

Survival of Mature CD4 T Lymphocytes Is Dependent on Major Histocompatibility Complex Class II-expressing Dendritic Cells

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Summary

Thymic T cell development is controlled by T cell receptor (TCR)-major histocompatibility complex (MHC) interactions, whereas a further dependence of peripheral mature T cells on TCR-MHC contact has not been described so far. To study this question, CD4 T cell survival was surveyed in mice lacking MHC class II expression and in mice expressing MHC class II exclusively on dendritic cells. Since neither of these mice positively select CD4 T cells in the thymus, they were grafted with MHC class II-positive embryonic thymic tissue, which had been depleted of bone marrow derived cells. Although the thymus grafts in both hosts were repopulated with host origin thymocytes of identical phenotype and numbers, an accumulation of CD4⁺ T cells in peripheral lymphoid organs could only be observed in mice expressing MHC class II on dendritic cells, but not in mice that were completely MHC class II deficient. As assessed by histology, the accumulating peripheral CD4 T cells were found to be in close contact with MHC class II⁺ dendritic cells, suggesting that CD4 T cells need peripheral MHC class II expression for survival and that class II⁺ dendritic cells might play an important role for the longevity of CD4 T cells.

Thymic positive selection is a process that generates mature CD4⁺ and CD8⁺ single-positive T lymphocytes from CD4⁺CD8⁺ double-positive thymocytes. The mechanistic control of positive selection is the interaction between TCR on thymocytes and MHC-encoded molecules on thymic epithelial cells. Mature CD4⁺ and CD8⁺ single-positive thymocytes, selected on MHC class II and I, respectively, subsequently leave the thymus and seed the peripheral lymphoid organs (1-3). Consequently, CD4⁺ single-positive thymocytes and CD4⁺ peripheral T cells are nearly absent in class II-deficient mice (4, 5).

The further survival of peripheral T cells seems not to be dependent on antigen-specific TCR-MHC interactions. Transfer experiments performed with T cells from TCR-transgenic mice in the presence or absence of antigen (6, 7) showed that specific Ag is not necessary for T cell survival. In another experimental model, Sprent et al. (8) demonstrated that when unseparated lymph node cell suspensions were injected into H-2 identical SCID hosts, they formed a self-sufficient pool of lymphocytes. T cells survived in this system without reduction in numbers in the absence of antigen. However, conflicting results have been reported on the survival of T cells in the absence of MHC molecules expressed on hematopoietic cells. When irradiated normal mice received bone marrow from class II-deficient mice,

normal CD4 T cell repopulation was observed in one study (9). Others doing the same experiment could not detect reconstitution of the CD4 compartment in the MHC class II-negative environment of such mice (10). Huss et al. speculated that this discrepancy could have been caused by the different time spans of bone marrow inoculum in the host mice used by the two groups or different bone marrow treatments (e.g., T cell depletion) before injection (10). Therefore, these experiments could not definitely clarify the question of whether peripheral CD4 T cell survival is dependent of peripheral MHC class II expression.

In a recent report, Takeda et al. (11) transplanted untreated fetal thymi from MHC class II⁺ mice under the kidney capsules of class II⁺, as well as class II-deficient, hosts. The authors observed an identical initial donor type CD4⁺ T cell accumulation in the periphery of both hosts. In comparison to the MHC class II⁺ mice, the class II-deficient hosts showed faster declining numbers of peripheral CD4⁺ T cells. These results suggested that interactions between CD4⁺ T cells and MHC class II⁺ peripheral cells are not necessary for short-term survival, but might be important for longevity of T cells. However, a potential contamination of the MHC class II-deficient peripheral organs of the hosts with MHC class II⁺ donor type cells originating from the transplanted thymi (thymic dendritic cells, B cells, mac-

rophages) cannot be excluded when the thymus grafts (TGs),¹ are not depleted of hemopoietic cells before transplantation. Furthermore, the initial export of large numbers of donor-type thymocytes from untreated grafts (12) might not reflect the actual kinetics of thymocyte export from a developing thymus.

To avoid the presence of donor-type thymocytes and to exclude the possibility of contamination of the hosts with thymus-derived MHC class II-positive cells, in this report MHC class II⁺ fetal TGs were depleted of hematopoietic cells before transplantation. Then survival of host-type CD4 T cells in a host lacking MHC class II expression completely (4) was compared to CD4 T cell survival in an environment where only dendritic cells (DCs) express MHC class II (13). Although TGs in both types of hosts were repopulated with similar numbers of phenotypically identical host-type thymocytes, only the mice expressing MHC class II on DCs, but not the conventional MHC class II-deficient hosts, showed accumulation of CD4 T cells in blood and peripheral lymphoid organs. The conclusion from these experiments is that CD4 T cells, after having left the thymus, do need interaction with MHC class II-positive peripheral cells for survival. MHC class II-positive DCs are sufficient to ensure this survival.

Materials and Methods

Mice. Transgenic mice expressing the MHC class II I-E_α^d transgene under control of the mouse CD11c promoter were bred to MHC class II^{-/-} (B6 I-A^{-/-}) mice (4, 13). The resulting mice have been named B6CD11c-E_α^dI-A^{-/-} in this study. Line 107.1 expressing I-E_α^d under the control of the MHC class II promoter has been described earlier (14) and was crossed onto the MHC class II^{-/-} background (B6-E_α^dI-A^{-/-}). All mice were bred in the animal colony of the Basel Institute for Immunology (Basel, Switzerland).

mAbs and Reagents. The mAbs specific for CD4 (No. 09005), CD8 (No. 01044), CD3 (No. 01085), heat stable antigen (HSA; No. 01575), CD69 (No. 01505), Vβ2 TCR (No. 01634), Vβ4 TCR (No. 01934), Vβ5.1/5.2 TCR (No. 01354), Vβ6 TCR (No. 01364), Vβ7 TCR (No. 01424), Vβ8.1/8.2 TCR (No. 01344), Vβ11 TCR (No. 01374), Vβ13 TCR (No. 01394), I-E_α (No. 09625), CD11c (No. 09705), CD62L (No. 01264), and CD11b (No. 01712) were purchased from PharMingen (San Diego, CA). Anti-CD4 R613 and streptavidin-R613 (GIBCO BRL, Gaithersburg, MD) were used for three-color fluorescence analysis. The rat anti-mouse mAbs 33D1 (TIB227; American Type Culture Collection, Rockville, MD) and NLDC-145 (reference 15; a kind gift of A. Schneeberger, Vienna, Austria) were used as culture supernatants. With these mAbs, flow cytometry was performed on a FACStar[®] (Becton Dickinson, Mountain View, CA) instrument. Single cell preparation, staining and FACS[®] analysis was done according to standard procedures.

