

Targeted Expression of Major Histocompatibility Complex (MHC) Class II Molecules Demonstrates that Dendritic Cells Can Induce Negative but Not Positive Selection of Thymocytes In Vivo

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Summary

It is well established that lymphoid dendritic cells (DC) play an important role in the immune system. Beside their role as potent inducers of primary T cell responses, DC seem to play a crucial part as major histocompatibility complex (MHC) class II⁺ “interdigitating cells” in the thymus during thymocyte development. Thymic DC have been implicated in tolerance induction and also by some authors in inducing major histocompatibility complex restriction of thymocytes. Most of our knowledge about thymic DC was obtained using highly invasive and manipulatory experimental protocols such as thymus reaggregation cultures, suspension cultures, thymus grafting, and bone marrow reconstitution experiments. The DC used in those studies had to go through extensive isolation procedures or were cultured with recombinant growth factors. Since the functions of DC after these in vitro manipulations have been reported to be not identical to those of DC in vivo, we intended to establish a system that would allow us to investigate DC function avoiding artificial interferences due to handling. Here we present a transgenic mouse model in which we targeted gene expression specifically to DC. Using the CD11c promoter we expressed MHC class II I-E molecules specifically on DC of all tissues, but not on other cell types. We report that I-E expression on thymic DC is sufficient to negatively select I-E reactive CD4⁺ T cells, and to a less complete extent, CD8⁺ T cells. In contrast, if only DC expressed I-E in a class II-deficient background, positive selection of CD4⁺ T cells could not be observed. Thus negative, but not positive, selection events can be induced by DC in vivo.

Signaling through the TCR/CD3 complex of immature CD4⁺CD8⁺ thymocytes can induce at least two different developmental events: negative selection resulting in cell death or positive selection resulting in maturation of thymocytes (1–3). This signaling, however, can have different outcomes depending on the type of stromal cell that presents MHC/peptide complexes to the developing thymocytes. Yet, some controversy still exists with respect to the relative capacities of epithelial- or bone marrow (BM)¹-derived cells in inducing apoptosis or positive selection. For negative selection, results varied according to the in vitro experimental system used. Thus, in suspension cultures, BM-derived cells were as efficient as epithelial cells in deleting self-reactive thymocytes (4, 5), whereas in the case of reaggregation cultures, they were shown to be much better deleters than epithelial cells (6, 7). The precise function of each BM-derived cell type (dendritic cells [DC], macrophages, or B cells), however, remains relatively unclear. While macrophages are probably not able to clonally eliminate self-reactive

thymocytes (8), it has been postulated that thymic DC could do so only in concert with B cells (9).

Early experiments involving MHC mismatched BM-chimeras suggested that thymic epithelial cells were responsible for inducing the maturation of immature thymocytes to mature single-positive T cells (reviewed in reference 1). These results were questioned by several experiments showing that many different cell types, such as fibroblasts (10, 11) or BM-derived cells (12), were able to mediate positive selection in some, but not in all cases (13), when injected intrathymically or used in BM reconstitution experiments. Interestingly, in more recent thymus reaggregation experiments, neither DC from spleen (14) nor thymic DC that was expanded in vitro with the help of recombinant GM-CSF (15, 16) were able to mediate positive selection. Differences in surface marker expression between thymic and splenic DC have been reported (17, 18), and it has been shown that short-term cultured DC differ phenotypically and functionally from their freshly isolated counterparts (19). Since the highly artificial experimental setups and extended handling of DC and thymocytes in vitro might have had an influence on the functions and properties of these cells (20), we wanted to investigate the role of thymic DC

¹Abbreviations used in this paper: BM, bone marrow; DC, dendritic cells; FS, forward scatter; SS, side scatter.

in an unmanipulated, non-invasive *in vivo* system. To this end, we developed a transgenic model in which transgene expression was directed exclusively to DC due to an appropriate promoter. We eventually selected the mouse CD11c promoter because the expression of CD11c β_2 integrin, as detected by the mAb N418 (21), was restricted to mouse DC in spleen (21) or other tissues (22) including thymus (22, 23), and therefore represented the best marker available for mouse DC.

Here we present our findings obtained with such a DC-specific expression system. We used the MHC class II I-E $_{\alpha}$ gene as a transgene in the I-E-negative C57Bl/6 background and describe a DC-specific expression pattern in the thymus. We could not detect transgene expression on thymic epithelium, B cells, nor macrophages and describe in this model that thymic DC were able to negatively select self-I-E-reactive T cells. The completeness of the deletion clearly demonstrates that thymic DC alone are sufficient to induce tolerance via clonal deletion. Further, when in an MHC class II-deficient background, only thymic DC express I-E, and CD4⁺ T cells are not positively selected, establishing that thymic DC *in vivo* are not able to induce positive selection.

Materials and Methods

Isolation of Genomic Clones, DNA Constructs, and Microinjection. A cDNA pool from four human T cell clones, all expressing human CD11c, was used as template for PCR amplification. The sense oligonucleotide corresponded to 40 bp of human CD11c-exon 7 (5'-TGCCACGATGATGAACTTCGTGAGAGCTGTGATAAGCCAG-3'), while the antisense oligonucleotide was located within human CD11c-exon 11 (5'-CCATGTCCAATTCGAAGGACTACTGCTTGTTGGTCTCCGT-3') (24). The amplified human CD11c gene fragment was sequenced and then used as a probe to screen a mouse genomic library (OLA 129- λ GEMTM-12-bank, gift of Dr. A. Berns, The Netherlands Cancer Institute, Amsterdam, Netherlands). We isolated two overlapping phages that were characterized by restriction mapping and partial sequencing of their inserts. DNA-sequence analysis of ~1,000 bp around the initiation codon showed 68.8% identity to the corresponding sequence of the human CD11c gene and were therefore accepted to represent mouse CD11c. We used a 5.5-kb Sst I-BspHI (blunt) fragment that contained the 5' region of mouse CD11c gene to drive the expression of the transgene. This fragment was cloned into a SmaI-SstI-opened pBSbluescript vector that contained a blunted 1.2-kb BamHI-XhoI fragment from pDOI-5 (25) in its blunt-ended EcoRI site. This rabbit β -globin gene fragment provided the transgene with an intron and a polyadenylation signal (25). The cDNA encoding I-E $_{\alpha}$ ^d was isolated from the B lymphoma A20 with the oligonucleotides 5'-ACCCAAGAAGAA-AATGGCCACAATT-3' and 5'-TACTTACCTCCAGGTATC-TCACAGGGCTCCTTG-3' and confirmed by DNA sequencing. The I-E $_{\alpha}$ ^d cDNA was cloned as a blunt-end fragment into the blunted EcoRI site of the rabbit β -globin gene. After digestion with XhoI and NotI, the transgene DNA was separated from the vector sequences by electrophoresis, extracted from agarose slices, and resuspended in Tris-EDTA buffer (10 mM Tris, pH 7.4, 0.1 mM EDTA). Fertilized oocytes were obtained from C57Bl/6 mice, and DNA injection was performed as previously described (26).

