

Selective Expression of the Cre Recombinase in Late-Stage Thymocytes Using the Distal Promoter of the *Lck* Gene¹

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Transgenic mouse lines were generated that express the Cre recombinase under the control of the distal promoter of the mouse *Lck* gene. Cre recombination in four of these lines of transgenic mice was characterized at the single cell level using ROSA26-regulated *loxP*-Stop-*loxP*- β geo and *loxP*-Stop-*loxP*-YFP reporter mouse lines. Two of the lines showed T cell-restricted Cre recombination, whereas the other two also expressed Cre in B cells, NK cells, and monocytes. Cre recombination began at a late stage of T cell development (at or after up-regulation of the TCR during positive selection) in the two T cell-restricted lines. Lines of mice that express the Cre recombinase at late stages of thymocyte development are of value for determining the impact of mutations on T cell function in the absence of complicating effects on early thymocyte selection. *The Journal of Immunology*, 2005, 174: 6725–6731.

The development of T lymphocytes is a well described multistage process. It begins with the seeding of the thymus by hemopoietic progenitor cells, and ends with the emergence of naive postthymic cells that can be activated by Ags to become effector and memory T cells (1, 2). While resident in the thymus, immature cells undergo successive episodes of V(D)J rearrangement that allow for surface expression of precursor, $\alpha\beta$ or $\gamma\delta$ TCRs. Signaling from these receptors is mediated by common signaling molecules such as the CD3 and TCR ζ chains, the Syk/ZAP-70 and Fyn/Lck protein tyrosine kinases, enzymes in the phosphatidylinositol and calcium pathways, downstream adaptors such as SLP-76, MAPK signaling relays, and transcription factors that include NFAT proteins and NF- κ B family members (3).

Although the signals delivered by T cell receptors through the above molecules are essential for immune responses, they are also critical for passage through several genetically defined developmental checkpoints (1). Furthermore, it is becoming increasingly clear that TCR signaling helps regulate the postdevelopmental proliferation and survival of mature T cells in the absence of infection (4–11). Thus, mutations that interfere with TCR function at any level have the potential to change the outcome of thymocyte development from an early stage and/or to alter peripheral T cell homeostasis. Systems that would allow for mutagenesis of T cells in a stage-specific fashion are therefore important for resolving the postdevelopmental functions of genes such as those that encode

core TCR signaling proteins, or other proteins that are important at multiple stages in T cell development.

One means to accomplish stage-selective mutagenesis of T cells involves the use of mice that express the Cre recombinase under the control of T cell-specific promoters (12, 13). Here, we describe a series of such transgenic mice that were generated using a *cre* transgene regulated by the distal promoter of the mouse *Lck* gene (14, 15). The four lines of transgenic mice we have characterized in detail show patterns of recombination that differ in their onset of expression and the range of cell types they affect. We show that two of these lines of mice have features that make them useful additions to the range of tools available for somatic mutagenesis of lymphocytes.

Materials and Methods

Mice and genotyping

dLck-icre transgenic mice were generated by pronuclear microinjection of DNA into C57BL/6xDBA/2 F2 eggs. The transgene was generated by insertion of a codon-optimized form of the *cre* open reading frame (referred to as *icre*) into a distal *Lck* promoter expression vector (14, 15). Transgenic founders were screened by Southern blot analysis, bred to mice carrying a conditional *Cd4* allele (*Cd4^{lox}* (16)) and either intercrossed, or backcrossed to *Cd4^{-/-}* mice to generate *Cd4^{lox/lox}* or *Cd4^{lox/-} dLck-icre* mice. ROSA26-*loxP*-Stop-*loxP*- β geo reporter mice (17) were obtained from Dr. S. H. Orkin (Harvard Medical School, Cambridge, MA) via Dr. R. Andino (UCSF, San Francisco, CA). ROSA26-*loxP*-Stop-*loxP*-YFP mice (18) were kindly provided by Dr. F. Costantini (Columbia University, New York, NY). All mice were housed in the Parnassus Heights pathogen-free barrier facility at University of California, San Francisco. *dLck-icre* transgenic mice and ROSA26-*loxP*-Stop-*loxP*- β geo reporter mice were typed by Southern blot and/or PCR using *icre* (5'-ATGGTGCCCAAGAAGAA-GAG and 5'-CAGGTGCTGTTGGATGGTCT) or *puro* (5'-GTCAC-CGAGCTGCAAGAACT and 5'-CGGGTCATGCACCAAGTG) primers, respectively. ROSA26-*loxP*-Stop-*loxP*-YFP mice were typed by multiplex PCR using primers originally designed in the laboratory of Dr. P. Soriano (Fred Hutchinson Cancer Research Center, Seattle, WA) (5'-GCGAAGAGTTTGTCTCTCAACC-3', 5'-GGAGCGGGAGAAATGGATATG-3', and 5'-AAAGTCGCTCTGAGTTGTTAT-3').

