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CD83 Expression Influences CD4+ T Cell Development in the Thymus

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Summary

T lymphocyte selection and lineage commitment in the thymus requires multiple signals. Herein, CD4+ T cell generation required engagement of CD83, a surface molecule expressed by thymic epithelial and dendritic cells. CD83-deficient (CD83−/−) mice had a specific block in CD4+ single-positive thymocyte development without increased CD4+CD8+ double- or CD8+ single-positive thymocytes. This resulted in a selective 75%–90% reduction in peripheral CD4+ T cells, predominantly within the naive subset. Wild-type thymocytes and bone marrow stem cells failed to differentiate into mature CD4+ T cells when transferred into CD83−/− mice, while CD83−/− thymocytes and stem cells developed normally in wild-type mice. Thereby, CD83 expression represents an additional regulatory component for CD4+ T cell development in the thymus.

Introduction

The process by which immature thymocytes undergo lineage commitment and selection is the subject of intense investigation. Precursor cells enter the thymus as double-negative (DN, CD4−CD8−) thymocytes but upregulate expression of both CD4 and CD8 coreceptor molecules to become CD4+CD8+ double-positive (DP) thymocytes. DP thymocytes develop into mixed transitional CD4+CD8low thymocytes before commitment to either the CD4+ or CD8+ lineages as single positive (SP) T cells (Lucas and Germain, 1996; Lundberg et al., 1995; Sant'Angelo et al., 1998; Suzuki et al., 1995). Immature DP thymocytes with a T cell antigen receptor (TCR) of high affinity for major histocompatibility complex (MHC) molecules and self-peptide complexes are deleted (referred to as negative selection), while those with a TCR of low affinity for self-peptide/MHC complexes are positively selected and survive (Jameson and Bevan, 1998). Stochastic and instructional models explain how MHC specificity and coreceptor expression are linked during development (von Boehmer, 1994). The stochastic model proposes that CD4 or CD8 lineage commitment occurs independent of TCR specificity, with either CD4 or CD8 expression terminated stochastically (Chan et al., 1993; van Meerwijk and Germain, 1993). In the instructional model, quantitative signals induce transitional CD4+CD8low thymocytes to become CD4+ or CD8+ T cells (Lundberg et al., 1995; Robey et al., 1991; Suzuki et al., 1995). CD4 coreceptor engagement contributes strong signals that induce CD4+ T cell generation, while weaker CD8 coreceptor signals generate CD8+ T cells (Basson et al., 1998; Bommhardt et al., 1997; Hernandez-Hoyos et al., 2000; Matechak et al., 1996; Suzuki et al., 1998; Yasutomo et al., 2000a). Additionally, an asymmetric commitment model postulates that CD4 T cell differentiation occurs by default rather than by instruction (Cibotti et al., 1997; Takahama et al., 1994). This complexity undoubtedly reflects the myriad of molecular interactions that influence thymocyte transcriptional regulation and development.

Regardless of stochastic or instructional lineage commitment, accessory signaling steps are required for the maturation of DP thymocytes into CD4+ or CD8+ SP thymocytes (Brugnera et al., 2000; Sant'Angelo et al., 1998). Although little is known about the signals and cell surface molecules involved, transitional CD4+CD8low thymocytes require signals from thymic stromal cells to become mature CD4+ or CD8+ T cells (Anderson et al., 1996; Cibotti et al., 1997; Wilkinson et al., 1995). Among stromal cells, cortical epithelial cells provide the signals required for positive selection and lineage commitment (Laufer et al., 1996; Matzinger and Guerder, 1989). Roles for dendritic cells (DCs) as antigen-presenting cells during thymocyte selection and survival have also been proposed, in addition to their roles in peripheral immune responses (Ardavin, 1997; Steinman, 2000; Yasutomo et al., 2000b). Human dendritic lineage cells include thymic DCs, skin Langerhans cells, circulating DC, interdigitating reticulum cells present in the T cell zones of lymphoid organs, and monocyte-derived DC, all of which express cell surface CD83 (Engel et al., 1995a; Kruse et al., 2000; Weissman et al., 1995; Zhou et al., 1992; Zhou and Tedder, 1995a, 1995b). CD83 is primarily a marker for mature DCs but is also expressed weakly by some germinal center lymphocytes and mitogen-activated lymphocytes (Zhou et al., 1992). Based on the pattern of CD83 expression, its structural similarity with other members of the immunoglobulin superfamily (Kozlow et al., 1993; Zhou et al., 1992), and the recent identification of potential ligands (Cramer et al., 2000; Lechmann et al., 2001; Scholler et al., 2001), CD83 may serve important roles during intercellular interactions. Although CD83 does not share significant sequence homologies with other known proteins, human and mouse CD83 are well conserved (~63%) in amino acid sequence (Berchtold et al., 1999; Twist et al., 1998). Therefore, CD83-deficient (CD83−/−) mice were generated to understand its function. Surprisingly, there was a specific block in thymocyte development in CD83−/− mice distinguished by a severe reduction in peripheral CD4+ T cells.

Results

Generation of CD83−/− Mice

CD83−/− mice were generated by homologous DNA recombination in embryonic stem cells. The targeting vec-

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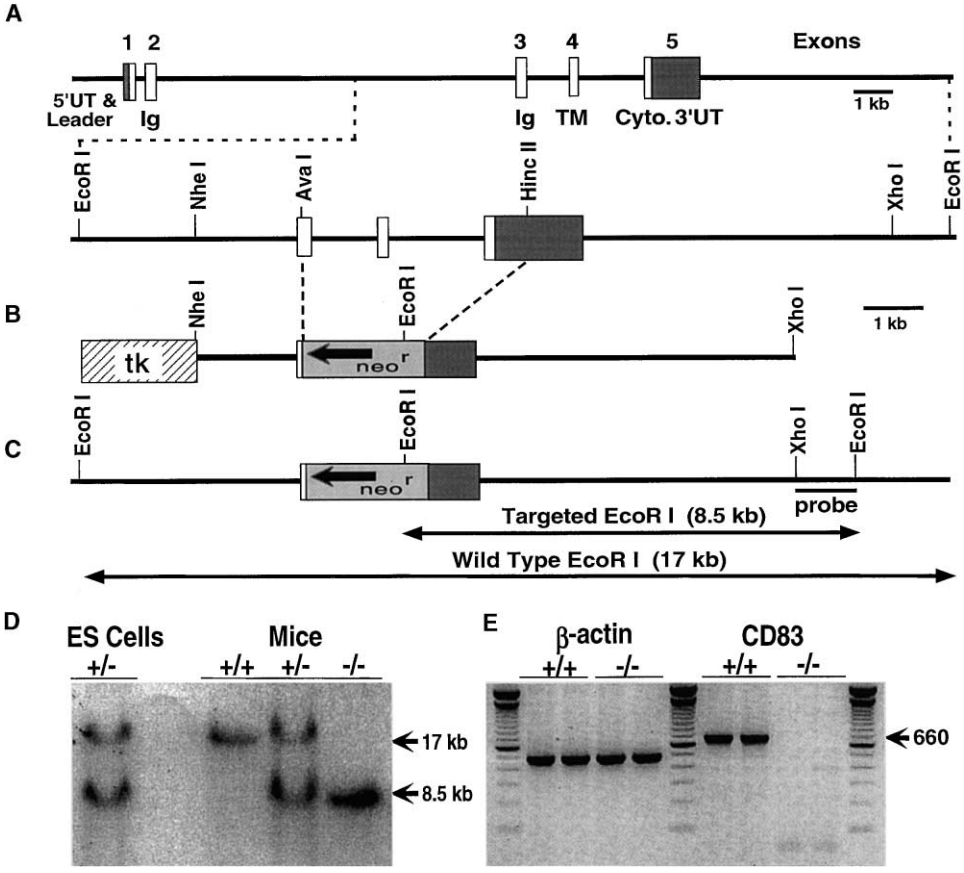

Figure 1. *CD83* Gene Disruption

(A) *CD83* exons encoding untranslated (filled squares) and translated (open squares) regions.

(B) The gene-targeting vector contained thymidine kinase (*tk*) and neomycin resistance (*neo*^{r}) genes.

(C) Gene targeting reduced a 17 kb EcoR I DNA cleavage product to 8.5 kb.

(D) Southern analysis of DNA from one targeted ES cell clone and littermate offspring from crosses of heterozygous CD83$^{+/-}$ mice. Genomic DNA was digested with EcoR I and hybridized with the DNA probe shown.