Monoclonal Antibodies and Reagents. The mAbs specific for CD4

(No. 09005), CD8 (No. 01044), V β 8.1/8.2 TCR (No. 01344), V β 5.1/5.2 TCR (No. 01354), V β 11 TCR (No. 01374), I-E $_{\alpha}$ ^k (No. 09625), CD11c (No. 09705), and CD19 (No. 09655) were purchased from PharMingen (San Diego, CA). Anti-CD8 R613 (GIBCO BRL, Gaithersburg, MD) was used for the three-color fluorescence analysis. Goat anti-rat IgG serum (No. 3010-02; Southern Biotechnology Assoc., Birmingham, AL) was used as second step reagent and MTS-10 ([27], gift of Dr. H.R. Rodewald, Basel Institute for Immunology, Basel, Switzerland), a mAb specific for medullary thymic epithelial cells, was used as culture supernatant. UEA-1, a fucose-specific lectin-staining medullary, but not cortical, epithelium (28) was purchased from Vector Labs., Inc. (Burlingame, CA).

Immunohistology. Fresh organs were embedded in OCT medium (No. 4583; Miles-Yeda Inc., Elkhart, IN), snap frozen, and 6 μ m sections were cut with a cryostat. Sections were air dried for 60 min, acetone-fixed for 2 min, and air dried for a minimum of 12 h. Sections were then rehydrated in PBS (containing 1% BSA and 10% normal mouse serum). mAbs diluted in PBS/BSA/normal mouse serum were added directly onto the sections and incubated for 60 min. After washing, the sections were either directly mounted in Fluoromount for immunofluorescence analysis or incubated with the second step reagent and then mounted and analyzed.

For immunohistological stainings, we used biotinylated mAbs and alkaline phosphatase-conjugated streptavidin (No. RPN 1234; Amersham Intl., Buckinghamshire, UK) as a second step reagent. The color reaction was then developed (Vector-Red Alkaline Phosphatase Substrate Kit No. SK-5100; Vector Labs., Inc.) according to manufacturer's instructions.

Isolation of Thymic DC and B Cells. Thymi of different mice were digested twice with collagenase (CLSPA; Worthington Biochemical Corp., Freehold, NJ) for 30 min at 37°C as described previously (29). Cells were then recovered by centrifugation at 300 *g* for 5 min, and resuspended in a 17% OptiprepTM (Nycomed Pharma, Oslo, Norway) solution diluted in Hank's balanced salt solution without Ca²⁺ and Mg²⁺. They were then overlaid with 12% OptiprepTM diluted in 0.88% (wt/vol) NaCl, 1 mM EDTA, 10 mM Hepes, 0.5% BSA, pH 7.4, and 2 ml Hank's without Ca²⁺ and Mg²⁺, respectively, and centrifuged at 600 *g* for 15 min at 20°C. The low density cells at the Hank's 12% OptiprepTM interface were harvested, washed twice, and used as a thymic DC enriched fraction.

Thymic B cells were enriched by complement depletion of CD4⁺ and CD8⁺ cells of the thymus. Briefly, thymus was teased and cells resuspended in PBS, 1% FCS at 30 \times 10⁶ cells/ml. They were incubated for 10 min at 37°C with 1:10 diluted culture supernatant of 31M (anti-CD8 mAb) and 172 (anti-CD4 mAb). 1 ml H₂O-reconstituted low-tox-M rabbit complement (Cedarlane, Westbury, NY) was then added to the cells and incubated at 37°C for another 45 min. Living cells were isolated after a Ficoll-Paque gradient (Pharmacia Biotech, Uppsala, Sweden).

Flow Cytometry. Expression of cell surface proteins was assayed by indirect immunofluorescence. Organs were teased through a mesh and cell suspensions of 1 \times 10⁵ viable cells were stained with 20 μ g/ml mAb that was directly labeled. After washing, cells were analyzed using a FACScan[®] (Becton Dickinson, Mountain View, CA).

Results and Discussion

Cloning of the Mouse CD11c Promoter Containing Sequences. To isolate the regulatory elements controlling the expression of CD11c in mice, we screened a mouse genomic li-

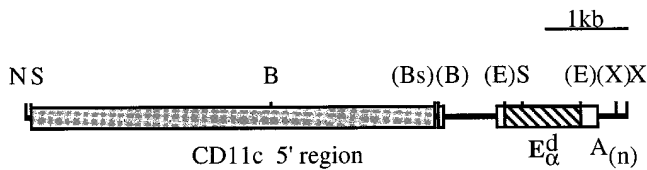


Figure 1. Restriction map of the transgenic construct CD11c-E α^d . MHC class II I-E α^d cDNA (striped box) was placed under the control of a mouse CD11c promoter-containing DNA segment (gray box). The rabbit β -globin gene fragment providing the cDNA with an intron and a polyadenylation signal (A_n) is displayed as a white box. B, BamHI; Bs, BspHI; E, EcoRI; N, NotI; S, SstI; X, XhoI; restriction sites in parentheses have been destroyed by blunt-end cloning.

brary with a probe corresponding to human *CD11c* exon 7 to 11 (see Materials and Methods). The clone we isolated was partially analyzed by DNA sequencing and showed a high homology to the published (24, 30) human *CD11c* genomic sequences (data not shown). We isolated a 5-kb fragment of the 5' region that we expected to contain the promoter region (see Materials and Methods) and used it as the controlling element to express the E α^d cDNA in C57Bl/6 mice (Fig. 1). Two founder lines were obtained and the transgene expression was monitored with 14.4.4S, a mAb specific for the α chain of the I-E MHC class II antigen (31). Thereafter, these mice are referred to as "B6CD11c-E α^d ."