Isolation of DCs from Spleen. Spleens of different mice were digested with collagenase (CLSPA; Worthington Biochemical Corp., Freehold, NJ) twice for 30 min at 37°C as described previously (13). Cells were then recovered by centrifugation at 300 g

for 5 min, resuspended in a 17% Optiprep[™] (Nycomed Pharma, Oslo, Norway) solution diluted in Hank's balanced salt solution without Ca²⁺ and Mg²⁺, overlaid with 12% Optiprep[™] 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. Cells were then centrifuged at 600 g for 15 min at 20°C. The low density cells at the Hank's 12% Optiprep[™] interface were harvested, washed twice, and used as a splenic DC-enriched fraction.

Transplantation of Fetal Thymus. Fetal thymi from C57BL/6 × DBA/2 crosses were taken at day 14 of gestation (vaginal plug was counted as day 0 of pregnancy). The thymic lobes were placed on culture plate inserts (Millipore No. PIHA01250; Bedford, MA) floating on culture medium containing 1.35 mM 2'-deoxyguanosine (dGuo; No. D-0901; Sigma Chemical Co., St. Louis, MO). Thymi were incubated at 37°C, 6% CO₂ for 5 d, and received an additional irradiation of 3,000 Rad when they were washed in a large volume of PBS to remove the drug. Each mouse received two thymic lobes under the kidney capsule.

Immunohistology. Fresh organs were embedded in O.C.T. medium (No. 4583; Miles Inc., Elkhart, IN), snap frozen, and 6-mm sections were cut with a cryostat. Sections were air dried (60 s), acetone fixed (2 s), and finally air dried for a minimum of 12 h. Sections were 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 s. After washing, sections were either incubated with the second step reagents or directly mounted in Fluoromount (Southern Biotechnology Assoc. Inc., Birmingham, AL). For immunohistological analysis, we used biotinylated mAbs and alkaline phosphatase-conjugated streptavidin (No. RPN 1234; Amersham, Buckinghamshire, UK). Color reaction was done with the Vector-Red Alkaline Phosphatase Substrate Kit (No. SK-5100; Vector Labs., Inc., Burlingame, CA) according to the manufacturer's instructions.

Isolation and Analysis of Peripheral Blood Lymphocytes. Mice were bled at their tail veins and the blood was mixed immediately with Heparin-containing PBS. Lymphocytes were then isolated over a ficoll gradient, washed twice, and 10⁵ viable cells were stained with 20 mg/ml mAb that was directly labeled. After washing, cells were analyzed using a FACScan[®].

Results

To investigate survival of CD4⁺ T cells in different environments, we studied CD4⁺ T cell repopulation of two different mouse strains lacking endogenous CD4⁺ T cells: conventional MHC class II-deficient mice (B6I-A^{-/-}; reference 4) or the same mice transgenically reconstituted for MHC class II I-E expression on DCs (B6CD11c-E_α^dI-A^{-/-}; reference 13).

MHC Class I-E Expression in B6CD11c-E_α^dI-A^{-/-} Mice Is Restricted to CD11c⁺ DCs. As described recently (13, 16), C57BL/6 mice expressing MHC class II I-E_α^d as a transgene under the control of CD11c regulatory elements, display class II I-E expression only on DCs. When bred to the class II-deficient (I-A^{-/-}) background (4), the presence of MHC class II I-E on DCs does not lead to positive selection of mature CD4⁺ thymocytes (13). By immunohistochemical (Fig. 1 a) and FACS[®] analysis of lymph nodes

¹ Abbreviations used in this paper: DC, dendritic cell; dGuo, 2'-deoxyguanosine; HSA, heat stable antigen; IDC, interdigitating cell; TG, thymus graft.

and spleen (Fig. 1 *b*), I-E transgene expression correlates with expression of the DC marker CD11c in B6CD11c- E_{α}^d I-A $^{-/-}$ mice. In lymph nodes of B6CD11c- E_{α}^d I-A $^{-/-}$ mice, transgenic I-E expression is restricted to DCs of the paracortical T cell area, whereas the B cells in follicles, as defined with an anti-IgM reagent, fail to express the transgene (Fig. 1 *a*). A similar I-E pattern is observed in the spleen, where again B cell areas are negative for transgene expression, whereas DCs in T areas of white pulp as well as marginal zone DCs express I-E (Fig. 1 *a*). These findings are in agreement with our recent reports, describing the absence of I-E transgene expression on CD19 $^{+}$ thymic B cells (13) and B220 $^{+}$ splenic B cells (16). Macrophages as detected with anti-CD11b (Mac-1) reagents can be located outside of the white pulp areas of the spleen and give a weak staining scattered all over these regions (Fig. 1 *a*, *Mac-1*). Furthermore, these cells can be localized in the outer regions of the marginal zone, whereas a weaker Mac-1 staining of the T areas of the white pulp might be due to Mac-1 expression on DCs, as reported previously (for review see reference 17). In lymph nodes, Mac-1-positive macrophages could be found predominantly in the lateral regions of the paracortex (Fig. 1 *a*). Since the I-E staining in lymph node and spleen is localized in those regions that also stain positive for CD11c, but not in those that are stained with IgM and Mac-1 reagents, it seems that the CD11c regulatory elements drive expression of the transgenic I-E in a restricted manner to CD11c-positive cells. Previously, we reported that 2–7% of peritoneal IFN- γ -treated cells of B6CD11c- E_{α}^d mice would express the I-E transgene (13, 16). Those cells were Mac-1 $^{+}$ and Fc γ III/II receptor $^{+}$, and could also be found in the B6CD11c- E_{α}^d I-A $^{-/-}$ mice used in this study (references 13, 16, and data not shown).