(E) CD83 expression by LPS-stimulated BM-DC generated from CD83$^{-/-}$ and wild-type (CD83$^{+/+}$) littermates. RNA was reverse transcribed and PCR amplified using primers within the 5′ and 3′ untranslated regions (internal to the Hinc II site in exon 5), which generated a 660 bp fragment when full-length CD83 transcripts were present. Actin was amplified as a control. Results in (D) and (E) represent three littermate pairs.

tor replaced parts of exons 3–5 with a neomycin resistance gene that introduced upstream stop codons within CD83 transcripts (Figures 1A–1C). The deleted DNA removes half the immunoglobulin-like domain (25 amino acids) of CD83 protein and the transmembrane and cytoplasmic domains. Targeted *CD83* gene disruption was confirmed by Southern blot analysis of genomic DNA from ES cells and littermate offspring generated from crosses of heterozygous CD83$^{+/-}$ mice (Figure 1D). Bone marrow-derived dendritic cells (BM-DC) from CD83$^{-/-}$ mice failed to produce full-length CD83 transcripts, which were easily detected in wild-type BM-DC (Figure 1E). CD83$^{-/-}$ mice were produced at the expected Mendelian frequency, thrived, and reproduced as well as their wild-type littermates. Developmental or morphological abnormalities were not obvious in CD83$^{-/-}$ mice up to 12 months of age.

Impaired CD4$^+$ Thymocyte Development in CD83$^{-/-}$ Mice

CD83$^{-/-}$ mice had profound reductions in thymic and peripheral CD4$^+$ T cells when compared with wild-type littermates. Although DN and DP thymocyte numbers were generally normal, CD83$^{-/-}$ littermates had a 68% reduction in CD4$^+$ SP thymocyte numbers (Figure 2A). The residual CD4$^+$ SP thymocytes in CD83$^{-/-}$ mice were predominantly CD8^{low} (Figure 2A), with a CD69^{hi}, CD62L^{low}, CD24^{hi}, and TCR^{int} phenotype, indicating that the CD4$^+$CD8^{low} thymocytes were predominantly transitional cells that had not committed to the CD4$^+$ lineage. Overall, CD8$^+$ SP thymocytes did not increase in CD83$^{-/-}$ mice, although numbers varied slightly between mice (Figure 2A). The phenotype of CD83$^{-/-}$ mice did not result from altered cell surface CD4 density, since DP thymocytes and CD4$^+$ T cells expressed wild-type levels of CD4 (Figures 2A and 2B). Therefore, CD83 deficiency selectively inhibited CD4$^+$ thymocyte development without a compensatory shift of DP thymocytes to the CD8 lineage.

CD83$^{-/-}$ littermates had a profound 75%–90% reduction in peripheral CD4$^+$ T cells (Figure 2B), with a significant increase in the proportion of T cells with the characteristics of memory cells (CD44^{high}, CD45Rb^{low}, and CD62L^{low}; Figure 2C, data not shown). The numbers of

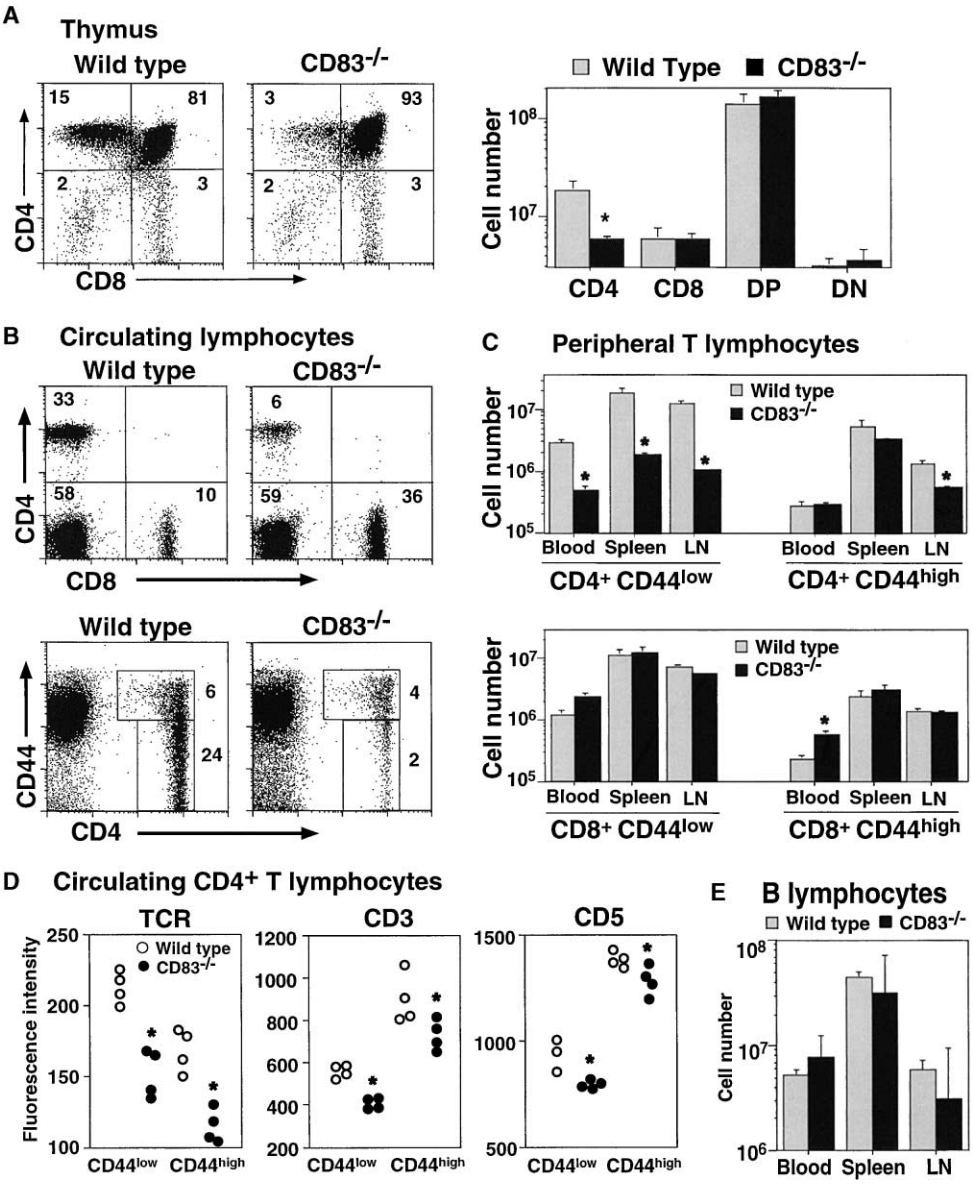

Figure 2. CD4+ T Cell Deficiency in CD83−/− Mice

(A) Immunofluorescence staining of thymocytes from CD83−/− and wild-type littermates. The percentage of cells with CD4+ SP, CD8+ SP, DN, or DP phenotypes are shown for each quadrant. Mean numbers of thymocytes in each subset are shown for six littermate pairs.

(B) Circulating lymphocytes from CD83−/− and wild-type littermates.

Mean numbers of (C) T cells and (E) B220+ B cells and within blood (cells/ml), spleen, and pooled inguinal, axial, and cervical lymph nodes (LN) from 5–8 littermate pairs.

(D) TCRβ chain, CD3, and CD5 cell surface expression by circulating CD4+ T cells as determined by three-color immunofluorescence analysis. Each symbol represents one mouse. Asterisks indicate significantly different sample means between littermate pairs, $p < 0.05$.

"naive" CD44lowCD4+ T cells in blood, spleen, and lymph nodes of CD83−/− littermates were reduced by 84%–93% (Figure 2C). Both CD4+ and CD8+ T cells from CD83−/− mice expressed wild-type levels of CD24 (HSA) and CD69, suggesting that they were not systemically activated. However, TCR β, CD3, and CD5 expression were significantly lower on circulating CD4+ T cells of CD83−/− mice (Figure 2D). TCR β chain expression was also lower on CD8+ T cells of CD83−/− mice, although this difference was more prominent on CD4+ T cells. CD8+ T cell numbers were increased in blood of CD83−/− mice, although their numbers were normal in peripheral lymphoid tissues (Figure 2B). Peripheral B220+ B cells (Figure 2E), bone marrow leukocytes, peripheral monocytes, neutrophils, and CD3−CD16+ NK cells developed normally in CD83−/− mice. Therefore, CD83−/− mice were selectively defective in CD4+ T cell generation due to blocked CD4+ SP thymocyte maturation.