Expression Pattern of the I-E α^d Transgene in the Thymus. Two different mouse strains were used as controls. An I-E transgenic line created by Widera et al. (32) was used as a "positive" control. This transgenic line 107.1 (throughout this study called B6-E α^d) expresses an I-E α^d transgene under the control of a segment of the I-E MHC class II promoter. The expression pattern previously described for the B6-E α^d line corresponded to wild-type I-E expression in I-E $^+$ strains including cortical and medullary thymic epithelial cells, as well as the BM-derived thymic fraction. Negative control mice were C57Bl/6 mice (B6) that did not express the I-E α genes, and therefore, no complementation with the β chain could occur, leading to absence of I-E MHC class II surface expression (33).

Immunohistological analysis of thymi of the three described mouse lines is shown in Fig. 2. Serial sections were incubated with UEA-1, a fucose-specific lectin that stains medullary epithelial cells (28; Fig. 2, *b*, *d*, and *f*), and 14.4.4S to monitor transgene expression (Fig. 2, *a*, *c*, and *e*). While B6 thymus does not show any I-E expression (Fig. 2 *a*) in medulla (as localized with UEA-1 in Fig. 2 *b*) or cortex, the control B6-E α^d thymus (Fig. 2, *c* and *d*) could be stained with 14.4.4S (Fig. 2 *d*) in medulla as well as in cortical regions. While the medulla (Fig. 2 *c*, *M*) showed a strong staining due to I-E expression on its BM-derived and epithelial components (32), the cortical area (Fig. 2 *c*, *C*) stained in a typical reticular lattice pattern due to transgene expression on cortical epithelial cells (32).

Our transgenic B6CD11c-E α^d thymus (Fig. 2, *e* and *f*), on the other hand, showed a clearly distinct thymic I-E expression pattern; the staining was confined to the thymic medulla and corticomedullary junctions (Fig. 2 *e*, *M*). This

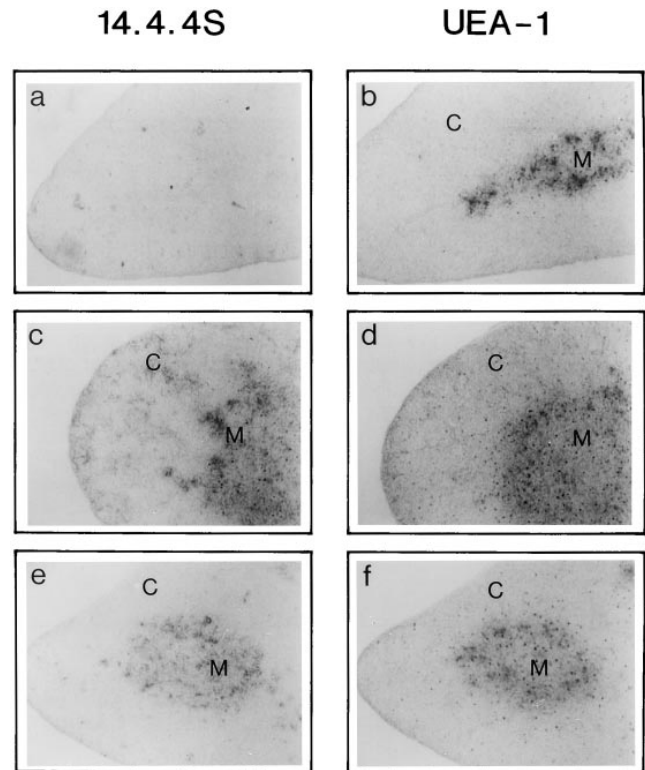


Figure 2. Serial cryostat sections of normal adult thymus stained with 14.4.4S specific for I-E (*a*, *c*, and *e*) and UEA-1 (*b*, *d*, and *f*), a lectin binding specific for medullary epithelial cells (28). (*a* and *b*) Serial sections of a wild-type B6 (I-E $^-$) thymus. (*a*) I-E, no I-E staining can be detected in the B6 background, (*b*) UEA-1, staining is restricted to dense aggregates of epithelial cells in the medulla (*M*), while no staining can be detected in the cortex (*C*). (*c* and *d*) Serial sections of B6-E α^d (I-E $^+$) thymus, class II I-E transgene staining is confluent on BM-derived and epithelial cells in the medulla (*M*), and reticular staining in the cortex (*C*) is typical of epithelial cells. UEA-1 staining (as in *b*) marking the medullary region (*M*) only. (*e* and *f*) Serial sections of B6CD11c-E α^d thymus. I-E (*e*) transgene under control of the CD11c promoter can be detected only in medulla (*M*) and medullary-cortical junctions. No I-E staining in cortex (*C*) was detectable. UEA-1 (*f*) staining is the same as for *b* and *d*. All sections were photographed at $\times 100$.

I-E expression pattern corresponded exactly to the CD11c expression pattern in the thymus when the CD11c-specific mAb N418 was used (data not shown) and was matching the thymic area where thymic DC had been localized before (34, 35). Furthermore, in sharp contrast to B6-E α^d transgenic thymi (Fig. 2 *c* and Fig. 3 *a*), in B6CD11c-E α^d mice no I-E expression was detectable on cortical epithelial cells (Fig. 2 *e* and Fig. 3 *b*) even when a higher magnification and more sensitive immunofluorescence technology was used (Fig. 3 *b*).