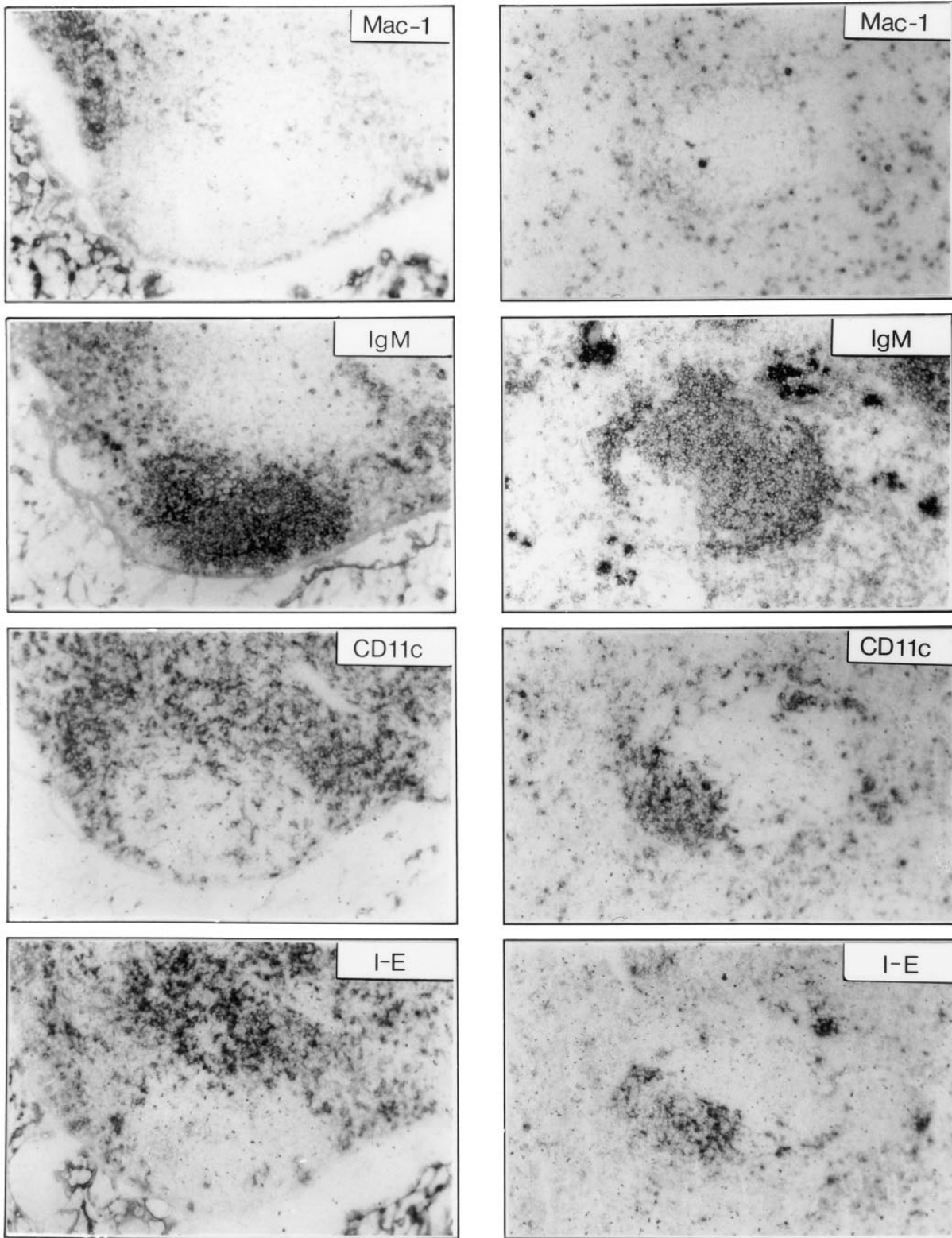
To further characterize the cells expressing the I-E transgene, we isolated the light density cell fraction from collagenase-treated spleens as described earlier (18) and performed a three-color flow cytometric analysis (Fig. 1 *b*). From all cells obtained in the low buoyant density fraction, ~80% were cells with relatively high forward scatter and intermediate side scatter signals (data not shown), whereas lymphocytes from the high density fraction had both low forward and side scatter signals (data not shown). Analysis of the cells from the low density fraction, which fulfilled the indicated forward/side scatter criteria, showed that ~70% were CD11c positive (Fig. 1 *b*). CD11c-positive cells derived from B6I-A $^{-/-}$ mice were obviously negative for the transgenic I-E (Fig. 1 *b*, *left*). In contrast, the corresponding cells derived from the transgenic B6CD11c- E_{α}^d I-A $^{-/-}$ mice showed expression of I-E on ~70–80% of the CD11c-positive fraction as previously reported (16). To identify more precisely the distribution of transgene expression in distinct splenic DC subpopulations, we used additional DC markers in a three-color FACS $^{\circ}$ analysis. The DC-restricted mAb 33D1 (19) has been described to react with at least 80% of all splenic DCs (20), whereas the remaining 33D1-negative DC fraction of the spleen corresponds to a minor subpopulation reacting with the mAb

NLDC-145 (20); NLDC-145 is a marker for interdigitating cells (IDC; reference 21). It has been proposed that the bulk of DCs in spleen are the less mature CD11c $^{+}$ NLDC-145 $^{-}$ (33D1 $^{+}$) cells and are located at the periphery of the white pulp nodule. Those cells would mature into the CD11c $^{+}$ NLDC-145 $^{+}$ (33D1 $^{-}$) IDC phenotype, and be then located in the central area of the periarteriolar sheath (17, 20, 22, 23). In concordance, the CD11c $^{+}$ I-E $^{-}$ DCs from the transgene negative B6I-A $^{-/-}$ mice (Fig. 1 *b*, *left*) were mostly NLDC-145 $^{-}$ or 33D1 $^{+}$, whereas a minor fraction was NLDC-145 $^{+}$ or 33D1 $^{-}$, respectively (Fig. 1 *b*, *left*, *lower histograms*). To further analyze DCs from the transgenic B6CD11c- E_{α}^d I-A $^{-/-}$ mice, the two gates shown in Fig. 1 *b* (*right upper plot*) were set on either transgene expressing (CD11c $^{+}$ I-E $^{+}$) or nonexpressing DC (CD11c $^{+}$ I-E $^{-}$). This analysis revealed that the transgenic I-E is expressed in all types of DCs, since the CD11c $^{+}$ I-E $^{+}$ subpopulation could be further subdivided into NLDC-145 $^{+}$ and NLDC-145 $^{-}$ cells. Similarly, the CD11c $^{+}$ I-E $^{+}$ DCs are splitting up into 33D1 $^{+}$ and 33D1 $^{-}$ cells, respectively (Fig. 1 *b*, *right*, CD11c $^{+}$ I-E $^{+}$). The smaller CD11c $^{+}$ I-E $^{-}$ subpopulation, which does not express the transgenic I-E, behaves identically to the CD11c $^{+}$ I-E $^{+}$ fraction, namely representing DCs from all phenotypes, NLDC-145 $^{+}$, NLDC-145 $^{-}$, 33D1 $^{+}$, and 33D1 $^{-}$ (Fig. 1 *b*, *right*, CD11c $^{+}$ I-E $^{-}$). Taken together, the immunohistochemical analysis (Fig. 1 *a*), the flow cytometric data (Fig. 1 *b*) and our previous report (16) demonstrate that expression of the transgenic I-E is found on ~70–80% of all DCs in lymph nodes and spleen. Transgene expression is not restricted to a certain DC subpopulation, since it can be found on all types of CD11c $^{+}$ DCs of the spleen including NLDC-145 $^{+}$ 33D1 $^{-}$ IDCs in the T areas as well as NLDC-145 $^{-}$ 33D1 $^{+}$ cells representing DCs from peripheral white pulp areas (20). The absence of transgene expression on 20–30% of CD11c $^{+}$ DCs might be due to missing regulatory elements that could be located further upstream of the promoter-containing DNA segment used in the transgenic construct (13). Another possibility is the absence of enhancer elements that could be located in introns of the CD11c gene and that are therefore not included in the transgenic construct.

