-level reactivity of the anti-IgM^a ELISA capture antibody with IgM^b allotype control serum and the complete lack of detectable IgM^a in sera from the three B cell-deficient mice.

intercrossed to obtain ΔJ_H-homozygous mutants (confirmed by Southern blot analysis; data not shown). The homozygous mutants appeared totally, and specifically, B-cell-deficient, as PBMCs did not express B220, IgM^a, or IgM^b (Fig. 6*A*) but did express Thy-1.2 (data not shown). In addition, serum obtained from B-cell-deficient mice lacked IgM (Fig. 6*B*). These results confirm the findings obtained from the DK207 chimeras, thus validating our evaluation of the effect of ΔJ_H in ES-derived homozygous mutant B cells.

DISCUSSION

Our report introduces an application of homozygous mutant ES cells for determining the mutant phenotype *in vivo* in chimeric mice. This approach can accelerate evaluation of the effect of site-directed mutations and provides a strategy to study the phenotype of potentially lethal mutations.

Using both chimeric and germ-line mutant mice, we have shown that homozygous deletion of J_H results in complete inhibition of antibody production and arrest of B-cell development at an early stage, thus providing additional evidence that B-cell maturation is linked to successful D–J_H rearrangement. The similarity between the phenotype of the B-cell precursors in DK207 ΔJ_H-homozygous chimeric mice and the SCID (*scid/scid*) and RAG-deficient mice (3–5) suggests that homozygous deletion of J_H region indeed leads to arrest of the B-lineage differentiation at an early stage in which V(D)J recombination is initiated. In the DK207 chimeric mice the vast majority of ES-derived, J_H-deleted B-cell precursors exhibited a phenotype characteristic of pro-B cells, which are large, $B220^+$ Thy-1.2^+ $CD43^+$ IgM^- cells, and very few cells resembled pre-B cells, which are small, $B220^+$ Thy-1.2^- $CD43^-$ IgM^- cells. The failure of these mutant IgM^- pro-B cells to mature to IgM^- pre-B cells suggests that heavy chain normally plays a critical role in this maturation. Our findings thus support the concept that membrane-anchored heavy chain complexed with surrogate light chains (7, 17) is critical to this developmental transition.

Although in phenotype the ΔJ_H mouse resembles other gene-targeted B-cell-deficient mice (4, 5, 7), the ΔJ_H mouse is uniquely suited for reconstitution with unrearranged Igh transgenes. Unlike the μMT mouse (7), Igh is not expressed in ΔJ_H mice, and due to the cis nature of ΔJ_H, it should be possible to reconstitute the B lineage with unrearranged autologous or heterologous Igh transgenes. Thus it is now possible to produce transgenic mice that, upon immunization, should produce antibody heavy chains from other species in the absence of endogenous mouse heavy chains. Finally, ΔJ_H mice represent a useful model for study of specific B-cell deficiency, of the role of antibodies in the immune response, and of T-cell/B-cell interactions.

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