To discriminate I-E expression between medullary epithelial cells and thymic DC we carried out double immunofluorescence analysis looking at the expression of the medullary epithelial marker UEA-1 alone (Fig. 4, *a*, *c*, and *e*) or its colocalization with the mAb specific for the transgenic I-E, 14.4.4S (Fig. 4, *b*, *d*, and *f*). UEA-1 positive medullary epithelial cells were stained green, while the cells expressing the I-E transgene stained red after detection with biotin-

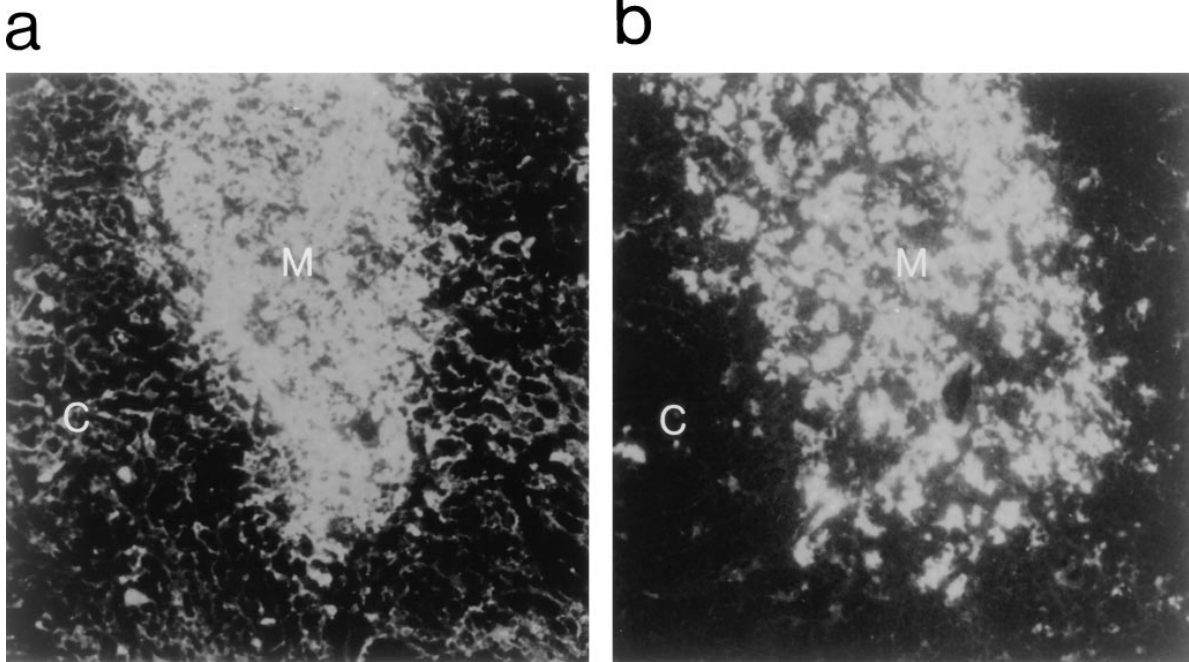


Figure 3. Immunofluorescence staining of B6- E_{α}^d (a) and B6CD11c- E_{α}^d (b) thymus with the mAb 14.4.4S specific for I-E. While B6- E_{α}^d (a) shows a typical reticular staining of cortical (C) epithelial cells, the B6CD11c- E_{α}^d thymus (b) does not display this pattern. Here, the I-E seems to be expressed only in the medulla (M) and on cells of the cortico-medullary junction. Photographs were taken at $\times 200$.