Impaired CD4+ Thymocyte Development in CD83−/− AND Mice

To further clarify the role of CD83 in CD4+ thymocyte development, CD83−/− mice were crossed with AND mice that carry MHC class II-specific TCR (Vα11, Vβ3)

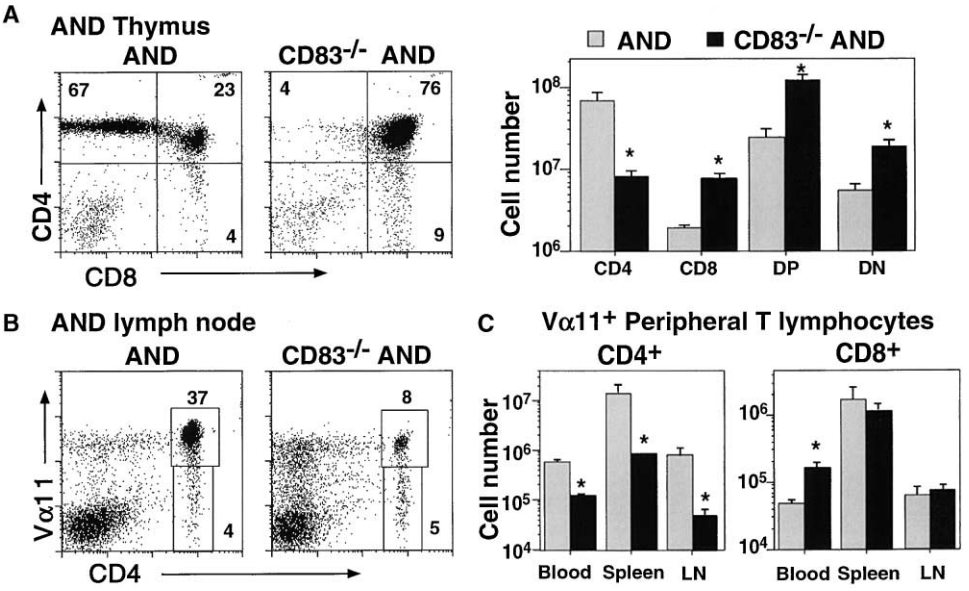

Figure 3. CD4$^+$ T Cell Deficiency in CD83$^{-/-}$ AND Mice

(A) Thymocyte subsets in representative CD83$^{-/-}$ AND and AND littermates. Mean numbers from four littermate pairs are shown.

(B) Vα11 and CD4 expression by lymphocytes from pooled inguinal, axial, and cervical lymph nodes.

(C) Mean numbers of lymphocytes from four pairs of CD83$^{-/-}$ AND and AND littermates. Asterisks indicate significantly different means between littermate pairs, $p < 0.05$.

transgenes, which positively select thymocyte development into the CD4 lineage when expressed in an H-2^b background (Kaye et al., 1989). Remarkably, very few CD4$^+$ SP thymocytes (<5%) were found in CD83$^{-/-}$ AND littermates, with the majority being CD8^{low} (Figure 3A). By contrast, 70% of thymocytes in AND mice were CD4$^+$ SP cells. Consistent with reduced CD4$^+$ thymocyte production in CD83$^{-/-}$ AND mice, CD4$^+$ T cell numbers were reduced by ~90% in the periphery (Figures 3B and 3C). DP and CD8$^+$ SP thymocyte numbers were increased >5-fold and 4-fold, respectfully, in CD83$^{-/-}$ AND mice compared with AND transgenic littermates (Figure 3A), suggesting that a small number of DP thymocytes were redirected to the CD8$^+$ lineage. Alternatively, these CD8$^+$ AND cells may be differentiating in response to MHC class I recognition, since the TCR in AND mice can direct MHC class I-dependent CD8$^+$ T cell maturation when CD4 expression is absent (Matechak et al., 1996). This explanation is consistent with normal CD8$^+$ T cell numbers in CD83$^{-/-}$ mice (Figure 2) and suggests that CD83 expression may promote CD4 signals in DP thymocytes. Although circulating CD8$^+$ T cell numbers were significantly increased in CD83$^{-/-}$ AND littermates, CD8$^+$ T cell numbers within the spleen and lymph nodes were normal.

Impaired CD4$^+$ SP thymocyte and T cell development in CD83$^{-/-}$ mice were stable phenotypic characteristics. CD83$^{-/-}$ mice crossed with C57BL/6 mice for four generations maintained the striking phenotypic characteristics of CD83$^{-/-}$ founder mice. This was also true for all CD83$^{-/-}$ AND$^+$ littermates. Moreover, CD83$^{-/-}$ genotypes and phenotypes remained 100% concordant, suggesting that the phenotype of CD83$^{-/-}$ mice resulted from selective gene targeting rather than genetic alterations independent of the *CD83* locus.

CD4$^+$ T Cell Development Requires CD83 Expression by Thymic Stromal Cells

Reflecting increased numbers of DP thymocytes and decreased SP thymocyte numbers, thymi from CD83$^{-/-}$ mice had a normal architecture with relatively large cortical regions and correspondingly smaller medullary regions. Differences in expression patterns of CD205, a specific marker for thymic DC and cortical epithelial cells (Kraal et al., 1986), were not apparent (Figure 4A). Numbers, morphology, and localization of CD205$^+$ DCs in the thymic medulla, lymph nodes, and spleens of CD83$^{-/-}$ and wild-type littermates were also similar (Figure 4A). In addition, thymic epithelial cells (CD205$^+$CD11c$^-$) were isolated in equal numbers from thymi of CD83$^{-/-}$ and wild-type littermates. Remarkably, in situ hybridization assays using CD83 antisense oligonucleotides demonstrated that cells throughout the cortex expressed CD83 transcripts, with fewer CD83-expressing cells in the medulla (Figure 4B). An antisense CD83 probe did not hybridize with tissues in CD83$^{-/-}$ mice (Figure 4B). Sense probes did not hybridize in wild-type and CD83$^{-/-}$ mouse tissues. Purified thymic epithelial cells (CD45$^-$CD205$^+$CD11c$^-$) and DC (CD205$^+$CD11c$^+$) expressed CD83 transcripts at similar levels, while DP thymocytes did not express detectable CD83 transcripts (Figure 4C). CD83 transcripts were expressed at >100-fold higher levels by BM-DC than total thymus tissue or purified splenic T cells and B cells (Figure 4D). Thus, mouse CD83 was expressed by both thymic DC and epithelial cells, in contrast to human thymus, where DC predominantly express CD83 (Zhou et al., 1992).

To assess whether CD83 on thymic DC or epithelial cells was required for CD4$^+$ T cell development, purified thymic or splenic DCs or cultured fetal thymic epithelial cells were transferred into the thymi of CD83$^{-/-}$ mice.