Recombined and unrecombined alleles of the ROSA26-*loxP*-Stop-*loxP*-YFP allele were detected in genomic DNA by multiplex PCR using the following three primers: 5'-AAGGGAGCTGCAGTG GAGTA-3' (UPS); 5'-GCCAGAGGCCACTTGTGTAG-3' (PGK); 5'-TGGTGCAGATGAACCTCAGG-3' (YFP).

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Flow cytometry

FITC-, PE-, or TC-conjugated Abs were purchased from CalTag Laboratories or BD Pharmingen. The fluorescent β -galactosidase substrate fluorescein digalactopyranoside (FDG)³ was obtained from Molecular Probes. Bone marrow cells (0.3×10^6), lymphocytes, or thymocytes were incubated for 30 min on ice in a volume of 25 μ l of FACS buffer (PBS with 0.3% BSA and 0.01% NaN_3) containing Abs at saturating concentrations. The cells were washed twice in FACS buffer, stained with secondary reagents as necessary, washed again, and then analyzed using a FACScan flow cytometer (BD Biosciences) and CellQuest or Flowjo software.

To detect β geo activity by flow cytometry, 3×10^6 cells were washed once with PBS, then resuspended in 60 μ l of PBS. FDG was diluted in water to 7.5 mM from a 100 mM stock solution. The cells and FDG were warmed to 37°C for 5 min then 40 μ l of FDG were added to 60 μ l of cells. The mixture was incubated at 37°C for 5 min before adding 1 ml of ice-cold PBS. The cells were placed on ice for 5 min, then transferred to a 15°C water bath for 15 to 30 min. Finally, the cells were pelleted by centrifugation, resuspended in FACS buffer, and stained with fluorescent Abs before FACS analysis.

To detect Cre in permeabilized cells by FACS, $2\text{--}5 \times 10^6$ cells were stained with appropriate antibodies, washed once in PBS, then resuspended in 1 ml of PBS. The cells were fixed by the addition of 1 ml of 4% paraformaldehyde in PBS and incubation at room temperature for 20 min. After washing twice with PBS, the fixed cells were resuspended in 50 μ l of saponin buffer (0.5% saponin in PBS with 0.3% BSA and 0.01% NaN_3 ; Sigma-Aldrich) containing 7% normal goat serum (Jackson ImmunoResearch Laboratories) and incubated at room temperature for 15 min. Anti-Cre mAb 7.23 (50 μ g/ml; Covance Research Products) was then added to the permeabilized cells, and they were incubated at room temperature for another 20 min. The cells were washed twice with saponin buffer and stained with FITC-goat-anti-mouse IgG (BD Pharmingen) in saponin buffer for 20 min at room temperature. Finally, the cells were washed twice in saponin buffer and resuspended in FACS buffer for analysis using the FACScan.

Detection of Cre by immunoprecipitation and Western blot

Either 50×10^6 thymocytes or lymphocytes were lysed in 250 μ l of buffer containing 20 mM Tris-HCl, 137 mM NaCl, 2 mM EDTA, 1 mM PMSF, 10 μ g/ml leupeptin, 10 μ g/ml aprotinin, and 1% Nonidet P-40. Insoluble material was removed from the lysates by centrifugation. After preclearing with 25 μ l of packed goat anti-mouse IgG agarose beads (Sigma-Aldrich), the Cre protein in the lysates was immunoprecipitated using 2.5 μ l of the anti-Cre mAb 7.23 (1 mg/ml; Covance Research Products) and 25 μ l of fresh beads. The immunoprecipitates were then fractionated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore) and blotted with a rabbit anti-Cre polyclonal Ab B4197 (gift of Drs. U. Muller and L. Reichardt, UCSF) using an HRP-conjugated donkey anti-rabbit Ig anti-serum (Amersham Biosciences) and ECL detection reagents (Amersham Biosciences).

Supernatants from the above Cre immunoprecipitations were re-cleared with 25 μ l of protein A-agarose beads (Invitrogen Life Technologies), and then re-immunoprecipitated with 2.5 μ l of anti-ZAP-70 polyclonal Ab R1600 (gift of Dr. A. Weiss, UCSF) and 25 μ l of fresh protein A-agarose beads. After SDS-PAGE and transfer to Immobilon-P membranes, the immunoprecipitated ZAP-70 protein was detected by blotting with R1600, HRP-conjugated donkey anti-rabbit Ig, and ECL reagents.