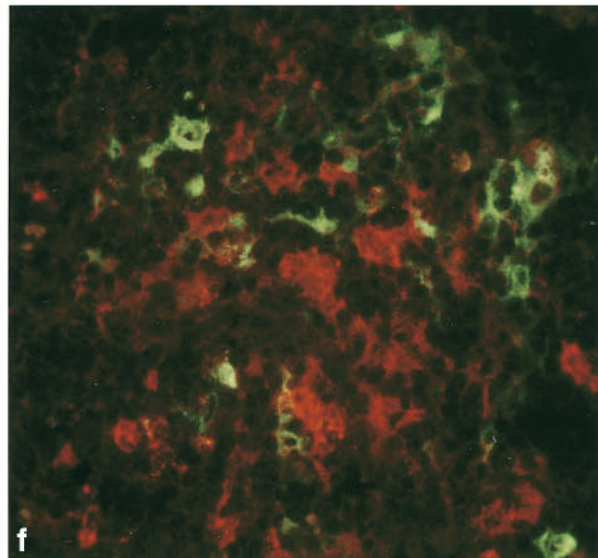
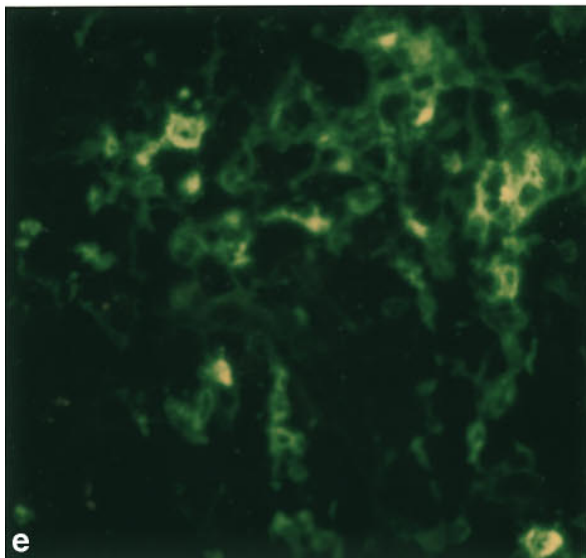
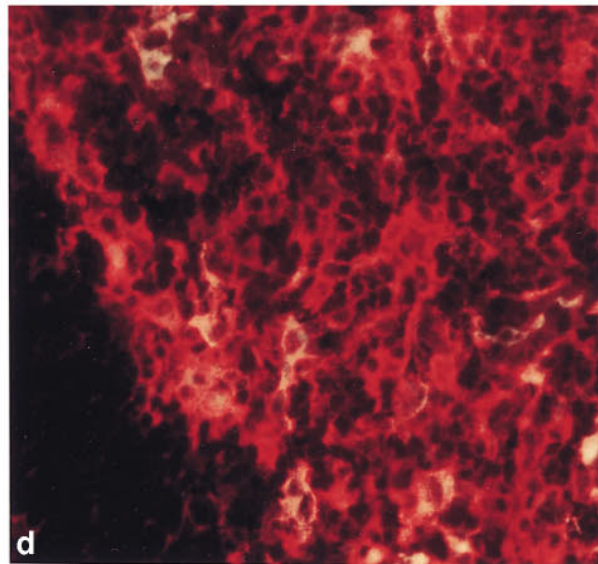
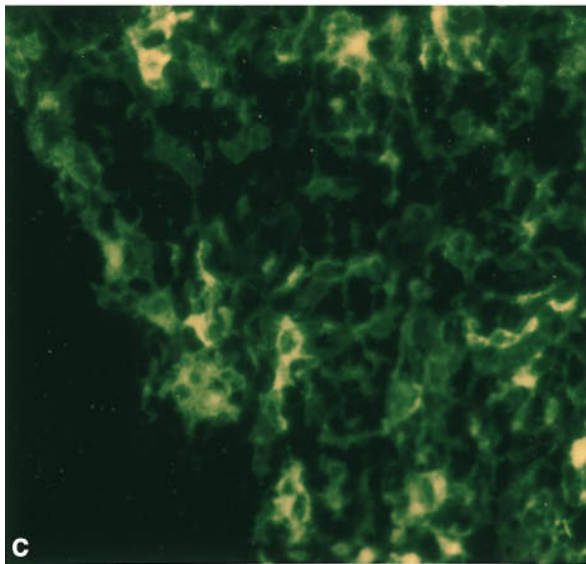
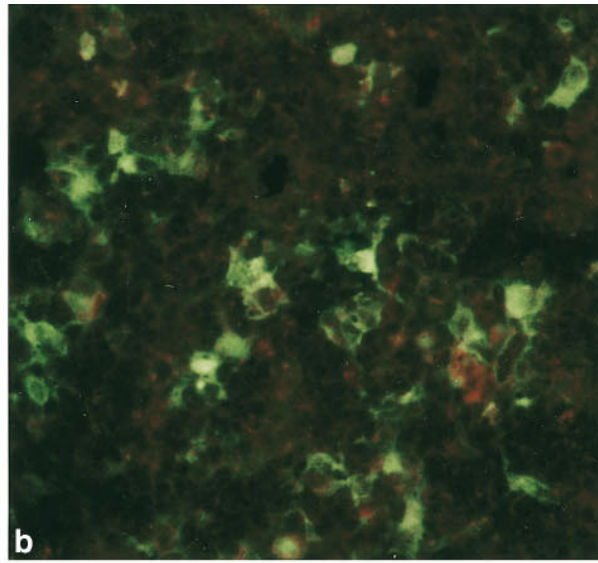
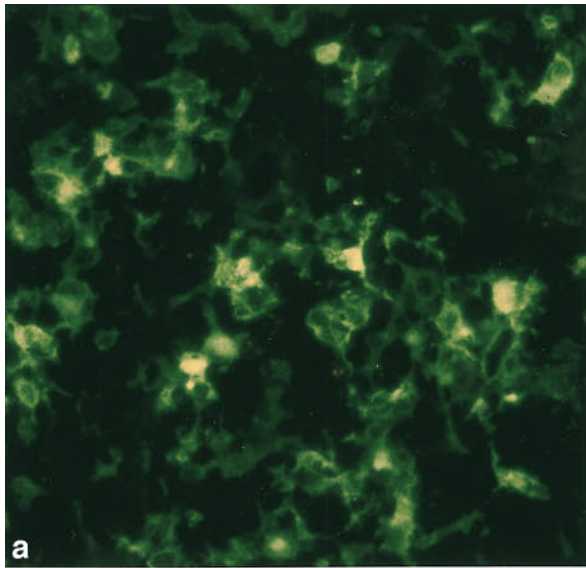
ylated mAb 14.4.4S plus avidin-TxR. While in the B6 thymus, the medulla does not show I-E expression (Fig. 4, a and b), and the B6- E_{α}^d medullary areas do express I-E (Fig. 4 d). In this case, the UEA-1-positive medullary epithelial cells (Fig. 4 c, green staining) coexpress both markers and consequently appear orange in the double fluorescence analysis (Fig. 4 d). Since UEA-1 produces very dense punctate stainings of the cell center but very weak stainings of the cell processes (36), only the central areas of epithelial cells appear orange and the cell processes of these cells appear red single positive (Fig. 4 d). In contrast, the UEA-1-positive medullary epithelial cells of the B6CD11c- E_{α}^d thymus do not express I-E and only stain green (Fig. 4, e and f), while red single positive cells of typical dendritic shape (extremely large elongated cells with long cell processes) express transgenic I-E (Fig. 4 f). An identical pattern has been revealed with MTS-10 (27), another mAb specific for medullary epithelial cells (data not shown) and confirmed the absence of transgenic I-E on medullary epithelial cells.

To further characterize the cells expressing the I-E transgene in the thymus, we isolated the light density cell fraction from collagenase-digested thymi as described earlier (29),

and performed flow cytometric analysis. The major cell type obtained in the low buoyant density fraction from thymus were cells with relatively high forward scatter (FS) and intermediate side scatter (SS) signals (data not shown); in contrast, thymocytes (from the high density fraction) had both low FS and SS (data not shown). When the low density fraction was stained for CD11c expression, 80–90% of the cells fulfilling the above described FS/SS criteria were CD11c-positive (see Fig. 5, *dendritic cells*). While more than 60% of the CD11c-positive cells from C57Bl/6 mice stained brightly for MHC class II I-A molecules, they were all negative for I-E (Fig. 5, B6). In contrast, the CD11c-positive cells from B6- E_{α}^d mice were positive for I-A, but as expected, also showed a remarkably high expression of transgenic I-E molecules (Fig. 5, B6- E_{α}^d).

When the B6CD11c- E_{α}^d mice were analyzed (Fig. 5, B6CD11c- E_{α}^d), we found a similar class II expression pattern to the B6- E_{α}^d mice pattern; >70% of the CD11c-positive cells were both I-E- and I-A-positive. All three mouse lines showed the presence of CD11c-positive thymic DC that are low or negative for MHC class II expression. This heterogeneous class II expression by DC has been described

Figure 4. Immunofluorescence staining of B6 (a and b), B6- E_{α}^d (c and d), and B6CD11c- E_{α}^d (e and f) thymus with the medullary epithelial marker UEA-1 labeled with FITC (a, c, and e) or double immunofluorescence analysis with UEA-1-FITC plus 14.4.4S specific for I-E (biotinylated and detected with Avidin-Texas red) (b, d, and f). The B6 thymus shows only green labeling (a and b) indicating the natural absence of I-E expression in this mouse strain. The B6- E_{α}^d thymus (c and d) expresses both antigens in the wild-type pattern and the green UEA-1 positive cells (c) become yellow-orange in the double fluorescence filter (d). In addition, red single positive cells become evident and are most likely nonepithelial cells expressing the I-E transgene as well as UEA-1 weak positive cell processes of epithelial cells (36). In the B6CD11c- E_{α}^d thymus (e and f), both mAbs clearly stain two different cell types and no I-E staining is visible on the UEA-1-labeled (green single positives) medullary epithelial cells (f), while other nonepithelial I-E positive cells (red single positives) of dendritic shape become evident in the medulla (f).