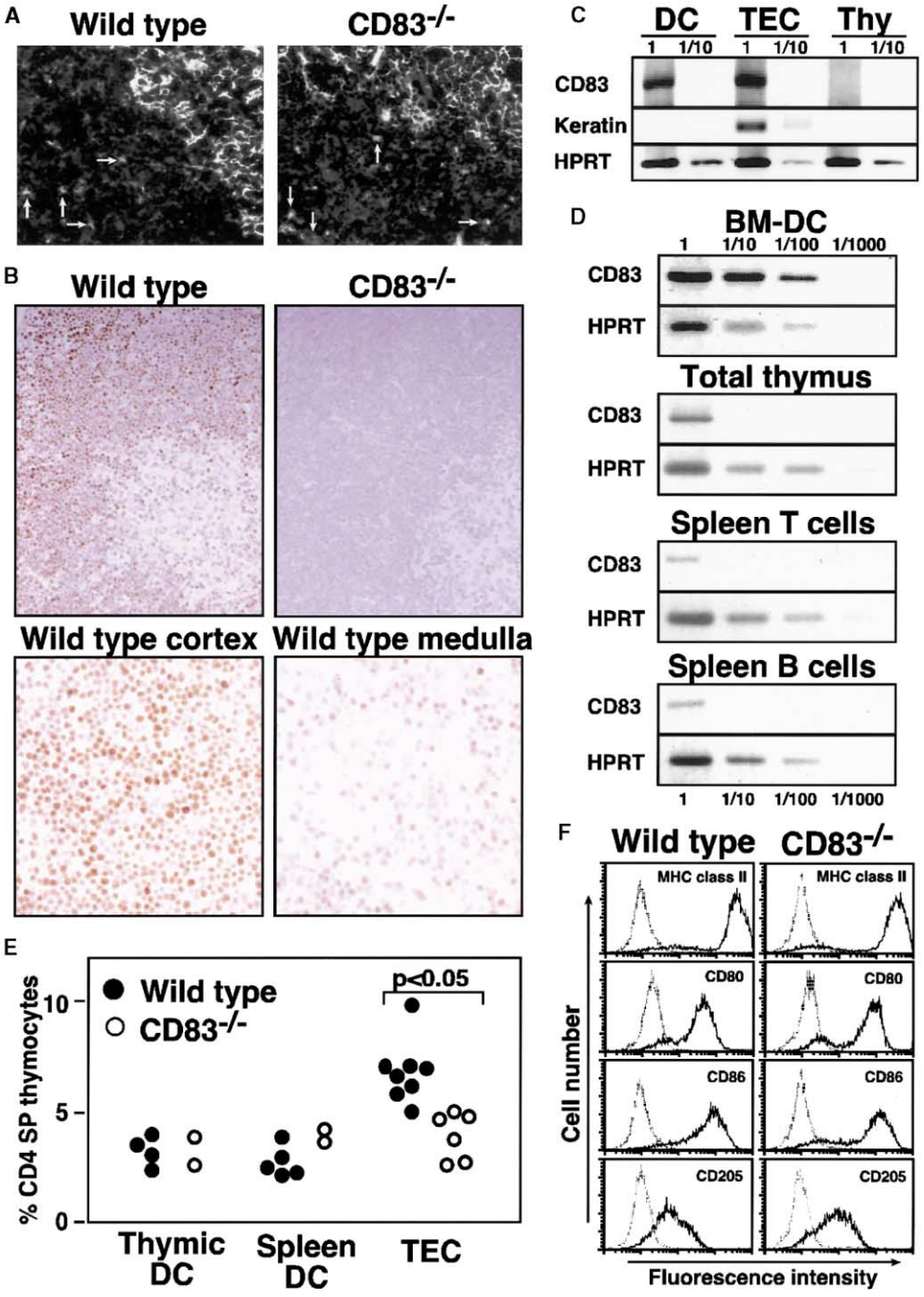

Figure 4. Epithelial and DC Expression of CD83

(A) Thymus sections from wild-type and CD83$^{-/-}$ littermates stained for CD205. Cortical epithelial cells were stained most intensely (upper right in images). Arrows indicate representative CD205^{+} medullary DCs (magnification, 400×).

(B) In situ hybridization of CD83 antisense oligonucleotide probe in thymus (40× and 400× magnification). Transcript-positive cells are stained red with hematoxylin (blue) counterstain.

(C) CD83 expression by purified thymic DCs (CD205^{+} CD11c^{+}), thymic epithelial cells (TEC; CD205^{+} CD11^{-}), and DP thymocytes (Thy). Equal amounts of RNA from each cell population was reverse transcribed, diluted in 10-fold increments, and PCR amplified using CD83-, keratin-, or HPRT-specific primers.

(D) CD83 expression by cultured BM-DC, whole thymus, and purified splenic T and B cells. Lanes labeled 1, 1/10, 1/100, and 1/1000 represent the PCR products from each dilution. (C) and (D) results represent those obtained using at least three littermate pairs of mice.

(E) CD4 SP thymocytes in thymic lobes of CD83$^{-/-}$ mice injected with cultured thymic epithelial cells or purified thymic or splenic DC from either wild-type or CD83$^{-/-}$ donors. Each symbol represents results from individual thymic lobes.

(F) Phenotypes of BM-DC generated from CD83$^{-/-}$ and wild-type littermates. Results represent at least three littermate pairs of mice.

The injection of wild-type epithelial cells into CD83$^{-/-}$ mice increased CD4$^+$ SP T cell production by 2-fold, while CD83$^{-/-}$ epithelial cells did not alter CD4$^+$ SP T cell numbers (Figure 4E). By contrast, the transfer of wild-type or CD83$^{-/-}$ DCs into CD83$^{-/-}$ mouse thymi did not alter CD4$^+$ SP T cell production. While these results do not rule out a role for DC CD83 in CD4$^+$ SP T cell development, they demonstrate that epithelial cell CD83 contributes substantially to this process. Because of this, thymic epithelial cell and DC expression of costimulatory molecules required for CD4$^+$ T cell development was assessed. BM-DC from CD83$^{-/-}$ mice expressed wild-type levels of MHC class II (IA), CD80, CD86, and CD205 (Figure 4F). Similarly, thymic DC (CD205$^+$CD11c$^+$) and epithelial cells (CD205$^+$CD45$^-$CD11c$^-$) purified from CD83$^{-/-}$ mice expressed wild-type levels of CD205 and near wild-type levels (~80%–85%) of MHC class II, CD80, and CD86.

CD83 Deficiency Does Not Affect Intrinsic T Cell Development

Whether abnormal CD4$^+$ T cell development in CD83$^{-/-}$ mice was due to intrinsic thymocyte defects or an altered thymic microenvironment was assessed in adoptive transfer experiments. Bone marrow cells from CD83$^{-/-}$ AND$^+$ and AND$^+$ littermates were transplanted into irradiated CD83$^{-/-}$ and wild-type littermates. Four weeks later, Vα11$^+$ thymocytes from both CD83$^{-/-}$ AND$^+$ and AND$^+$ donors had developed normally into CD4$^+$ SP thymocytes in wild-type recipients (Figure 5A, Table 1). By contrast, Vα11$^+$ thymocytes from either CD83$^{-/-}$ AND or AND donors failed to develop into CD4$^+$ SP cells in CD83$^{-/-}$ littermates. Moreover, there was a blockade of Vα11$^+$ thymocyte development at the DP stage for bone marrow cells transplanted into CD83$^{-/-}$ littermates, resulting in a prominent accumulation of DP Vα11$^+$ thymocytes relative to those present in wild-type recipients (Table 1). These results did not reflect differences in the transplanted bone marrow cells, since similar numbers of each thymocyte subset developed in wild-type mice injected with either CD83$^{-/-}$ AND or AND bone marrow cells (Table 1).

Peripheral Vα11$^+$ CD4$^+$ T cell numbers were equivalent in wild-type recipients transplanted with either CD83$^{-/-}$ AND or AND bone marrow cells (Figure 5A, Table 1). In addition, the Vα11$^+$ T cells expressed Vα11, CD5, and CD3 at wild-type densities. By contrast, Vα11$^+$ CD4$^+$ T cell numbers were significantly reduced in CD83$^{-/-}$ recipients transplanted with either CD83$^{-/-}$ AND or AND bone marrow. A slight increase in the frequency of CD8$^+$ SP thymocytes in CD83$^{-/-}$ mice transplanted with either CD83$^{-/-}$ AND or AND bone marrow cells (Table 1) was consistent with the phenotype of CD83$^{-/-}$ AND mice (Figure 3). However, peripheral Vα11$^+$ CD8$^+$ T cell development was not significantly induced by transplanting CD83$^{-/-}$ mice with either CD83$^{-/-}$ AND or AND bone marrow cells (Table 1).

To further confirm that impaired CD4$^+$ T cell development in CD83$^{-/-}$ mice was due to an intrinsic thymic microenvironment defect, thymocytes from CD83$^{-/-}$ AND and AND mice were directly transferred into the thymi of irradiated CD83$^{-/-}$ and wild-type littermates. Vα11$^+$ thymocytes from both CD83$^{-/-}$ AND and AND donors failed to develop into CD4$^+$ SP thymocytes in CD83$^{-/-}$ recipients by 1 and 3 weeks, but developed in normal proportions in wild-type littermates (Figure 5B, Table 1). Peripheral Vα11$^+$ CD4$^+$ T cell numbers were equivalent when wild-type recipient mice were injected with either CD83$^{-/-}$ AND or AND thymocytes (Figure 5B, Table 1). In addition, the peripheral Vα11$^+$ T cells in wild-type recipients expressed Vα11, CD5, and CD3 at normal densities. Collectively, these results demonstrate that thymocytes from CD83$^{-/-}$ mice retain their potential to develop normally in a CD83$^+$ microenvironment.