Taken together, these data indicate that the majority of macrophages (>90%) do not express the transgene and that the CD11c promoter used in this study drives expression of the I-E transgene specifically to most of the DC in spleen and lymph nodes. Due to absence of mature CD4 $^{+}$ T cells, the B6CD11c- E_{α}^d I-A $^{-/-}$ mice and the nontransgenic class II-deficient B6I-A $^{-/-}$ mice offer a unique system to study the role of MHC class II $^{+}$ DCs in survival of mature CD4 $^{+}$ T cells.

Fetal Thymic Grafts Are Repopulated Identically in Both Hosts. To reconstitute B6CD11c- E_{α}^d I-A $^{-/-}$ and B6I-A $^{-/-}$ mice with mature CD4 $^{+}$ T cells, both types of mice were engrafted with fetal thymi from MHC class II $^{+}$ mice. To ensure that the fetal thymus would not release any MHC class II $^{+}$ cells, the grafts were treated with 2-dGuo plus an additional 3,000 Rad irradiation before transplantation,

A



Lymph node

Spleen

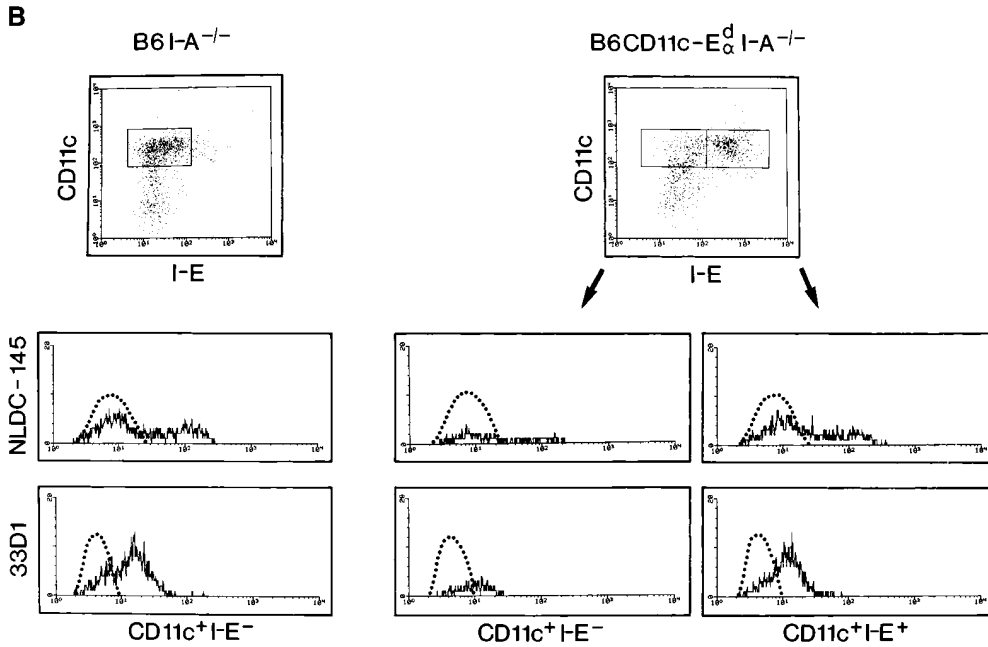


Figure 1. (a) MHC class II I-E transgene expression correlates to CD11c expression in B6CD11c-E α^d I-A $^{-/-}$ transgenic mice. Immunohistochemical analysis of normal adult lymph nodes (*left*) and spleen (*right*) from B6CD11c-E α^d I-A $^{-/-}$ mice (13). Biotinylated antibodies specific for CD11b (Mac-1), CD11c, MHC class II I-E, or IgM were used on serial cryostat sections of frozen organs and developed with streptavidin-alkaline phosphatase. Transgenic I-E expression correlates to CD11c staining in lymph node and spleen, but spares IgM-positive B cells and most of the Mac-1-positive macrophages. All sections were originally photographed at $\times 200$. (b) Transgenic I-E expression correlates with CD11c expression on DCs and can be found on less as well as more mature DC subpopulations. Three-color flow cyto-

metric analysis of low density spleen cells from a low buoyant density gradient was performed. Only cells meeting high forward scatter and intermediate side scatter criteria are shown in this analysis. Cells falling within the gates shown in the top panels (CD11c, I-E) were further analyzed with the mAb NLDC-145 and 33D1 (lower histograms, *solid lines*). The controls for the second step reagent are shown as dotted lines (no first step mAb).

procedures which have separately been described to efficiently and completely remove bone marrow-derived cells from the thymus (24–26). Survival and function of the TG was monitored every 3 wk by killing one animal per experimental group, reisolating the grafted thymic tissue from the kidney capsule, and submitting the thymic cell suspension to FACS[®] analysis. The three-color FACS[®] analysis shown in Fig. 2 was performed with reisolated thymic grafts at 17 wk after transplantation. First, the thymocytes were analyzed according to their CD4 and CD8 expression and gated in distinct developmental stages between CD4⁺CD8⁺ double-positive and CD4⁺ single-positive thymocytes (Fig. 2, *left, dot histograms*, gates 1–3). The cells in gates 1–3 were then further analyzed according to their expression of the classical thymocyte maturation markers CD3, HSA, and CD69 (27–29). Although CD3^{dull} thymocytes could only be detected in gate 1 within the more immature CD4⁺CD8⁺ double-positive population (see Fig. 2, *right panel histograms*, CD3 gate 1), CD3^{high} thymocytes did accumulate with ongoing maturation (Fig. 2, *right panel histograms*, CD3 gate 2 and CD3 gate 3).