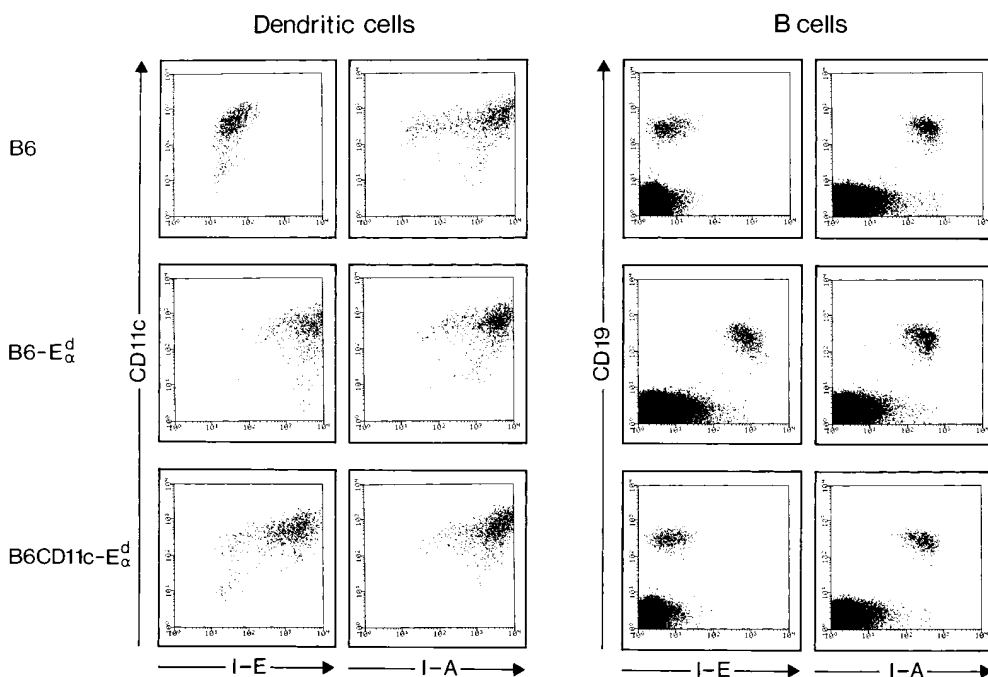


Figure 5. Expression of the CD11c-E α^d transgene leads to I-E expression on thymic DC, but not on thymic B cells. DC: Thymi of B6, B6-E α^d , and B6CD11c-E α^d mice were collagenase digested and DC were isolated after a low density gradient as described in Materials and Methods. Cells were stained with mAbs specific for CD11c (PE) and I-E or I-A (FITC), respectively. B cells: thymi of the three mouse types were complement depleted for CD4 and CD8 to enrich non-T cells (see Materials and Methods) and then stained with mAbs specific for the pan B cell antigen CD19 (PE) and I-E or I-A (FITC), respectively.

earlier (23) and was then interpreted as indicating the presence of immature DC expressing lower levels of class II MHC. In contrast to the I-A expression pattern of all three mouse strains (Fig. 5, right), all CD11c-negative cells in the B6CD11c-E α^d mice were negative for I-E (Fig. 5, B6CD11c-E α^d) indicating the strictly controlled I-E expression pattern by the CD11c promoter. Further analysis showed that the I-E transgene in the B6CD11c-E α^d strain is not expressed on CD19 $^+$ thymic B cells (Fig. 5, B cells, B6CD11c-E α^d), in contrast to the B6-E α^d strain (Fig. 5, B cells, B6-E α^d) where thymic B cells express I-E and I-A.

Further, when we analyzed peritoneal macrophages after IFN- γ treatment for class II expression, only 2–7% of the cells in these preparations showed I-E transgene expression, while the majority of macrophages were I-E-negative like the B6 control (Fig. 6, B6, B6CD11c-E α^d). Analysis of these I-E-positive cells showed that they were Mac-1 $^+$, Fc γ III/II receptor $^+$, B220 $^-$, CD19 $^-$, and CD5 $^-$ (data not shown). This indicates that, most likely, some peritoneal macrophages, but not peritoneal B cells, partially express the I-E transgene. Since macrophages are equally distributed in thymic cortex where we could not detect I-E staining in the B6CD11c-E α^d mice (see Fig. 2 e) and medulla (36), the cells expressing the I-E transgene in the thymus of the B6CD11c-E α^d transgenic animals are most likely thymic DC.

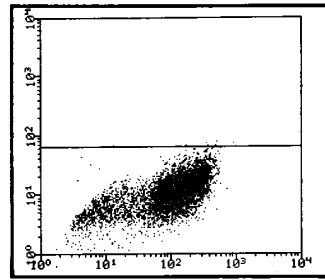
When we analyzed other lymphoid organs for transgene expression, we found a selective I-E expression pattern that was restricted to areas where DC are usually localized: in the T cell areas of the splenic white pulp, in areas at the margin of white and red pulp that are macrophage-free, and in the paracortical T cell-rich areas of LN (data not shown). In contrast, we could not detect I-E expression in B cell follicles of spleen or LN, or in epidermal Langerhans cells.

Taken together, these results indicate, that the mouse

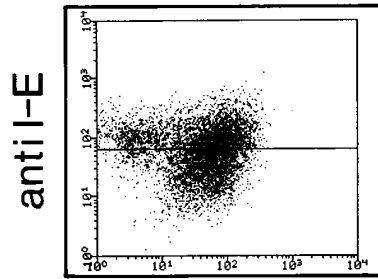
CD11c promoter, used to drive transgene expression in B6CD11c-E α^d mice, was in fact specifically active in CD11c-positive dendritic cells. This transgenic model allowed us to study the function of thymic DC in vivo.