Impaired Hypersensitivity Responses and Humoral Immunity in CD83$^{-/-}$ Mice

Contact hypersensitivity responses depend in part on the antigen-presenting capacity of Langerhans cells within skin that induce antigen-specific CD4$^+$ T cells. Human Langerhans cells express CD83 (Zhou et al., 1992), and CD83 transcripts are found in mouse skin (Twist et al., 1998). The ability of CD83$^{-/-}$ mice to generate hypersensitivity responses was therefore assessed by applying a hapten, oxazolone, to the skin with subsequent ear lobe challenge. CD83-deficiency resulted in a significant 22% reduction in swelling of oxazolone-challenged ears compared with wild-type littermates that was detected by 12 hr, peaked by 24 hr, and began to subside by 48 hr (Figure 6A). Despite these differences, the ability of CD83$^{-/-}$ DC to induce allogeneic T cell proliferation in vitro was normal. In mixed lymphocyte reaction (MLR) assays, BM-DC generated from CD83$^{-/-}$ and wild-type littermates induced allogeneic T cell proliferation to the same extent (Figure 6B). In addition, CD4$^+$ T cells from CD83$^{-/-}$ mice proliferated normally in response to allogeneic BM-DC in MLR assays (Figure 6C). Both BM-DC and CD4$^+$ T cells from CD83$^{-/-}$ littermates were also normal in their ability to stimulate or proliferate, respectively, during syngeneic MLR assays. T cells from CD83$^{-/-}$ mice also proliferated normally in response to stimulation with a mitogenic anti-TCR or CD3 antibody, phytohemagglutinin (PHA) and Concanavalin A (Figures 6D and 6E). Therefore, while peripheral DC or CD4$^+$ T cell function may be impaired by CD83 deficiency, in vitro DC and T cell function was normal in CD83$^{-/-}$ mice.

Reflecting a significant decrease in the number of CD4$^+$ T cells exiting the thymus, the number of bromodeoxyuridine (BrdU)-labeled cells among the CD44^{low}CD4$^+$ T cell subpopulation in blood, lymph nodes, and spleen was significantly lower in CD83$^{-/-}$ mice than in wild-type littermates following 10 or 30 days of BrdU labeling (Figure 6F, data not shown). The number of BrdU-labeled cells among the CD44^{high}CD4$^+$ population was also reduced but was more similar to wild-type littermates (Figure 6F, data not shown). Consistent with this, CD83$^{-/-}$ littermates had reduced humoral immune responses following challenge with a T cell-dependent antigen, 2,4-dinitrophenol-conjugated keyhole limpet hemocyanin (DNP-KLH). Primary immune responses of all isotypes were significantly reduced in CD83$^{-/-}$ littermates (Figure 7A). Nonetheless, immune responses normalized following a secondary challenge (Figure 7A), and serum immunoglobulin levels were similar in CD83$^{-/-}$ and wild-type littermates (Figure 7B).

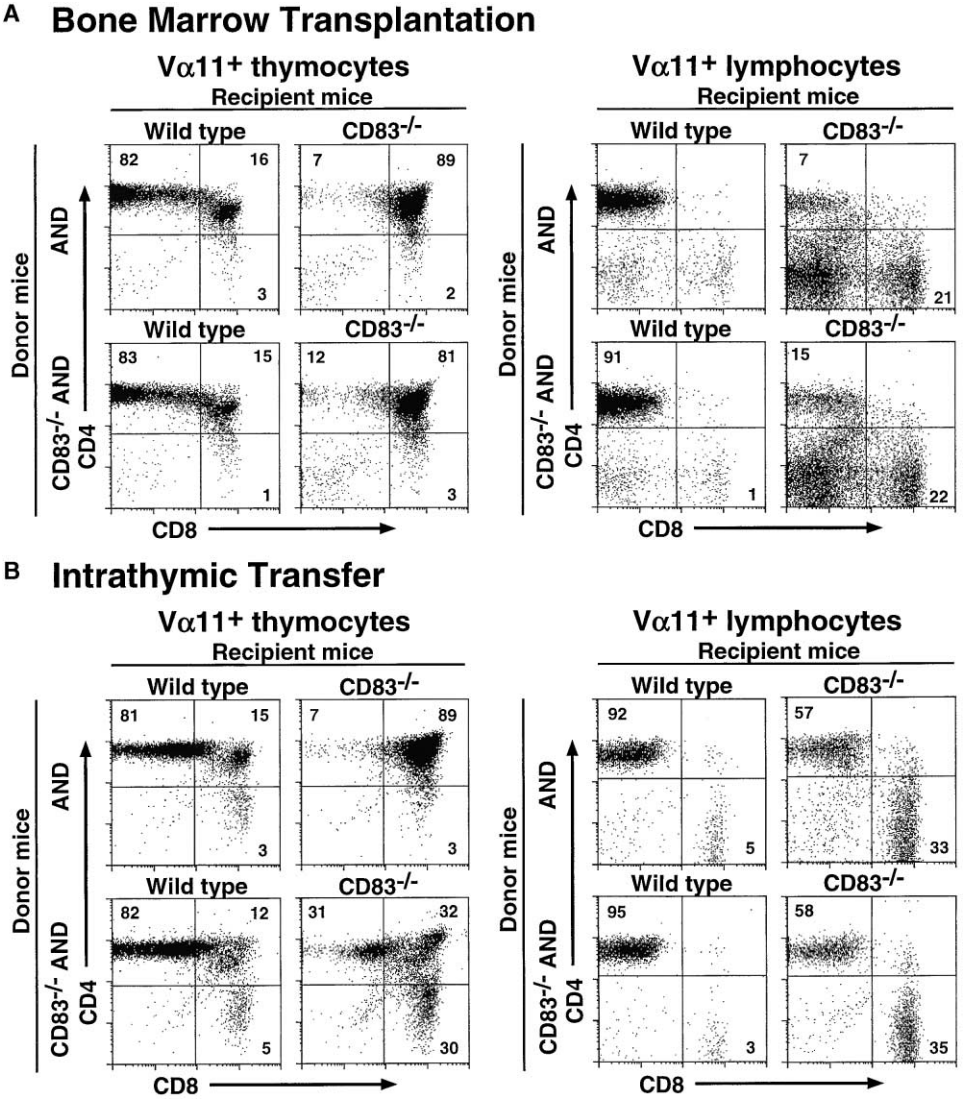

Figure 5. CD83$^{-/-}$ Thymocytes Develop Normally in Wild-Type Mice

(A) Bone marrow cells from CD83$^{-/-}$ AND and AND littermates were transplanted into irradiated CD83$^{-/-}$ and wild-type littermates.

(B) Thymocytes from CD83$^{-/-}$ AND and AND littermates were transplanted into the thymus of irradiated CD83$^{-/-}$ and wild-type littermates.

(A) Four and (B) three weeks later, the relative percentages of Vα11^{+} cells expressing CD4 and/or CD8 were assessed in the thymus and spleen. These results represent those shown in Table 1.

Decreased immune responses in CD83$^{-/-}$ mice did not result from defective B cell maturation since IgM, IgD, and CD19 expression were normal on splenic B cells. Similarly, CD83$^{-/-}$ B cells proliferated normally following lipopolysaccharide (LPS) treatment and IgM crosslinking in vitro (Figure 7C). However, splenic B cells from CD83$^{-/-}$ mice expressed cell-surface MHC class II antigens and CD86 at ~50% and ~20% lower levels than in wild-type littermates, respectively (Figure 7D). Class II and CD86 expression by CD83$^{-/-}$ B cells remained 3- to 4-fold below normal levels following activation with LPS, IL4, IgM, or interferon. Despite this, class II expression levels by blood and tissue monocytes from CD83$^{-/-}$ mice were wild-type. Therefore, CD83 deficiency significantly affected the activation status of peripheral B cells and inhibited antigen-specific humoral immune responses but did not affect intrinsic B cell proliferative function.