Results

In an attempt to direct expression of the Cre recombinase to late stages of T cell development, we generated a *cre* transgene regulated by the distal promoter of the mouse *Lck* gene (14, 15) (Fig. 1A). This construct was used to make fourteen transgenic founders that were test mated to mice carrying a *loxP*-containing conditional allele of the *Cd4* gene (16). The pattern and extent of Cre recombination in these mice was then assessed by Southern blot using a *Cd4* probe on DNA taken from their tails, spleens, lymph nodes, and thymuses. As shown in Fig. 1B, a spectrum of effects was observed: in some cases (e.g., lines 4423, 4425, and 3780), there was evidence of extensive recombination in the thymus and in secondary lymphoid tissue; in other cases (e.g., lines 3800 and

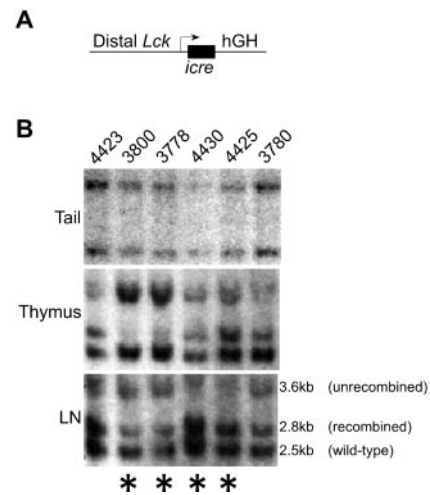


FIGURE 1. A, Design of the *dLck-icre* transgene. A codon-optimized *cre* open reading frame (*icre*) was placed downstream of the distal *Lck* promoter. Human growth hormone (hGH) exons in the construct allow for splicing to improve transgene expression (24, 25) and also to provide a transcriptional terminator. B, Representative Southern blot assay for recombination at the *Cd4* locus in *dLck-icre* transgenic lines. DNA was prepared from the tails, thymuses, and lymph nodes of *dLck-icre* transgenic *Cd4^{lox/+}*, digested with *SacI* and hybridized with a probe from the first intron of the *Cd4* gene, as described (16). The asterisks identify some of the lines that were selected for further breeding based on this assay.

3778), recombined alleles were infrequent in the thymus but clearly detectable in the lymph nodes or spleens. Seven of the lines were selected for further breeding based on this preliminary screen. One of the lines was later shown to contain two transgene insertion sites that segregated independently (data not shown), and these were maintained as independent stocks (3778 and 3779). Of the six other lines, we eventually focused on two (4430 and 3785) that demonstrated a high level of recombination in T cells by the assays described below.

In addition to the Southern blot assay just mentioned, we also followed Cre expression in the lines of mice by four other procedures. One was to monitor loss of CD4 expression on T cells in mice that had two copies of a conditional null *Cd4* allele, or one copy of the conditional allele balanced by a null allele (16). Although these experiments provided useful data that were entirely consistent with other assays (data not shown), the loss of CD4 expression was evident only in cells that normally would have expressed CD4. Moreover, the loss of CD4 also changed the outcome of thymocyte development and peripheral T cell homeostasis (16) limiting the value of this allele for detailed analysis of Cre expression patterns. We therefore crossed the *cre* transgenic lines to mice carrying either a *loxP*-Stop-*loxP*- β geo or a *loxP*-Stop-*loxP*-YFP element inserted into the ROSA26 locus (17, 18) in search of a more general means of assessing when the Cre recombinase was expressed. Cre recombination at these ROSA26 reporter alleles causes excision of a *loxP*-flanked disruption (the "Stop" element) and allows for β geo or YFP to be produced under the control of the broadly active ROSA26 *cis*-acting sequences (19). β geo- or YFP-expressing cells in the *cre* transgenic reporter mice could then be identified by FACS analysis (using FDG as a fluorescent substrate in the case of β geo) (20). Cre expression was also monitored directly in the transgenic mice by immunoprecipitation and immunoblotting using Cre-specific monoclonal and polyclonal Abs, and by intracellular FACS using a Cre-specific mAb (21).

³ Abbreviations used in this paper: FDG, fluorescein digalactopyranoside; HSA, heat-stable Ag.

Of the various techniques we used, FACS analysis of cells from the *cre* transgenic ROSA26 reporter mice was the most informative because it gave clear single-cell resolution concerning the onset of Cre activity. Western blot analysis suggested that there was enhanced expression of Cre in the lymph nodes vs thymuses of lines 4430 and 3778 (data not shown), but the magnitude of this effect was difficult to judge, and the experiments we performed did not involve purification of the different cell types present in peripheral lymphoid tissue. In-

tracellular FACS analysis for Cre showed clear recombinase expression in several lines and allowed for limited single-cell discriminations to be made (data not shown). However, the sensitivity of this assay was not high, and by contrast, the Cre-recombined ROSA26 reporter loci reproducibly gave much stronger fluorescent signals in all of the cell types we examined (Figs. 2–4).