We then analyzed the expression of HSA. As described previously, immature thymocytes express high levels of HSA and lose this marker on their way to mature single positive cells (27). Accordingly, thymocytes from TG of B6I-A $^{-/-}$ as well as B6CD11c-E α^d I-A $^{-/-}$ hosts were HSA^{high} only in their immature stage (Fig. 2, *right panel histograms*, HSA gate 1) and lost this marker gradually with maturation (Fig. 2, *right panel histograms*, HSA gates 2 and 3).

When CD69 expression in the thymocytes of different origins was compared, we found the expected transient

CD69 upregulation (Fig. 2, *right panel histograms*, CD69 gate 2; reference 28). With further maturation, the percentage of CD69^{high} thymocytes was decreasing again (Fig. 2, *right panel histograms*, CD69 gate 3).

These data demonstrate that the TG in B6I-A $^{-/-}$ as well as in B6CD11c-E α^d I-A $^{-/-}$ hosts were accepted, functional, and had been efficiently repopulated by T cell precursors, which gave rise to mature thymocytes as defined by a CD4^{high}, CD3^{high}, HSA^{low}, CD69^{low} phenotype (Fig. 2). Thymocyte origin from host bone marrow was confirmed by absence of staining for MHC class I K^d molecules (data not shown). From weeks 3 to 21 after transplantation, a normal development of thymocytes in the grafted embryonic thymi of the different host animals was observed as described in Fig. 2 for week 17 post-transplantation thymi. At the beginning of the experimental period, the TG contained $\sim 3 \times 10^6$ thymocytes (3–10 wk after transplantation) with a tendency to decrease in cell numbers down to 1×10^6 in wk 17 (data not shown).

Because all mice ($n = 2 \times 20$) in this experiment received the same identically treated embryonic thymi and repopulated them with thymocytes with a similar efficiency, this experimental system seems to be ideal to study the further development and survival of CD4 lineage T cells in MHC class II disparate host environments.

Repopulation of Peripheral Lymphoid Organs and Blood with Mature CD4 T Cells Is Observed Only in B6CD11c-E α^d I-A $^{-/-}$ Mice. In the B6CD11c-E α^d I-A $^{-/-}$ group, 16 mice received embryonic thymus transplants under their kidney capsules. Seven of these mice were killed during the course of the experiment and their thymic transplants re-

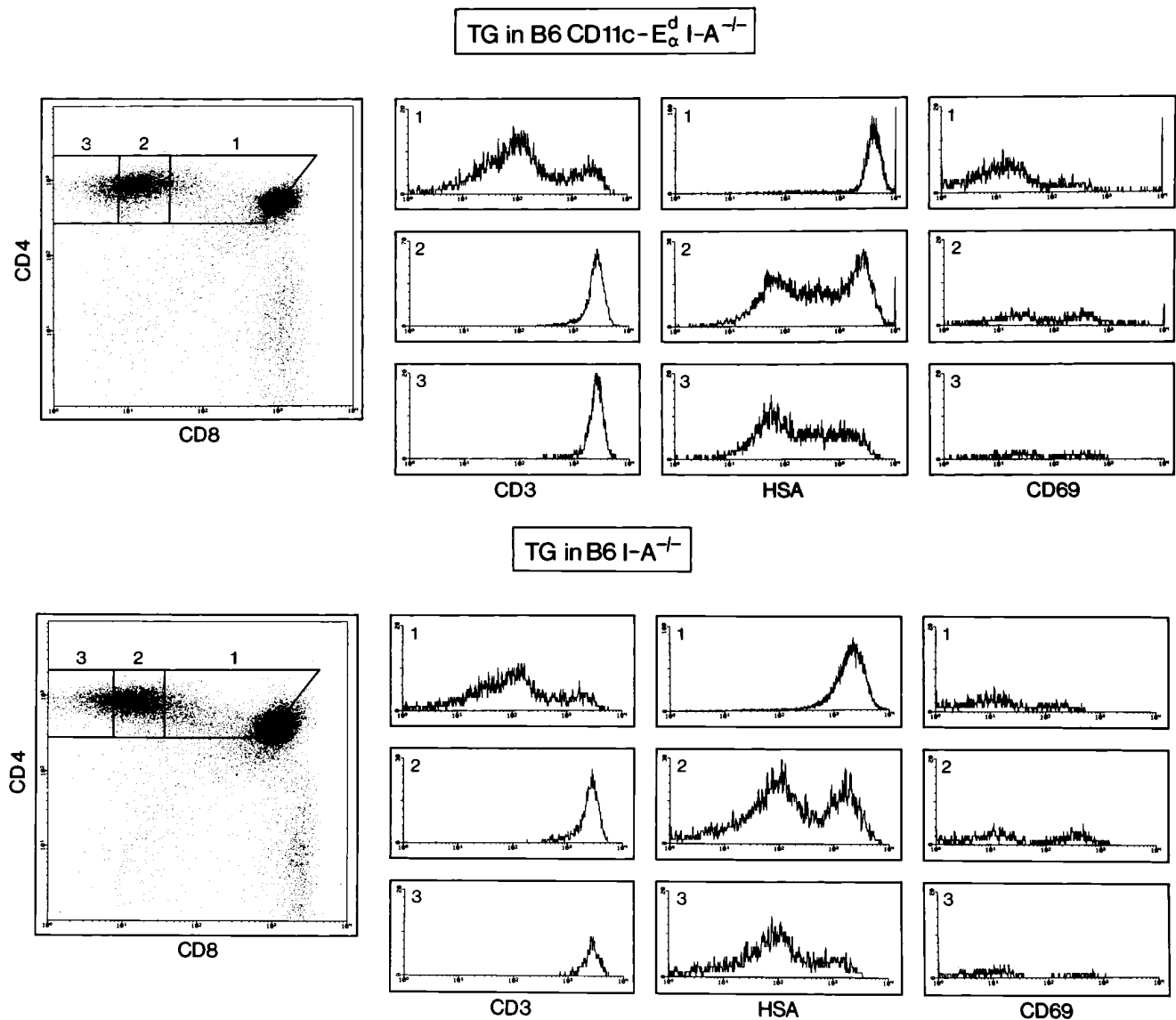


Figure 2. Identical thymocyte repopulation of thymic grafts in B6CD11c-E α^d I-A $^{-/-}$ and B6I-A $^{-/-}$ mice. Three-color FACS[®] analysis of fetal thymic grafts at wk 17 after transplantation. CD4 lineage thymocytes were divided into the indicated gates 1, 2, and 3 and further analyzed for expression of CD3, HSA, and CD69. In TG from both hosts, total thymocyte numbers were $\sim 3 \times 10^6$ from wk 3–8 and $\sim 10^6$ in wk 17–21 after transplantation.

isolated and analyzed by FACS[®] as shown in Fig. 2. Nine mice from this group were kept during the whole experimental period of 21 wk and their peripheral blood lymphocytes analyzed as shown in Fig. 3 (Fig. 3, B6CD11c-E α^d I-A $^{-/-}$). Taken together, all 16 thymus-transplanted B6CD11c-E α^d I-A $^{-/-}$ mice showed a substantial peripheral reconstitution with CD4⁺ peripheral T cells in blood (Fig. 3), lymph nodes (see Fig. 4) and spleen (data not shown).