Discussion

These studies reveal a significant and unexpected role for CD83 in CD4^{+} T cell development. A profound reduction in CD4^{+} SP thymocyte development in CD83$^{-/-}$ mice translated into a striking reduction in naive CD4^{+} T cells in the periphery (Figure 2). Identical defects were also found in CD83$^{-/-}$ AND transgenic mice, a model system for examining positive selection (Figures 3A–3C). Despite the dramatic effects of CD83 deficiency on CD4^{+} DP thymocyte development, CD8^{+} T cell development and numbers were generally normal (Figure 2). Blocked CD4^{+} T cell development resulted from an in-

Table 1. Normal CD83$^{-/-}$ AND T Cell Development in Wild-Type Mice after Adoptive Transfer

	Bone Marrow Transplantation				Thymocyte Transfer			
Donor	AND		CD83$^{-/-}$ AND		AND		CD83$^{-/-}$ AND	
Recipient	Wild-Type	CD83$^{-/-}$	Wild-Type	CD83$^{-/-}$	Wild-Type	CD83$^{-/-}$	Wild-Type	CD83$^{-/-}$
Thymus (total number $\times 10^{-6}$)					Thymus (% of total)			
CD4$^+$ SP	36 ± 3	3 ± 1**	39 ± 4	3 ± 1**	80 ± 4	24 ± 17**	82 ± 3	39 ± 3**
CD8$^+$ SP	0.5 ± 0.1	1.4 ± 0.2**	0.5 ± 0.1	1.7 ± 0.4**	4 ± 1	11 ± 8	5 ± 1	27 ± 1**
DP	18 ± 2	51 ± 9**	17 ± 1	39 ± 9**	12 ± 3	63 ± 26*	13 ± 3	32 ± 2**
Blood (cells/ml $\times 10^{-3}$)								
CD4$^+$	226 ± 71	32 ± 7**	387 ± 82	18 ± 13**	43 ± 6	12 ± 1**	54 ± 16	5 ± 2
CD8$^+$	23 ± 4	27 ± 5	28 ± 6	13 ± 4	4 ± 1	4 ± 1	5 ± 1	2 ± 1
Spleen ($\times 10^{-5}$)								
CD4$^+$	68 ± 9	27 ± 6**	102 ± 18	2 ± 0.1**	1.9 ± 0.3	1.6 ± 0.1	2.2 ± 0.8	1.0 ± 0.6
CD8$^+$	4.0 ± 0.1	0.7 ± 0.2**	3.8 ± 0.4	1.0 ± 0.2**	<0.1	<0.2	<0.1	<0.1
ILN ($\times 10^{-3}$)								
CD4$^+$	291 ± 63	7 ± 3**	270 ± 88	2.4 ± 1.0**	59 ± 21	9 ± 3	22 ± 3	<1.0**
CD8$^+$	15 ± 4	6.2 ± 2.2*	13 ± 3	4.2 ± 1.6	3 ± 1	5 ± 2	<1.0	<1.0

Results represent those obtained with at least four sets of mice of each phenotype. ILN refers to inguinal lymph node. Asterisks indicate significantly different sample means between CD83$^{-/-}$ and wild type littermates; single asterisk indicates $p < 0.05$, double asterisk indicates $p < 0.01$.

trinsic defect in the thymic microenvironment, since bone marrow stem cells and thymocytes from CD83$^{-/-}$ mice developed normally into CD4$^+$ T cells when transplanted into wild-type mice, but not CD83$^{-/-}$ littermates (Figure 5, Table 1). By contrast, wild-type thymocytes and bone marrow stem cells failed to differentiate into CD4$^+$ T cells when transplanted into CD83$^{-/-}$ mice. Consistent with this, CD83 was expressed by mouse thymic DC and epithelial cells (Figure 4). In addition, wild-type epithelial cells transplanted into the thymi of CD83$^{-/-}$ mice significantly increased CD4$^+$ SP T cell production, while CD83$^{-/-}$ epithelial cells did not (Figure 4E). CD83 deficiency did not affect the phenotypes, generation, or distribution of thymic DC and epithelial cells in vivo (Figure 4) or in vitro or affect the ability of DCs to stimulate T cell proliferation in MLR assays (Figure 6B). Therefore, the lack of CD4$^+$ T cells in CD83$^{-/-}$ mice primarily results from a deficiency in CD83 expression by nonlymphoid cells within the thymus. Thus, CD83 deficiency reveals an important but heretofore unrecognized developmental step in T cell maturation.

The gross reductions in CD4$^+$ thymocytes and T cells in CD83$^{-/-}$ mice are characteristics reminiscent of CD4- and MHC class II-deficient mice (Cosgrove et al., 1991; Grusby et al., 1991; Killeen et al., 1993; Rahemtulla et al., 1991). However, CD4 expression was normal on DP thymocytes and the few peripheral CD4$^+$ T cells found in CD83$^{-/-}$ mice (Figures 2 and 3). Likewise, class II antigen expression by CD83$^{-/-}$ BM-DC, circulating and tissue monocytes, thymic DC, and thymic epithelial cells was near wild-type levels (Figure 4F), and reduced class II expression in heterozygous class II-deficient mice does not significantly affect CD4$^+$ T cell development (Cosgrove et al., 1991; Grusby et al., 1991). Interrupting CD4 and class II molecule interactions also results in increased TCR and CD4 expression by DP thymocytes (McCarthy et al., 1988; Nakayama et al., 1990), while CD4 expression was normal in CD83$^{-/-}$ mice (Figure 2C). The CD83$^{-/-}$ phenotype was also distinct from that of HD (helper T cell-deficient) mice with an intrinsic block in thymocyte development (Dave et al., 1998). In contrast with CD83$^{-/-}$ mice, CD4$^+$CD8^{low} transitional thymocytes are markedly increased in HD mice, suggesting that class II-restricted thymocytes are arrested at this stage. In addition, HD mice have normal numbers of peripheral T cells, but they are all CD8$^+$. CD83-deficient mice had near normal numbers of transitional thymocytes and reduced peripheral T cell numbers, but no increase in CD8$^+$ T cells. Therefore, the CD83$^{-/-}$ phenotype is distinct from previously described mutations that affect thymocyte development.

In human thymus, CD83 is expressed predominantly by DC (Zhou et al., 1992). Comparable CD83 expression patterns were predicted in mouse and human since their transcription regulatory sequences are similar (McKinsey et al., 2000; Twist et al., 1998). As in humans, CD83 transcripts were not detected in purified thymocytes (Figure 4C) and were modest in spleen T and B cells (Figure 4D). However, mouse thymic epithelial cells expressed CD83, which was most obvious in the thymic cortex (Figure 4B). Epithelial cell expression of CD83 explains why the transplantation of wild-type bone marrow stem cells into irradiated CD83$^{-/-}$ mice did not restore CD4$^+$ T cell generation, despite the introduction of CD83$^+$ DC (Figure 5A). This also explains why purified thymic epithelial cells from wild-type mice increased CD4$^+$ T cell production when transplanted into CD83$^{-/-}$ mice (Figure 4E). Thus, epithelial cells are likely to be the major source of CD83 expression in the thymus, which is consistent with the rarity of thymic DCs (Ardavin, 1997; Zhou et al., 1992). Nonetheless, CD83 deficiency did not affect the production of CD205$^+$ DCs in thymus (Figure 4A), spleen, and lymph nodes or the in vitro production and maturation of DCs (Figure 4F). It is therefore possible that a thymocyte receptor(s) for CD83 expressed by epithelial cells and/or DCs provides a pro-

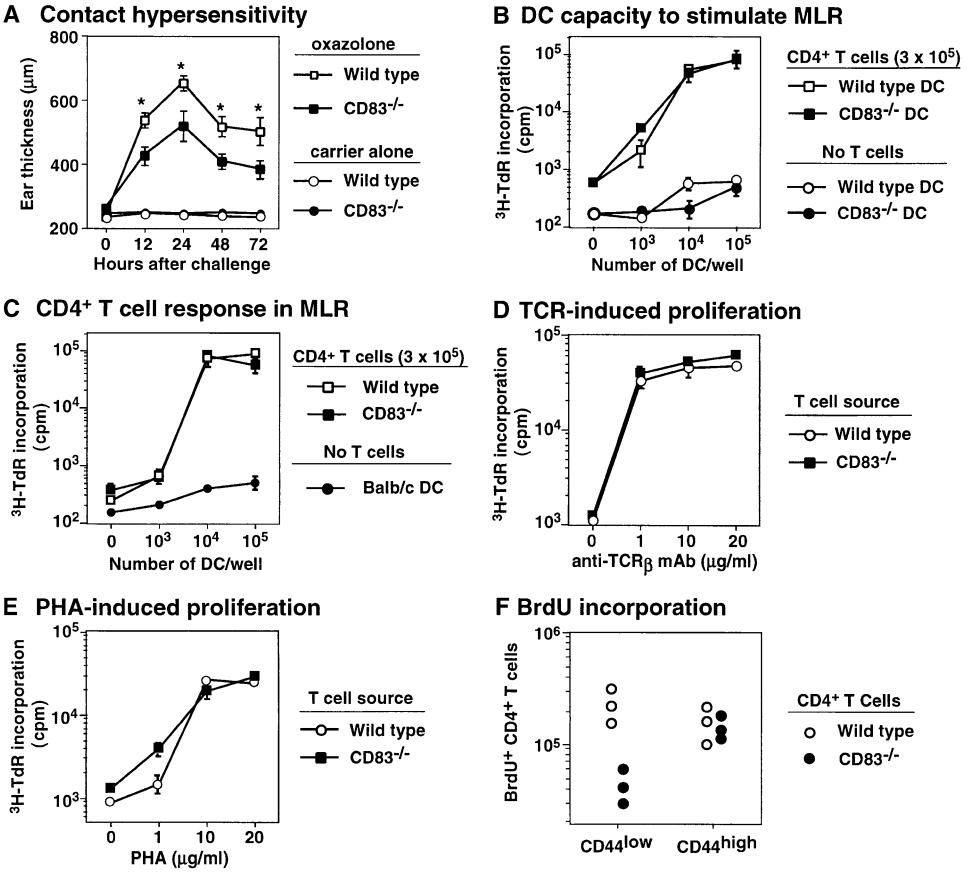

Figure 6. Intrinsic DC and T Cell Function in CD83$^{-/-}$ Mice

(A) Contact hypersensitivity in CD83$^{-/-}$ and wild-type littermates. Values represent mean thickness of oxazolone- or carrier-challenged ear lobes in six CD83$^{-/-}$ and eight wild-type littermates. Significant differences between sample means are indicated; asterisks indicate $p < 0.05$.