Thymocyte populations from the four *cre* transgenic/reporter mouse lines were analyzed by multicolor flow cytometry as shown

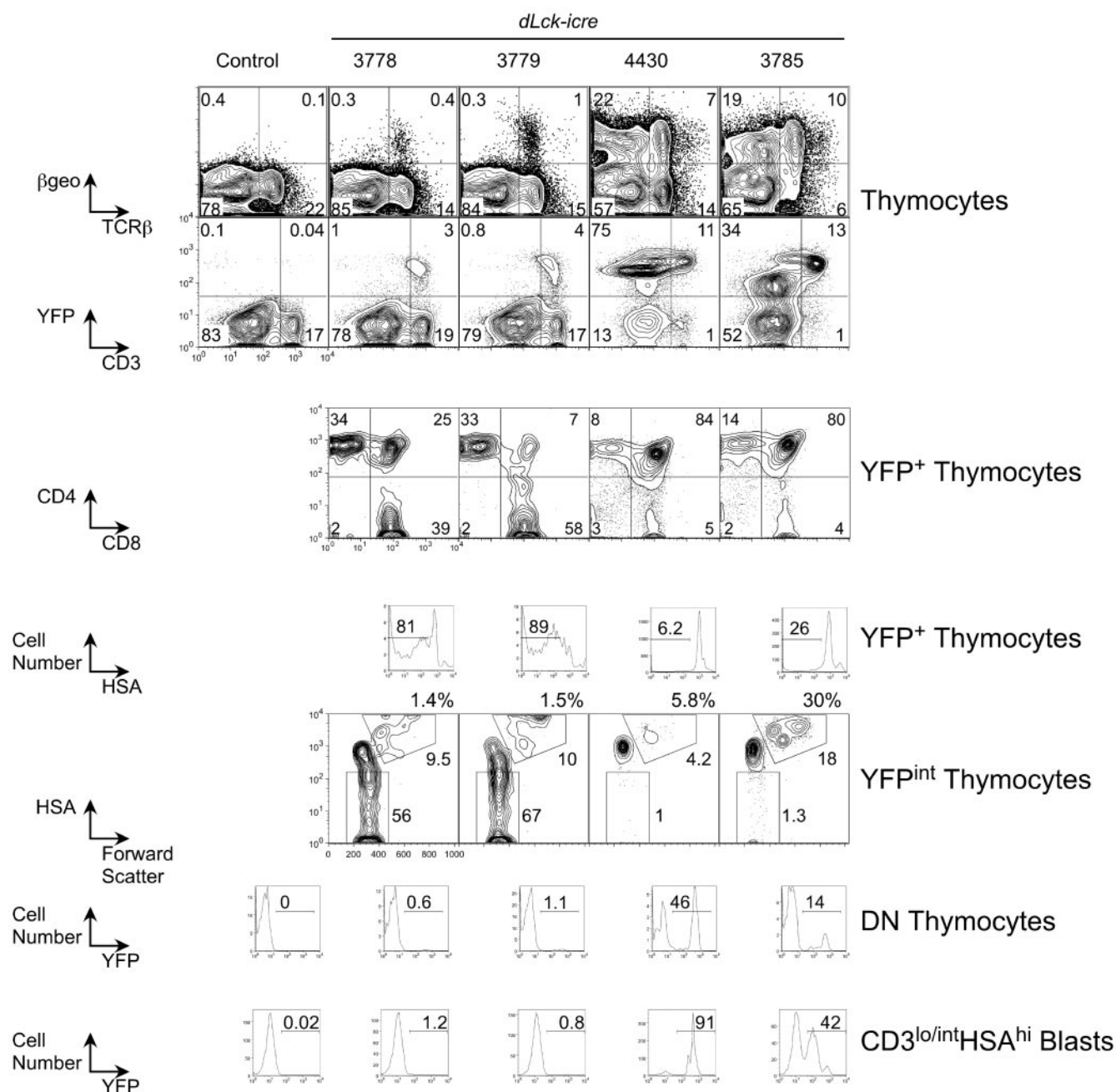


FIGURE 2. Flow cytometric analysis of β geo or YFP expression in thymocytes from *dLck-icre* transgenic mice carrying the ROSA26-*loxP*-Stop-*loxP*- β geo or ROSA26-*loxP*-Stop-*loxP*-YFP reporters, respectively. β geo expression was detected by loading cells with FDG as described in *Materials and Methods*. In the *top panel* (labeled “Thymocytes”), cells from the two types of reporter mice were costained with an Ab specific for the β chain of the TCR or an Ab specific for CD3 ϵ to identify cells at different stages of development (cell surface expression of TCR β /CD3 increases with the developmental maturity of thymocytes). The panel labeled “YFP⁺ Thymocytes” shows the frequencies of HSA⁺ cells in the YFP⁺ populations in the different lines of *icre* mice (HSA expression decreases with thymocyte developmental maturity). The panel labeled “YFP^{int} Thymocytes” shows the frequencies of HSA^{high} blasts and HSA^{low} small cells that express intermediate levels of the YFP reporter; intermediate expression of the YFP reporter identifies cells that have recently undergone Cre recombination. The frequencies of YFP^{int} thymocytes in the different thymuses are shown at the top of the contour plots (i.e., 1.4%, 1.5%, 5.8%, and 30% for lines 3778, 3779, 4430, and 3785, respectively). YFP expression in CD4⁺CD8⁺ thymocytes is shown in the panel labeled “DN Thymocytes,” and similarly, YFP expression in CD3^{lo/int}HSA^{high} blasts is shown in the panel labeled accordingly.