In the other experimental group, 20 MHC class II-negative B6I-A $^{-/-}$ mice had received thymic transplants under their kidney capsule. 11 mice from this group, the data of which are not included in Fig. 3, were killed at different time points after transplantation to ensure repopulation of the thymic graft with thymocytes. The data shown in Fig. 3 were derived from nine transplanted B6I-A $^{-/-}$ mice that

were killed after 21 wk and analyzed for presence of TGs. As a result, all 20 transplanted B6I-A $^{-/-}$ mice analyzed had accepted and repopulated the TGs with thymocytes as shown in Fig. 2. Nevertheless, the percentage of CD4⁺ T cells among the peripheral blood lymphocytes of all 20 thymus-transplanted class II-deficient B6I-A $^{-/-}$ mice did at no time differ from that observed in untransplanted B6I-A $^{-/-}$ mice (2–5%; Fig. 3, B6I-A $^{-/-}$ references 4, 5); neither could CD4⁺ T cells be found in spleen (data not shown) or lymph nodes (see Fig. 4). Surprisingly, the identical development of mature CD4⁺ thymocytes in the TG of both host types did not lead to a comparable accumulation of peripheral CD4⁺ T cells.

To check the possibility that the surviving CD4⁺ T cells were an accumulation of (self) antigen-specific cells that had

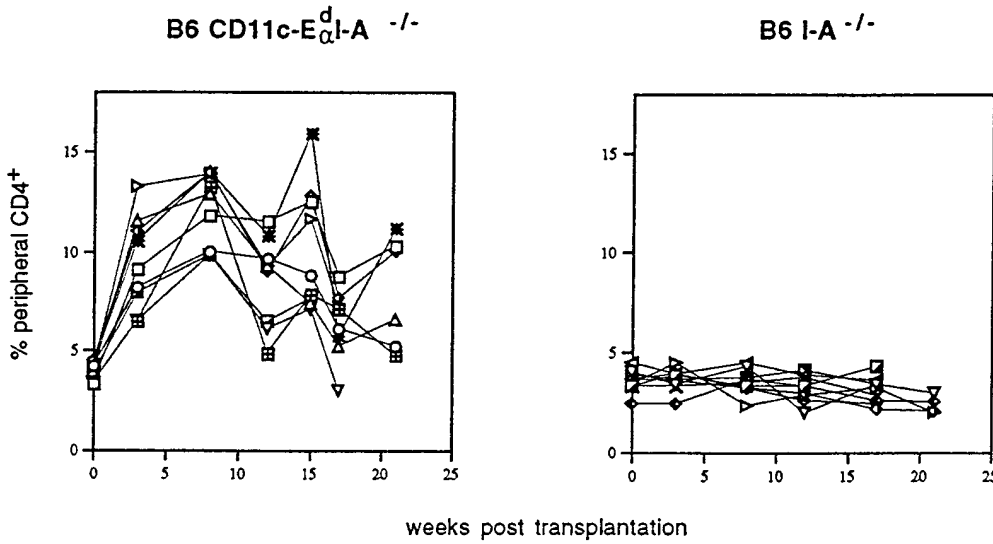


Figure 3. Accumulation of peripheral CD4⁺ T cells can be detected in thymus-grafted B6CD11c-E_α^dI-A^{-/-} mice, but not in nontransgenic thymus-grafted B6I-A^{-/-} mice. Animals were bled, leukocytes purified by ficoll gradient centrifugation, stained with mAbs specific for CD4 and CD8, and analyzed by flow cytometry. The percentages of CD4⁺ cells of total blood leukocytes from 9 individual mice/group are shown.

been stimulated by I-E on DCs in B6CD11c-E_α^dI-A^{-/-} mice, we performed FACS[®] analysis with markers discriminating between activated and resting T cells (Fig. 4). CD4/CD8 staining of lymph node cells (Fig. 4) and splenocytes (data not shown) confirmed the presence of CD4⁺ T cells in transplanted B6CD11c-E_α^dI-A^{-/-}, but not in transplanted B6I-A^{-/-} mice. CD4⁺ T cells from TG⁺ and TG⁻ B6I-A^{-/-} mice (and untransplanted B6CD11c-E_α^dI-A^{-/-} mice, data not shown) showed relatively low α/β-TCR surface expression levels, as has been reported for the few atypical CD4⁺ T cells of class II-deficient mice (5). In con-

trast, the CD4⁺ T cells from transplanted B6CD11c-E_α^dI-A^{-/-} mice expressed normal TCR levels, resembling CD4⁺ T cells from B6E_α^dI-A^{-/-} control mice, which express MHC class II I-E under the control of a class II promoter and do positively select CD4⁺ T cells in their own thymus (references 13, 14; Fig. 4, *bottom row*). Furthermore, analysis of total CD4⁺ T cell numbers within the various subsets as defined by staining with mAb-specific for Mel-14 and CD69 demonstrates that the accumulating CD4⁺ T cells in transplanted B6CD11c-E_α^dI-A^{-/-} mice distribute equally into naive (Mel-14⁺/CD69⁻) and activated (Mel-