(B) Capacity of BM-DC from CD83$^{-/-}$ and wild-type littermates to stimulate allogeneic CD4$^+$ T cells in MLR assays.

(C) Proliferative capacity of splenic CD4$^+$ T cells from CD83$^{-/-}$ and wild-type littermates in response to allogeneic DC in MLR assays.

(D and E) Proliferation of splenic CD4$^+$ T cells from CD83$^{-/-}$ and wild-type littermates in response to PHA and anti-TCR antibody.

(B–E) Values represent mean counts obtained from triplicate cultures and represent those obtained in four independent experiments.

(F) BrdU uptake by circulating naive and memory CD4$^+$ T cells. Each value represents the number of BrdU$^+$ CD4$^+$ T cells per milliliter of blood in individual mice after BrdU uptake over a 30 day period.

gression signal in addition to MHC interactions that allows T cells to enter or survive in the thymic medulla and complete CD4 lineage development (Cibotti et al., 1997; Iwata et al., 1996; Ohoka et al., 1996; Yasutomo et al., 2000b). Since CD83 appears to provide a selective progression signal for the CD4$^+$ but not to the CD8$^+$ lineage, these results reveal an additional step in CD4$^+$ lineage development.

Decreased CD4$^+$ T cells in CD83$^{-/-}$ mice did not result from intrinsic T cell defects. Bone marrow stem cells and thymocytes of CD83$^{-/-}$ mice developed normally when transplanted into wild-type littermates (Figure 5, Table 1). Moreover, CD4$^+$ T cells from CD83$^{-/-}$ mice proliferated normally in response to mitogens and during MLR assays (Figures 6C–6E). Nonetheless, peripheral CD4$^+$ and CD8$^+$ T cells in CD83$^{-/-}$ mice expressed TCR, CD3, and CD5 at low levels (Figure 2D). In fact, CD4$^+$ SP thymocytes and peripheral CD4$^+$ T cells from CD83$^{-/-}$ mice expressed TCR at levels characteristic of CD4$^+$CD8^{low} thymocytes (Lucas and Germain, 1996; Suzuki et al., 1995). This may explain decreased allergic reactions to oxazolone (Figure 6A) and antibody responses to a T cell-dependent antigen (Figure 7A) in CD83$^{-/-}$ mice. Low MHC class II antigen expression by resting B cells from CD83$^{-/-}$ mice and reduced MHC class II and CD86 induction following activation also suggest additional requirements for CD83 expression in B cell activation. These findings are concordant with significant roles for DC in thymic and peripheral T cell homeostasis (Brocker, 1997; Steinman, 2000). Consistent with this, peripheral CD4$^+$ T cell numbers were lower when CD83$^{-/-}$ mice were transplanted with bone marrow from CD83$^{-/-}$ donors rather than wild-type littermates (Table 1). Nonetheless, CD83$^{-/-}$ and wild-type DCs generated equivalent levels of T cell proliferation in allogeneic and syngeneic MLR assays (Figure 6B), and serum immunoglobulin levels were normal in CD83$^{-/-}$ mice (Figure 7B). Thus, CD83 expression by peripheral DC may support interactions between DCs and naive T cells in the periphery and thereby influence lymphocyte maturation, function, and turnover.

In conclusion, CD83$^{-/-}$ mice revealed a requirement

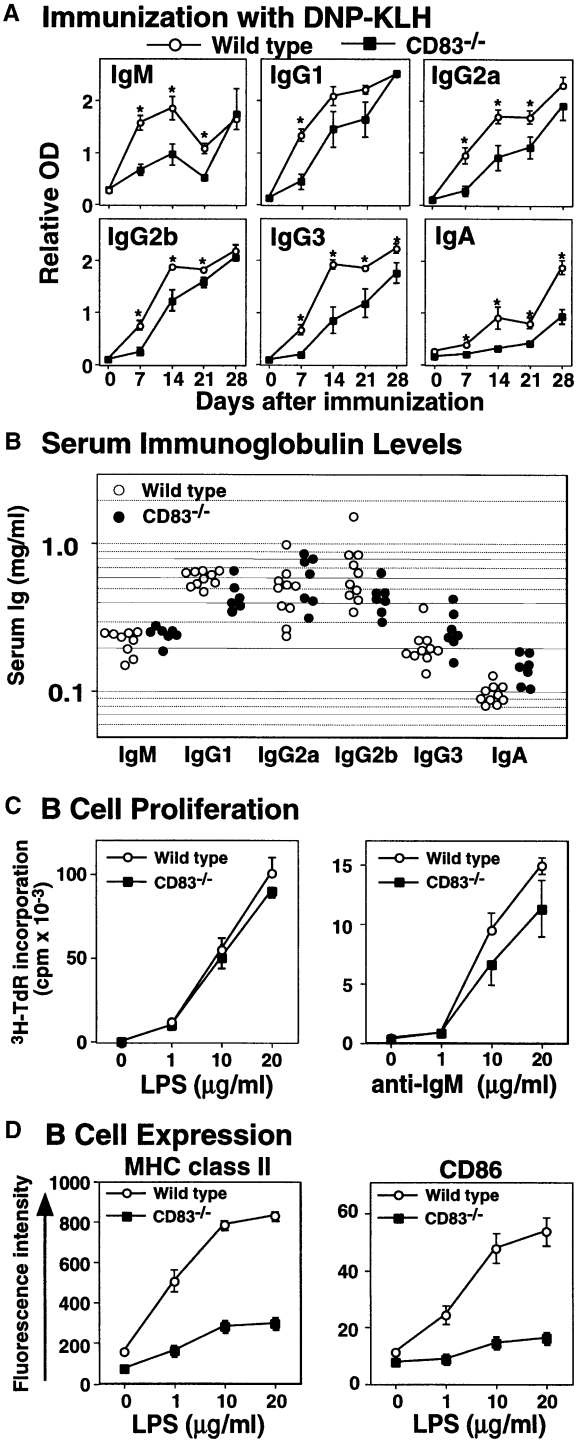

Figure 7. B Cell Function in CD83$^{-/-}$ Mice

(A) Humoral immune responses of CD83$^{-/-}$ and wild-type littermates after immunization with a T cell-dependent antigen. Six mice in each group were immunized on day 0 and boosted on day 21. Significant differences are indicated; asterisks indicate $p < 0.05$.

(B) Serum immunoglobulin levels in CD83$^{-/-}$ and wild-type littermates. Symbols represent results from individual mice as determined by ELISA.

(C) Proliferation of splenic B cells in response to LPS and anti-IgM antibody. Values represent mean counts obtained from triplicate cultures and represent those obtained in four independent experiments.

(D) MHC class II antigen and CD86 expression by resting and LPS-stimulated splenic B cells after 24 hr. Values represent the mean fluorescence intensity of antigen expression as determined by flow cytometry analysis of cells from three CD83$^{-/-}$ and wild-type littermates.

for CD83 expression in CD4$^+$ T cell development and an additional regulatory step during the complex network of molecular interactions that controls thymocyte and peripheral T cell generation. Whether defective CD4$^+$ T cell development in CD83$^{-/-}$ mice is due to defective lineage commitment or aborted positive selection is unknown. It remains possible that CD83 deficiency reduces TCR signaling during DP thymocyte selection, although CD83 deficiency did not enhance differentiation toward the CD8 lineage. Reduced TCR complex signaling in CD83$^{-/-}$ mice could result from lower avidity interactions between the TCR and its accessory molecules with MHC and other ligands or to reduced levels of cytoplasmic signaling. Identification of the thymocyte ligand(s) for CD83 will help untangle these possibilities. Nonetheless, the accumulation of DP cells in CD83$^{-/-}$ AND mice (Figure 3A) and the normal numbers of CD8$^+$ cells in CD83$^{-/-}$ and CD83$^{-/-}$ AND mice (Figures 2 and 3) suggest that early events in positive selection are not disrupted. Since DCs and CD4$^+$ helper T cells are central to the immune system and the generation of immune responses, it is understandable that CD4$^+$ T cell homeostasis is precisely controlled. In fact, reduced CD4$^+$ T cell numbers is the major factor determining morbidity and mortality in AIDS patients. While CD83 deficiency may abrogate normal CD4$^+$ T cell development, it is possible that augmenting CD83 expression or the engagement of CD83 receptors on developing thymocytes will provide signals that enhance CD4$^+$ T cell production. Thus, CD83-deficient mice may provide insight into the development of new therapies to manipulate the development of helper T lymphocytes in vivo.