in Fig. 2. In general, the same qualitative conclusions could be drawn using data from the analysis of either the β geo or YFP reporter lines. The YFP data were found to be more reproducible because in contrast to the β geo mice, the generation of the fluorescent signal in cells from these mice was not dependent on the sometimes-variable penetration of the FDG substrate into cells.

Line 4430 showed recombination of the reporter alleles from an early stage of thymocyte development (in $CD4^-CD8^-$ thymocytes), and reporter expression remained high in all subsequent stages of development. Recombination appeared to occur at a later time point in line 3785 because a lower fraction of $CD4^-CD8^-$ cells in mice of this line expressed YFP. Moreover, there were more cells expressing intermediate levels of YFP in line 3785 than in line 4430 (see “YFP^{int} thymocytes” in Fig. 2). Intermediate expression of YFP in mice such as these identifies cells that have recently undergone recombination at the reporter locus. This is apparent because as shown in line 4430, cells at all stages of thymocyte development have the capacity to express YFP at similar levels when the allele is recombined at an early stage of development. Of the thymocytes of line 3785, 30% expressed intermediate levels of YFP, and all of these expressed low/intermediate levels of CD3 on their cell surfaces. Approximately one-fifth of the YFP^{int} cells also showed high forward scatter indicating that they were actively proliferating. These characteristics indicated that the onset of recombination in the thymuses of line 3785 mice was primarily during the expansion of clones of TCR β -expressing cells after deliverance of the pre-TCR signal. In contrast, the frequency of YFP^{int} cells was 5-fold lower in line 4430 than in line 3785 and a lower fraction of these recently recombined cells were blasts (4% compared with 18%). Thus, recombination occurred primarily before the pre-TCR signal in line 4430 and after it in line 3785. We have not performed a more detailed analysis to identify the precise stage of development at which recombination begins in line 4430,

and it remains possible that some (if not all) of the recombination occurs before entry into the thymus.

Lines 3778 and 3779 showed similar patterns of recombination. In these mice, β geo or YFP expression could only be detected in thymocytes that expressed high levels of the TCR, the majority of which were $CD4^+$ or $CD8^+$ single-positive cells (Fig. 2). These reporter-expressing cells were enriched for low expression of the heat-stable Ag (HSA or CD24), again consistent with them being mostly mature single-positive cells. As would be expected, YFP^{int} cells in lines 3778 or 3779 expressed much less HSA compared with the YFP^{int} cells from lines 4430 or 3785. Double-negative cells, or HSA^{high} CD3^{low/int} blasts were negative for YFP expression. Cumulatively, these data indicated that recombination in lines 3778 and 3779 occurred at a late stage in T cell development after acquisition of the TCR α chain and expression of high levels of cell surface CD3 following positive selection.

Of the four lines we examined in detail, peripheral T cells from lines 3785 and 4430 showed the highest proportions of YFP⁺ cells (Fig. 3). Line 3779 was next with recombination being more penetrant across the $CD8^+$ population of cells than the $CD4^+$ population ($\sim 90\%$ vs $\sim 75\%$ YFP⁺ cells, respectively). Consistent with the thymic data (Fig. 2 and data not shown) line 3778 showed the lowest frequencies of recombined alleles in its peripheral T cell populations.

Although *Lck* is a T cell-restricted gene, line 4430 showed clear evidence of recombination in almost all $CD4^-CD8^-$ lymph node cells using either the β geo or the YFP reporters (data not shown). We therefore examined reporter expression in non-T cells (specifically NK cells, B cells, and monocytes) in all four lines of mice. Whereas recombination in lines 3778 and 3779 was specific to T cells, lines 4430 and 3785 exhibited a more promiscuous pattern of recombination (Fig. 4A). Both NK cells and B cells were mutated with high efficiency in line 4430, whereas line 3785 allowed for