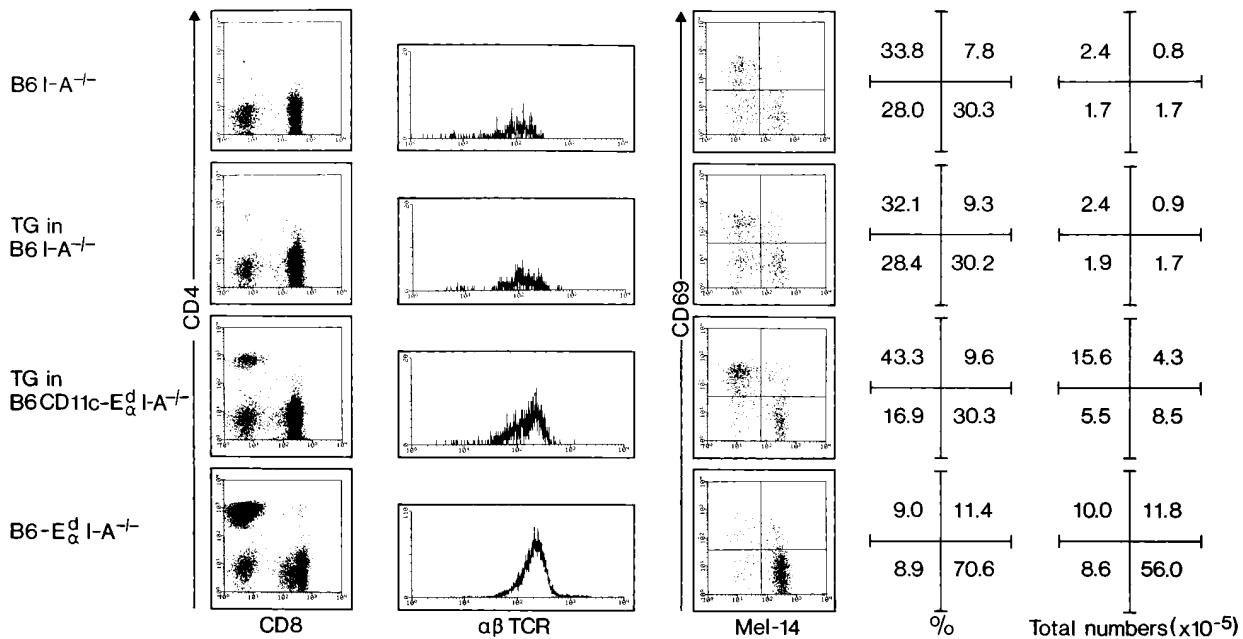


Figure 4. Analysis of lymph node cells from TG⁻ and TG⁺ B6I-A^{-/-}, TG⁺ B6CD11c-E_α^dI-A^{-/-}, and B6-E_α^dI-A^{-/-} mice (control mice expressing transgenic I-E under control of the MHC class II promoter; references 13, 14). Cell suspensions from lymph nodes of the indicated mice were analyzed by flow cytometry. Three-color analysis with mAbs specific for CD4, CD8, and α/β-TCR is shown in the two left sets of panels. The histograms for α/β-TCR staining and the Mel-14/CD69 staining was performed on gated CD4⁺ T cells. The percentages and total cell numbers falling within the indicated regions from the Mel-14/CD69 stainings are shown on the right side and are an average of 3 animals/group (percentage, total cell numbers).

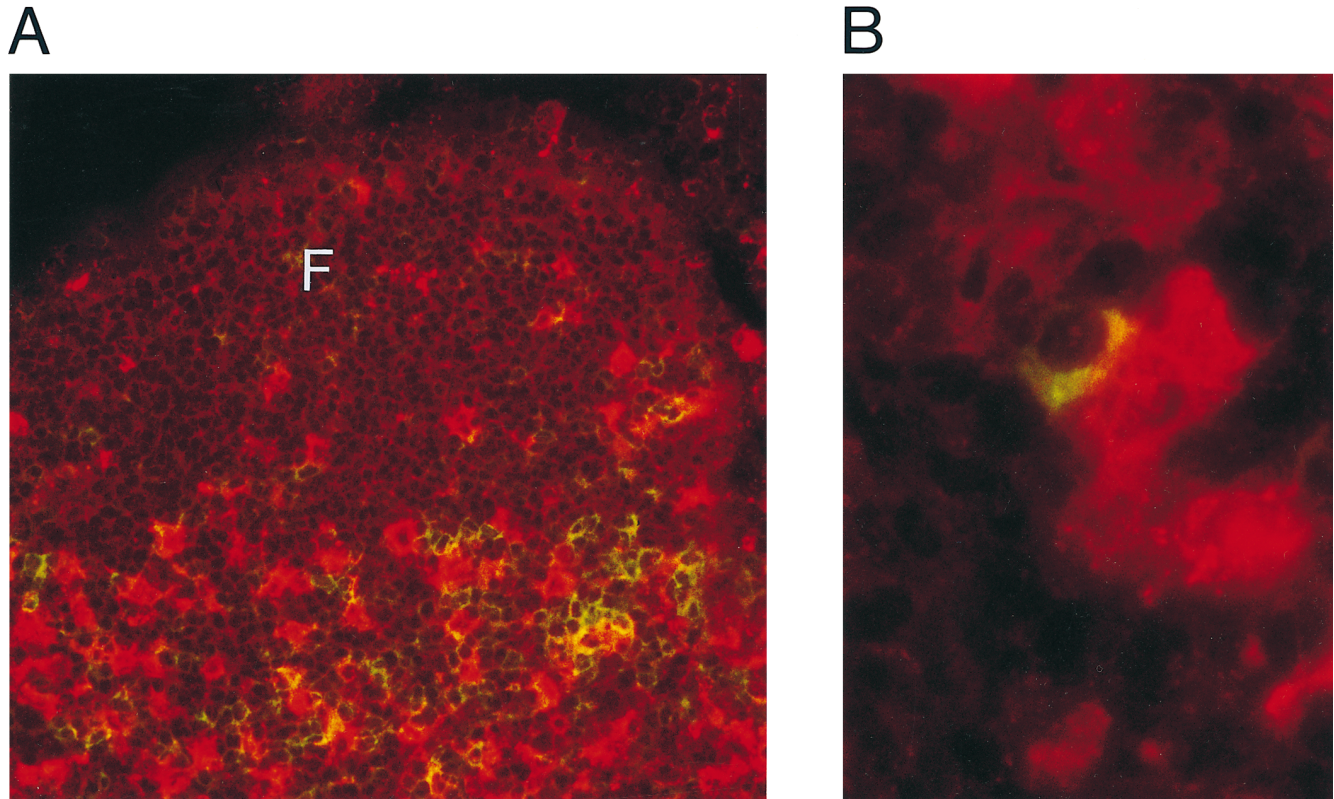


Figure 5. Peripheral CD4⁺ T cells accumulate in T cell areas of lymph nodes in close proximity to I-E⁺ DCs. Double immunofluorescence analysis of a lymph node from a TG⁺ B6CD11c-E_α^dI-A^{-/-} mouse at 8 wk after thymus transplantation. (A) CD4⁺ T cells (anti-CD4-FITC, *green*) are found in the paracortical area of the lymph node, whereas I-E-expressing DCs (anti-I-E-PE, *red*) are localized, but not in the primary B follicle (*F*) (original magnification: 200). (B) Higher magnification (1,000) of the same area.