I-E Expression Restricted to Thymic DC Mediates Clonal Deletion of V β 5 $^+$ and V β 11 $^+$ T Cells In Vivo. The selective expression of I-E on thymic DC, but not on cortical or medullary epithelial cells, B cells, or macrophages, offered an opportunity to investigate whether thymic dendritic cells in vivo would be capable of inducing clonal deletion as reported for DC from spleen (6, 9, 37) or GM-CSF-cultured thymic DC (16) in vitro. On the other hand, we were now able to test if they really could not induce negative selection in the absence of I-E $^+$ thymic B cells as initially postulated by Mazda et al. (9). To assess in vivo clonal deletion, we measured the frequency of I-E reactive V β 5 $^+$ and V β 11 $^+$ T cells in B6, B6-E α^d , and B6CD11c-E α^d mice. T cells expressing V β 5 and V β 11 genes were deleted in certain mouse strains that expressed I-E (38, 39) and retrovirally encoded superantigens in the thymus (40). Because B6 mice do not express functional I-E molecules, the frequency among total CD4 $^+$ cells of both V β 5- and V β 11-expressing CD4 $^+$ T cells is ~4%, and ~15 and 6%, respectively, in the CD8 $^+$ population (Fig. 7). In I-E transgenic mice, the efficiency of clonal deletion was reported to be maximal when the transgene was expressed on both thymic epithelium and BM-derived cells (41, 42). This situation was reflected in the case of the B6-E α^d mouse. In comparison with B6 mice, and as reported earlier (43) in this mouse strain, 65% of CD4 $^+$ V β 5 $^+$ T cells and >95% of V β 11 $^+$ CD4 $^+$ T cells were deleted (Fig. 7). In the CD8 compartment, a strong but less complete deletion was also observed (83% deletion of V β 5 $^+$ and 64% deletion of V β 11 $^+$ T cells). In T cells from the B6 CD11c-E α^d strain, we found a similar degree of deletion in the CD4 (63% V β 5 $^+$ deletion,

B6



B6- E_{α}^d



B6CD11c- E_{α}^d

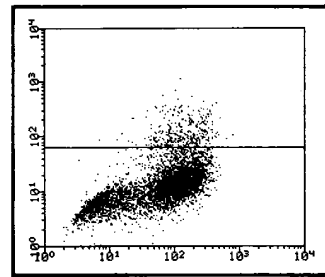


Figure 6. The majority of peritoneal lavage cells do not express the CD11c- E_{α}^d transgene. Peritoneal washes of the mice indicated were performed 5 d after an initial intraperitoneal injection of 2 ml 3% thioglycolate. The isolated cells were incubated for 48 h in IFN- γ -containing medium (1,000 U/ml) to induce MHC class II expression, and then stained with mAbs specific for I-E (PE) and I-A (FITC). Shown are all cells from the cultures with the gates set on live cells only. The percentages of I-E positive cells in B6CD11c- E_{α}^d mice varied from 2–7% in the four experiments done.

85% V β 11⁺ deletion) and significant, but less complete, deletion in the CD8 subset (75% V β 5⁺ deletion and 32% V β 11⁺ deletion) (Fig. 7). The efficiency of deletion in the CD4 compartment seems to be identical for the two situations studied. It is important to note that the absence of I-E expression on both thymic epithelium and thymic B cells in the B6CD11c- E_{α}^d thymus does not markedly reduce the efficiency of clonal deletion. This correlates with earlier studies using reaggregated organ cultures containing splenic DC as BM-derived cells, showing that professional APC clonally delete T cells much more efficiently than thymic epithelium (6). Also, in BM reconstitution experiments, Burkly et al. (43) showed that when I-E is expressed only on medullary epithelium, negative selection is only partial.

Our results support the notion that thymic DC are the most potent cell type in eliminating self-reactive T cells and

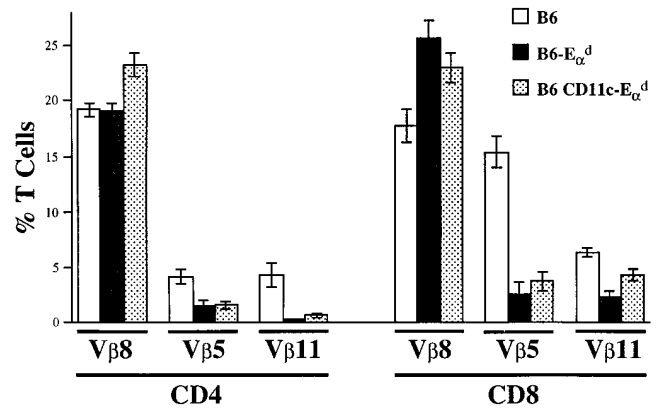


Figure 7. Clonal deletion of T cells as a consequence of E_{α}^d transgene expression. Flow cytometric analysis of V β 8, V β 5, and V β 11 expression among CD4⁺ and CD8⁺ LN T cells. B cell-depleted LN cells of the three indicated mouse strains ($n = 8$ /strain) were triple stained with anti-CD4-PE, anti-CD8-R613, and either anti-V β 8.1,8.2-FITC, V β 5.1,5.2-FITC, or V β 11-FITC, respectively. Results are expressed as a percentage of V β ⁺CD4⁺ and V β ⁺CD8⁺ cells.

that I-E expression on the epithelium or other BM-derived cells seems not to be necessary for complete clonal elimination of I-E-reactive T cells in the CD4⁺ compartment. Since the deletion observed in the CD8 T cell compartment of B6CD11c- E_{α}^d mice is less efficient than in the control situation (B6- E_{α}^d), other cell types might be involved in deletion of CD8⁺ I-E reactive T cells, while BM-derived DC are sufficient to eliminate CD4⁺ I-E reactive T cells.

Thymic DC Are Not Able to Mediate Positive Selection In Vivo. In earlier reports, targeted expression of class II molecules in transgenic mice showed that positive selection occurred only if the appropriate MHC molecules were present on cortical epithelium (and some medullary DC) (44), whereas if class II was only expressed on medullary epithelium and medullary BM-derived cells, no (45) or very little (46) positive selection was observed. Since in all of these experiments the transgene was expressed on multiple cell types, the B6CD11c- E_{α}^d strain provided the possibility to investigate the precise role of DC in positive selection in vivo.