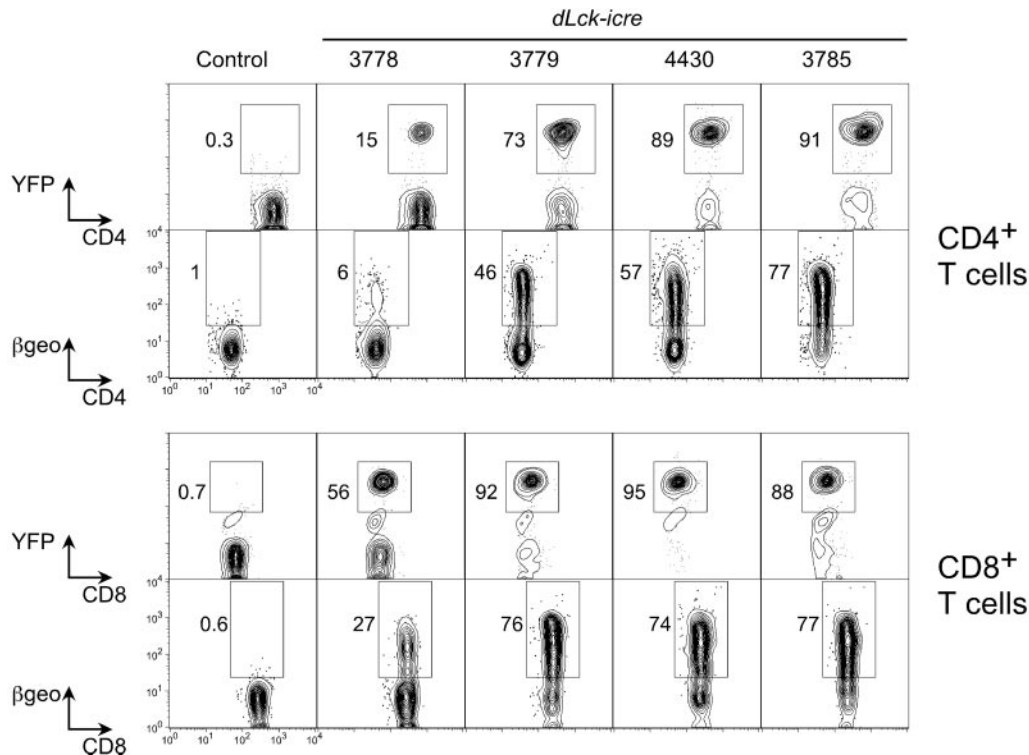


FIGURE 3. Flow cytometric analysis of β geo or YFP expression in T cells from *dLck-icre* transgenic mice carrying the ROSA26-*loxP*-Stop-*loxP*- β geo or ROSA26-*loxP*-Stop-*loxP*-YFP reporters, respectively. Frequencies of reporter-expressing $CD4^+$ or $CD8^+$ lymph node T cells are shown in the contour plots of representative *icre*⁺ reporter⁺ mice.