Experimental Procedures

Generation of CD83$^{-/-}$ Mice

DNA encoding *CD83* was isolated from a 129/Sv strain genomic library (Twist et al., 1998). The targeting vector 5′ end contained the pMC1-HSVTK gene ligated to a 1.1 kb DNA fragment that ended at the Ava I site in *CD83* exon 3 (Figure 1A). The region between the Ava I restriction site located in *CD83* exon 3 and the Hinc II site located in the 3′ untranslated region of exon 5 was replaced with the PGK neomycin resistance gene in the 3′ to 5′ orientation. The 3′ end of the targeting vector used a 6.3 kb DNA fragment that started at the Hinc II site in the 3′ untranslated region of the *CD83* gene. The vector was linearized using a unique Xho I site in the genomic DNA and electroporated into AK-7 ES cells (Selfridge et al., 1992). Four gene-targeted ES cell clones were injected into 3.5-day-old C57BL/6 blastocysts before transfer into foster mothers. One ES cell clone provided germline transmission of the disrupted allele. CD83$^{+/-}$ offspring were backcrossed with C57BL/6 mice (The Jackson Laboratory) and AND mice (provided by S.M. Hedrick, U. California-San Diego) with genotypes determined by Southern blot analysis of tail DNA. Heterozygous mice were intercrossed to generate homozygous CD83$^{-/-}$ mice and wild-type littermate controls. Mice were housed in a specific pathogen-free barrier facility and used at 2 months of age. All procedures were approved by the Duke Animal Care and Use Committee.

Generation of BM-DC

BM-DCs were generated as described (Inaba et al., 1992). Briefly, bone marrow cells (1×10^6/ml) from ~6- to 8-week-old mice were

cultured at 37°C in 6-well Falcon culture plates containing enriched RPMI 1640 culture medium supplemented with mouse GM-CSF (200 U/ml; PharMingen). On days 3 and 5 of culture, nonadherent cells were gently removed by rinsing, and fresh medium and GM-CSF were added. On day 7, the nonadherent cells were collected by gentle pipetting, resuspended in 10 ml of culture medium containing GM-CSF, and transferred to 100 mm tissue culture dishes. DCs were collected for use in MLR assays on day 9, while DCs for phenotypic analysis or RNA preparation were cultured until day 11.

Reverse Transcription-Polymerase Chain Reaction (PCR) Assays

DP thymocytes, thymic DC ($CD205^+$ $CD11c^+$), and cortical thymic epithelial cells ($CD205^+$ $CD11^-$) were purified from single-cell suspensions of collagenase-treated thymi by fluorescence-based cell sorting. RNA was reverse transcribed and PCR amplified using a sense primer representing 5′ untranslated sequence (TGT CGC AGC GCT CCA GCC) and antisense primer representing 3′ untranslated sequence (GCA TTC AGG CAC ACT GAT C). Keratin 5 was amplified as a marker for thymic epithelial cells. Primers specific for actin and HPRT transcripts were used to verify the efficiency of cDNA synthesis and PCR amplification.

Immunofluorescence Analysis, In Situ Hybridization, and BrdU Labeling

Single-cell suspensions were stained with biotin- or fluorochrome-conjugated antibodies (from PharMingen, Caltag, or Southern Biotechnology Associates) as described (Engel et al., 1995b). Biotin-conjugated antibodies were revealed by fluorochrome-conjugated streptavidin. Labeled cells were analyzed on a FACScan flow cytometer (Becton Dickinson).

Thymi were flash-frozen for immunohistology or fixed in paraformaldehyde for in situ hybridization and sliced into 5-μm-thick sections. For in situ hybridization, sections were treated with proteinase K before hybridization with biotinylated CD83 oligonucleotide probes. CD83 RNA was detected using a biotinylated antisense probe (TGA AAG TTG ACT CTG TAG CTT CCT TGG GGC ATC CTG). A sense oligonucleotide (CAG GAT GCC CCA AGG AAG CTA CAG AGT CAA CTT TCA) served as a negative control. Probe hybridization was detected using horseradish peroxidase-conjugated streptavidin and visualized using the DAB+ Liquid System (DAKO).

Mice were provided with drinking water containing BrdU (0.8 mg/ml, Sigma). The frequency of $BrdU^+$ cells within the blood and tissues was determined 10–30 days later using FITC-labeled anti-BrdU antibody (Becton Dickinson) as described (Tough and Sprent, 1994).

Adoptive Transfer Procedures

For bone marrow transplantation experiments, bone marrow cells were isolated from $CD83^{-/-}$ AND and AND littermates. $Thy1.2^+$ cells were removed (<2% $Thy1.2^+$) using antibody-coated magnetic beads (Dynal). Recipient mice were irradiated (1200 rads) 12 hr before i.v. injection of 5×10^6 donor bone marrow cells. $V\alpha11^+$ cells in recipient mice were assessed 4 weeks after transplantation. For thymocyte transfer experiments, recipient mice were sublethally irradiated (500 rads) 16 hr prior to intrathymic cell transfer. Unfractionated, single-cell thymocyte preparations isolated from $CD83^{-/-}$ AND and AND littermates (2×10^6) were injected into each thymic lobe of anesthetized $CD83^{-/-}$ and wild-type littermates as described (Guidos et al., 1989). $V\alpha11^+$ cells in recipient mice were assessed 1 and 3 weeks after thymocyte transfer. All recipients showed appropriate reconstitution of $CD8^+$ cells.

In some experiments, purified thymic or splenic DC or cultured thymic stromal cells were used for intrathymic cell transfers. DCs from $CD83^{-/-}$ and wild-type littermates were isolated from single-cell suspensions of tissues using anti-CD11c antibody-coated magnetic microbeads (Miltenyi Biotec). Purified cells (5×10^5; >95% $CD205^+$) were injected into the thymic lobes of $CD83^{-/-}$ mice. Epithelial cells were isolated from day 14 or 15 fetal thymi and cultured as described (Anderson et al., 1993; Robinson and Owen, 1977). After 6 or 7 days, $CD45^+$ cells were depleted from the cultured cells using antibody-coated magnetic beads (Miltenyi Biotec). Epithelial cells (5×10^6) were microinjected into each thymic lobe of $CD83^{-/-}$ littermates. Ten days later, thymocytes were isolated from each lobe for analysis.

Lymphocyte Function Assays

$CD4^+$ T cells and $B220^+$ B cells were isolated (>95% purity) from single-cell spleen suspensions using antibody-coated magnetic beads (Dynal). For MLR assays, $CD4^+$ T cells (4×10^5) were mixed with BM-DC from syngeneic or allogeneic (BALB/c) mice and cultured for 5 days. For mitogen assays, $CD4^+$ T cells were cultured with anti-mouse TCR or CD3 antibody (PharMingen), PHA (Sigma) or Concanavalin A (Sigma), while B cells were stimulated with LPS (*E. coli* serotype 0111;B4, Sigma) or anti-IgM antibody (Cappel) for 3 days. Proliferation during the last 18 hr of culture was assessed by [^{3}H]thymidine incorporation. For contact hypersensitivity assays, mice were sensitized on the flank with oxazolone (Sigma) and challenged on one ear 5 days later (Tedder et al., 1995). Carrier was administered to the other ear as a control. Each day after challenge, the thickness of the central portion of ear lobes was measured three times using a constant force, digital thickness gauge with means used for analysis.

Mice were immunized i.p. with 100 μg of DNP-KLH (Calbiochem-Novabiochem) in complete Freund's adjuvant and were boosted 21 days later as described (Engel et al., 1995b). Serum antibody levels and DNP-specific antibody titers were measured as described (Engel et al., 1995b).

Statistical Analysis

All data are shown as mean values ± SEM. The Student's t test statistic was used to determine the level of significance of differences in sample means.

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