To this end we crossed the above described mouse strains to the I-A^{-/-} line described earlier (47). When the I-A-deficient background was combined with the natural defect of I-E expression in C57BL/6 mice, this strain showed absence of all MHC class II molecules, and consequently, the absence of CD4⁺ mature T cells in thymus and periphery (47; Fig. 8, B6I-A^{-/-}). When the I-E gene was reintroduced into these mice under the control of the class II promoter, the reexpression of class II (I-E) on thymic epithelium and BM-derived cells led to the full restoration of the mature CD4⁺ T cell compartment (Fig. 8, B6- E_{α}^d I-A^{-/-}) as it has been described before (46). In contrast, when I-E expression was restricted to thymic DC (Fig. 8, B6CD11c- E_{α}^d I-A^{-/-}), restoration of the CD4⁺ compartment was not observed; the percentages of CD4⁺ T cells were identical to those of B6I-A^{-/-} mice (see legend

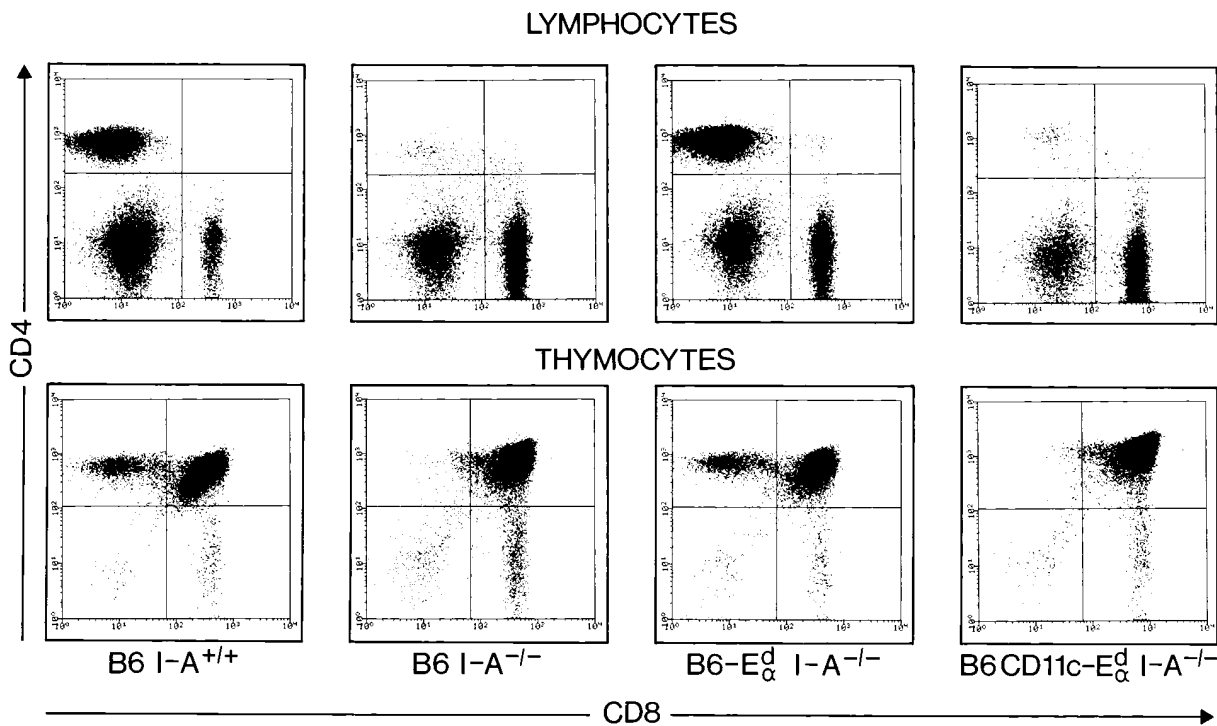


Figure 8. Influence of the E_{α}^d transgenes on the number of $CD4^+$ T cells. LN and thymus cell suspensions were stained with PE-labeled anti-CD4 and FITC-labeled anti-CD8 mAbs. The CD4/CD8 ratios in the LN T lymphocyte populations were determined from 5 mice per group and the following values were obtained: B6I-A^{+/+}, 3.03 ± 0.07 ; B6I-A^{-/-}, 0.031 ± 0.004 ; B6-E_α^dI-A^{-/-}, 2.94 ± 0.05 ; B6CD11c-E_α^dI-A^{-/-}, 0.04 ± 0.006 . The percentages of $CD4^+CD8^-$ thymocytes in the thymi of the same animals were: B6I-A^{+/+}, $8.61 \pm 0.71\%$; B6I-A^{-/-}, $1.06 \pm 0.03\%$; B6-E_α^dI-A^{-/-}, $6.96 \pm 0.51\%$; B6CD11c-E_α^dI-A^{-/-}, $0.77 \pm 0.132\%$.

to Fig. 8). Cosgrove et al. (46) have described that their ΔX mice, expressing I-E on epithelial and BM-derived cells in the medulla but not in the cortex, showed a slight increase in $CD4^+$ T cells when bred with the I-A^{-/-}-deficient background. The authors interpreted their findings by invoking a leaky expression pattern of the I-E transgene which would eventually be expressed at an albeit low, histochemically undetectable, level on cortical epithelium. Since we do not see such an increase in $CD4^+$ T cells when I-E was expressed exclusively on thymic DC, our findings argue (a) in support of the strictly defined expression pattern of the CD11c-E_α^d construct to DC and (b) that medullary epithelium could theoretically be responsible for the weak positive selection effect of $CD4^+$ T cells de-

scribed by others (46). Furthermore, the complete absence of positively selected $CD4^+$ T cells in our B6CD11c-E_α^dI-A^{-/-} strain confirms previous observations (14, 15) obtained using different in vitro experimental protocols, indicating that thymic dendritic cells in vivo are not able to induce MHC restriction of $CD4^+$ T cells.

The 5.5-kb fragment of the mouse CD11c 5' region seems to provide us with regulatory elements that drive expression of transgenes specifically in DC and not in other cell types. It might be a powerful tool, enabling us to study the roles of DC at their natural locations, avoiding complex DC isolation/culture methods, and artificial experimental setups.

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