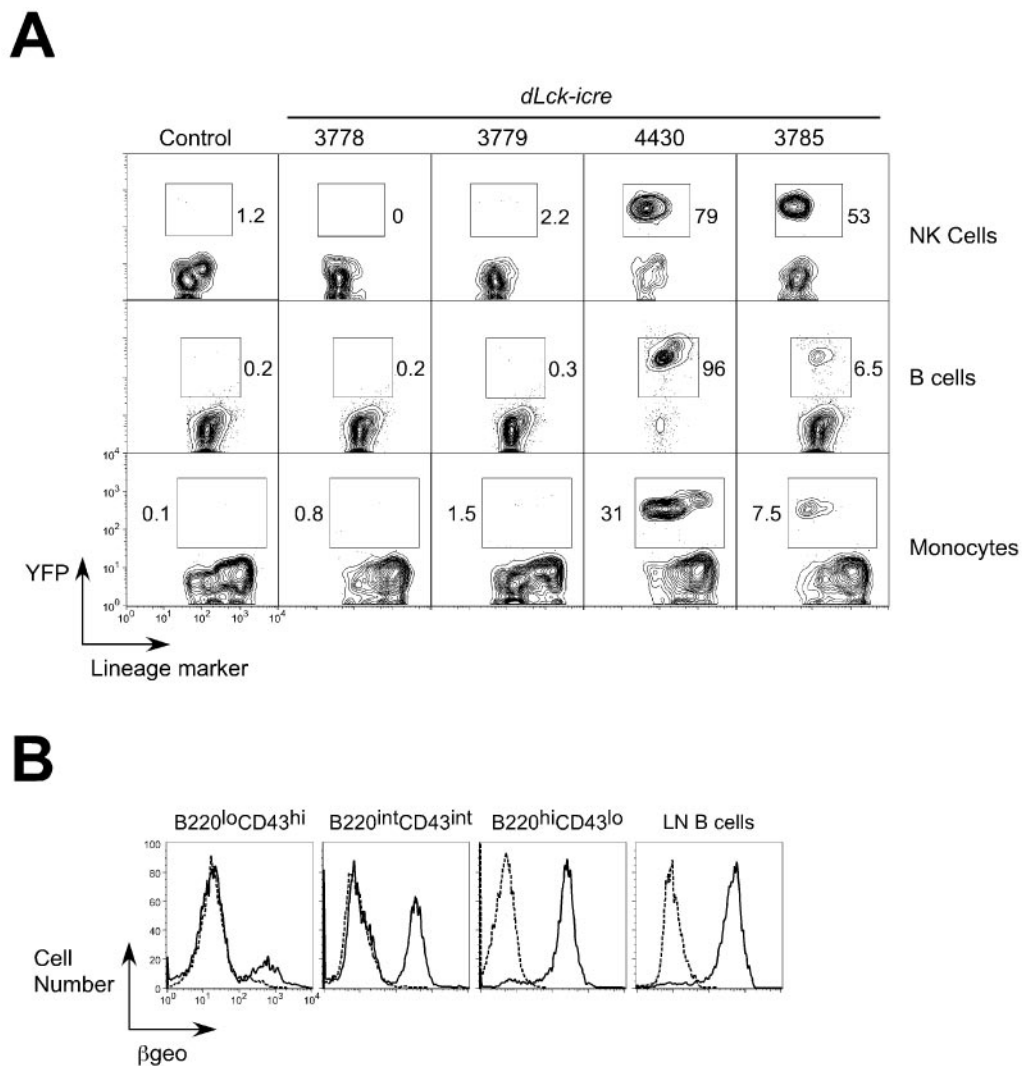


FIGURE 4. Flow cytometric analysis of β geo or YFP expression in non-T cells from *dLck-icre* transgenic mice carrying the ROSA26-*loxP*-Stop-*loxP*- β geo or ROSA26-*loxP*-Stop-*loxP*-YFP reporters, respectively. **A**, The contour plots show expression of YFP in NK cells, B cells, and monocytes from the spleens of the different lines of mice. NK cells were identified by expression of DX5 and absence of CD3. B cells were identified by expression of B220 and monocytes by expression of Mac-1. **B**, The histograms show the frequencies of β geo-expressing B cell populations (solid lines) in the bone marrow and lymph nodes of line 4430. The dashed curves show staining for β geo in cells from an *icre*⁻ littermate. B cell populations in the bone marrow were defined on the basis of varying expression of the B220 and CD43 markers.

good recombination in NK cells but only a minor fraction of B cells became YFP⁺. Approximately one-third and less than one-tenth of monocytes were YFP⁺ in lines 4430 and 3785, respectively.

The high frequency of YFP⁺ B cells evident in line 4430 prompted us to look more carefully at the onset of recombination during B cell development in the bone marrow. As shown in Fig. 4B, the frequency of reporter-expressing cells (in this case β geo-expressing cells) increased in parallel with the developmental maturity of the B cells, rising from ~20% in pre-B cells to close to 100% in peripheral B cells. These data indicate that line 4430 is useful for efficiently inducing Cre recombinase-dependent mutations in B cells as they develop in the bone marrow.

In light of the promiscuous pattern of Cre recombinase expression in hemopoietic cells of lines 4430 and 3785, it was important to look more generally for evidence of recombination in some of the other tissues in the transgenic mice. For this purpose, we isolated genomic DNA from the livers, hearts, kidneys, and brains of mice of the different lines. We then tested the DNA using a PCR assay that could distinguish between recombined and unrecombined alleles of the YFP reporter allele (Fig. 5). As expected, con-

trol DNA lacking the YFP reporter allele did not give a signal using this assay. Tail DNA from a reporter transgenic mouse lacking a *cre* transgene gave one product of ~520 bp as a consequence of amplification between the *UPS* and *PGK* primers. As a positive control, spleen DNA from a 3779/reporter mouse gave two bands, one of ~520 bp from unrecombined alleles in this tissue, and another of ~620 bp from recombined alleles (due to amplification between the *UPS* and *YFP* primers). Whereas lines 3779 and 3778 reproducibly failed to show evidence of significant recombination in these other tissues, kidney and liver DNA from lines 3785 and 4430 both allowed for weak amplification of the ~620-bp product derived from recombined alleles. Further work is required to determine whether the recombined alleles in these tissues are present in cells of hemopoietic or other origins. The hearts and brains of all transgenic mice were negative for recombination using this PCR assay indicating an absence of generalized Cre recombination in the mice.