14⁻/CD69⁺) subsets (Fig. 4). Similar results were obtained, when a mAb-specific for ppg-1 was used (data not shown).

We also examined the frequency of T cells expressing various TCR V β chains (V β 2, 4, 5, 6, 7, 8, 11, and 13; data not shown). This analysis confirmed that the CD4⁺ T cells from transplanted B6CD11c-E_α^dI-A^{-/-} mice were a polyclonal population, and therefore an oligoclonal expansion of CD4⁺ T cells (e.g., in response to environmental antigen presented in context of I-E on DCs) as the reason for peripheral CD4⁺ T cell reconstitution in B6CD11c-E_α^dI-A^{-/-} mice, could be excluded.

Peripheral CD4⁺ T Cells in TG⁺B6CD11c-E_α^dI-A^{-/-} Mice Are Located in T Cell Areas of Lymph Nodes. Immunohistochemical analysis of lymph nodes from transplanted B6CD11c-E_α^dI-A^{-/-} mice shows that the CD4⁺ T cells are located in the paracortical area close to the I-E⁺ DCs (Fig. 5 A). This is in contrast to the remnant CD4⁺ T cells of class II-deficient mice, which had been reported to be localized in the B cell follicles (5). Higher magnification reveals close T cell-DC contact (Fig. 5 B).

Discussion

In this report, two different mouse strains were used to study CD4⁺ T cell survival in presence or absence of MHC class II expression. Besides conventional MHC class II-defi-

cient mice (B6I-A^{-/-}, (4)), a transgenic strain was used that is expressing an MHC class II I-E transgene under the control of the CD11c promoter in the class II-deficient background (B6CD11c-E_α^dI-A^{-/-}; references 13, 16). Both mouse types used as thymus graft recipients were equally devoid of mature peripheral CD4⁺ T cells (4, 13). When groups of both mice were transplanted with MHC class II-positive fetal thymic grafts, only in the B6CD11c-E_α^dI-A^{-/-} group was an accumulation of mature CD4⁺ T cells detected, whereas in none of the transplanted B6I-A^{-/-} mice was a significant increase of peripheral CD4⁺ T cells found. Further analysis demonstrated that the failure of reconstituting B6I-A^{-/-} mice with mature CD4⁺ T cells was not due to inefficient repopulation of the transplanted thymi, since in all 20 transplanted B6I-A^{-/-} animals, the thymic grafts could be reisolated at different time points and had been repopulated with host-type thymocytes. The total numbers of thymocytes and their phenotype as analyzed by several thymic markers (CD3, CD4, CD8, HSA, and CD69) was normal and identical in both experimental groups. One possibility for the absence of reconstitution could be a very rapid decay of CD4⁺ T cells when interactions with class II MHC in the periphery are not possible. These results are in contrast with the reported survival of CD4⁺ T cells for more than 6 mo in mice lacking MHC class II (11). However, this discrepancy might be caused by

different experimental strategies; while Takeda et al. (11) transplanted embryonic thymi without pretreatment, the thymi in the present study were irradiated and dGuo treated. Placing untreated embryonic thymi under the kidney capsule of normal mice has been reported to result in considerable enlargement of the transplanted thymi for prolonged periods (12). These enlarged thymic grafts contain up to 2×10^7 donor-derived thymocytes during the initial 3 wk, and are releasing important numbers of mature donor-type T cells into the periphery (11, 12). The pretreated thymi in this study at no point of the experiment contained $>3 \times 10^6$ thymocytes and showed, in accordance with previous studies, optimal thymocyte production after ~ 3 wk of repopulation by host-type cells (12). These relatively low cell numbers allow to survey more precisely the subtle repopulation kinetics than an initially high, one "wave" thymocyte output. As demonstrated in the case of the B6CD11c-E $_{\alpha}^d$ I-A $^{-/-}$ recipients, the final repopulation efficacy of this system is comparable to that reached with the high output of untreated thymic grafts (11). Furthermore, when untreated thymi are transplanted, thymic cells of hemopoietic origin (thymic DCs, B cells, macrophages, or precursors of those) might theoretically as well emigrate from the TG and therefore render the initially class II-deficient environment at least partially MHC class II positive. In the presented experiments, such a possibility could be excluded by irradiation and dGuo treatment of the TG. This was leading to a complete absence of MHC class II-expressing cells in the transplanted B6I-A $^{-/-}$ recipients which might be responsible for the fast decay of recent CD4 $^{+}$ thymic emigrants. When this paper was submitted, another report on similar findings was published (30, 31). Tanchot et al. (30) demonstrated that naive CD8 $^{+}$ T cells are disappearing within a few days after transfer into a class I-deficient environment, but survive for an extended period when the correct MHC molecules are expressed. A

very fast death of CD4 $^{+}$ T cells due to complete absence of MHC class II-expressing cells together with the relatively low output of T cells from the treated TG in B6I-A $^{-/-}$ mice might explain why not even a low level reconstitution with CD4 T cells was observed.

Benoist and Mathis (31) raised the question of which peripheral cells need to express MHC molecules to enhance T cell survival. As shown in this and previous reports (13, 16), the CD11c promoter drives expression of the MHC class II I-E transgene specifically to DCs of thymus (13), spleen, and lymph nodes. Approximately 70–80% of all subpopulations of DCs do express the transgenic I-E, whereas B cells and the majority of macrophages ($\sim 90\%$) are transgene negative. Relatively high levels of MHC class II expression, together with costimulatory or adhesion molecules on DCs (32), may predestine this cell type for supporting peripheral T cell survival. Immunohistochemical analysis of frozen lymph node sections from the TG transplanted B6CD11c-E $_{\alpha}^d$ I-A $^{-/-}$ recipients revealed a close proximity of the repopulating CD4 $^{+}$ T cells and DCs in the paracortex. Whether other MHC class II $^{+}$ cell types have similar capacities remains to be explored. The localization of DCs in T cell areas of spleen and lymph nodes might specialize them for such T cell survival-inducing functions, whereas, for example, MHC class II-expressing B cells are located in separate follicles and might therefore have fewer opportunities to interact with T cells in the absence of antigen.

The presented data suggest that mature CD4 $^{+}$ T cells, once they have left the MHC class II-rich thymic environment, have to perpetually recognize MHC molecules in the absence of specific antigen to survive. This peripheral TCR-MHC interaction might create a baseline activation, in the absence of which CD4 $^{+}$ T cells are extremely short lived.

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