Discussion

The two most widely used T cell-specific *cre* transgenic lines feature Cre expression controlled by the proximal promoter of the *Lck*

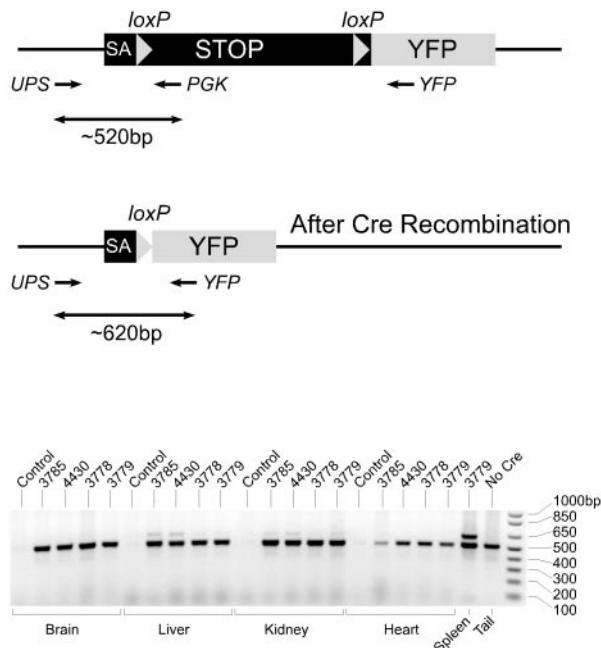


FIGURE 5. Detection of recombined and unrecombined forms of the ROSA26-loxP-Stop-loxP-YFP allele in tissues of *dLck-icre* transgenic mice. The diagram shows the design of the three-primer PCR assay used to detect the two forms of the reporter allele. The *bottom panel* shows a representative image of an ethidium bromide-stained agarose gel in which the PCR products from the indicated tissues of the different lines of mice were separated. The right-most track shows the banding pattern of a DNA sizing ladder.

gene or regulatory elements from the mouse *Cd4* gene (12, 13). In the former, Cre recombinase expression begins at a very early stage of development, whereas in the latter, the recombinase is induced at a later stage: at or just after the pre-TCR signal has been delivered. In both of these lines, as is also true for *hCD2-icre* mice (22), Cre-dependent mutations are induced before positive selection occurs. Line 3785 described here showed a pattern of recombination that was similar to that of *Cd4-cre* in terms of the timing of its onset (13), but it also showed a more promiscuous pattern of recombination than is likely to be the case for *Cd4-cre* (Figs. 4 and 5). For these reasons, breeding of line 3785 was not continued.

In contrast to lines 3785 and 4430, line 3779 showed much delayed onset of Cre expression compared with the existing strains of T cell-restricted *cre* transgenic mice, with there being little evidence of recombination before transition to the TCR^{high} stage of thymocyte development. Line 3778 showed a similar pattern of Cre expression to that of 3779, although fewer cells underwent recombination in these mice presumably because Cre was expressed at lower levels than in line 3779. Only line 3779 was therefore retained for continued breeding.

The frequency of mature T cells that had undergone Cre recombination in line 3779 was high: ~75% in CD4⁺ T cells and >90% in CD8⁺ T cells. Hence, line 3779 allows for highly penetrant but nonetheless incomplete recombination in the T cell lineage. A lack of complete penetrance of recombination is obviously a disadvantage in circumstances where a phenotype (e.g., a defective response to a pathogen) can only be revealed clearly by inducing a mutation in all T cells. However, it can be an advantage in circumstances where single-cell assays allow for mutant and wild-type cells present in the same animal to be distinguished from one another. Chimerism due to a lack of complete penetrance of recombination generates internal control populations against which

mutant cells can be compared while also allowing for selective effects of mutations to be revealed.

The high penetrance of recombination evident in multiple hemopoietic cell types in line 4430 may make this line attractive for some experimental purposes. The efficiency of Cre recombination in the B cell lineage in these mice was equivalent to that previously described for *Cd19-cre* mice (23), but of course line 4430 showed a more promiscuous pattern of recombination. The expression of Cre in NK cells may be a useful and somewhat distinctive attribute of this line, though at this point it is unclear whether recombination occurs in multipotential precursors of NK cells or in the differentiated cells themselves.

In conclusion, by screening a collection of *dLck-icre* transgenic lines, we have identified one in particular (line 3779) that is attractive for mutating *loxP*-modified genes selectively from a late stage in thymocyte development. This line should allow for loss or gain of function effects to be induced after positive selection has occurred: in single positive cells or in peripheral T cells in the absence of complicating effects of the induced mutations on most of thymocyte development. However, it remains possible that this line could induce effects that might compromise negative selection or other medullary processes, including the exit of mature thymocytes from the thymus.

On a final note, it is worth emphasizing that in this study we observed a spectrum of expression patterns using the *dLck* promoter, with only two of 15 founders showing the type of delayed onset of recombinase expression in the thymus that we sought. This observation suggests that transgenes using the *dLck* promoter are likely to be very sensitive to regulatory influences from the sites in the genome into which they integrate. Thus, although the *dLck* promoter retains obvious appeal for restricting transgene expression to late stages of development, care should be exercised in screening *dLck* transgenic lines for promiscuous or otherwise unexpected patterns of expression.

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Disclosures

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