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TCR–MHC class II interaction is required for peripheral expansion of CD4 cells in a T cell-deficient host

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Abstract

It is well established that T cell-deficient nude and SCID mice can be reconstituted by i.v. injection of small numbers of purified peripheral CD4$^+$ T cells; however, the requirements for expansion of the transferred T cells in such systems are not clear. We show here that blood and lymphoid organs of MHC class II-deficient mice (which selectively lack mature CD4$^+$ T cells) cannot be reconstituted by transfer of purified splenic CD4$^+$ T cells, whereas TCRα-deficient mice (which lack both CD4$^+$ and CD8$^+$ mature T cells) are readily reconstituted. The failure of CD4$^+$ T cell reconstitution in MHC class II-deficient mice was not due to the presence of CD8$^+$ T cells, since similar results were obtained in TCRα–MHC class II double-deficient mice. Consistent with most previous studies CD4$^+$ T cells in reconstituted TCRα-deficient mice had a diverse TCR V$_\beta$ repertoire and were predominantly of an activated/memory (CD44^{high}) phenotype. Collectively our data demonstrate that the expansion of peripheral CD4$^+$ T cells in a T cell-deficient host is dependent upon interactions of the TCR with MHC class II.

Introduction

T cells found in the periphery of humans or mice have an enormous potential of expansion and self renewal. If the thymus, the source of newly developing T cells, is removed after puberty in man or only >2 days after birth in mice, the size of the peripheral T cell pool stays constant, shows diversity and can respond to environmental antigens (reviewed in 1). Very little is known about how this peripheral T cell homeostasis is maintained in the absence of T cell emigration from the thymus. The classical experiments to analyze the repopulation potential of peripheral T cells involved reconstitution of T cell-deficient nude or lymphocyte-deficient SCID mice with total splenocytes or sorted CD4$^+$ T cells (reviewed in 2,3). These experiments showed that small numbers of CD4$^+$ T cells (10^2 to 10^3 sorted cells) were already sufficient to repopulate the periphery. Nevertheless, the CD4$^+$ T cell repertoire (judged by the expression of various V$_\beta$ genes) was quite diverse and similar to the distribution in the donor mice. Thus, the expansion of transferred peripheral CD4$^+$ T cells is polyclonal and presumably some regulatory mechanism is responsible for maintaining a broadly distributed T cell repertoire.

One obvious candidate for such a regulatory system would be the MHC molecules of the recipient. According to such a scenario peripheral CD4$^+$ T cells with a certain minimal TCR affinity for self MHC class II molecules would be allowed to survive and/or proliferate following transfer, whereas T cells with affinities below this threshold would die. In essence, peripheral T cells would undergo a process similar to the MHC-mediated positive selection mechanism operating in the thymus.

To test this hypothesis we used the classical approach of reconstituting immunodeficient mice with sorted CD4$^+$ splenic T cells. However, instead of nude or SCID recipients, we used the better defined TCRα- and MHC class II-deficient mice derived by gene targeted mutation. As expected T cell-deficient TCRα$^{-/-}$ mice could be easily reconstituted with peripheral CD4$^+$ T cells. However MHC class II-deficient mice (which lack CD4$^+$ T cells in the periphery) could not be reconstituted. To exclude a regulatory role of CD8$^+$ T cells present in the MHC class II-deficient mice we generated TCRα-deficient mice which do not express MHC class II. No

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significant CD4$^+$ T cell reconstitution was observed in these 'double-deficient' mice, indicating a critical role for TCR–MHC class II interactions in this model system.

Methods

Mice

MHC class II-deficient (MHC II$^{-/-}$) mice were obtained from RCC-BRL (Füllinsdorf, Switzerland). These mice were generated on the genetic background of C57BL/6 mice which do not express MHC class II I-E molecules. The MHC class II I-Aa chain gene was inactivated by gene targeted mutation leading to a total loss of MHC class II expression in these mice (4). TCRα-deficient (TCR$\alpha^{-/-}$) mice (backcrossed 12 generations to C57BL/6) (5) and B6.PL-Thy-1^a/Cy (Thy-1.1$^+$) mice were obtained from the Jackson Laboratories (Bar Harbor, ME). MHC II$^{-/-}$ female mice were crossed with a TCR$\alpha^{-/-}$ mouse and the offspring was inbred to generate MHC II$^{-/-}$ TCR$\alpha^{-/-}$ double-deficient mice. Double-deficient mice were identified either by PCR or by FACS analysis of peripheral blood lymphocytes (PBL) using anti-MHC class II (M5/114; Boehringer Mannheim, Mannheim, Germany) and anti-CD4 (H129.19; Boehringer) antibodies. All mice were kept under specific pathogen-free conditions.

Cell transfer

One or two spleens from male B6.PL (Thy-1.1) mice were homogenized through a steel mesh and red blood cells were removed by hypoosmotic shock. Cells were incubated in 0.5–1.0 ml cold PBS containing 5% FCS and anti-CD4–phycoerythrin (PE) (Boehringer) for 30 min. Cells were washed twice and diluted to 2×10^6 cells/ml, before CD4$^+$ T cells were sorted using a FACStar Plus (Becton Dickinson, San Jose, CA). Purity of the sorted cells was $>$98%. Sorted cells were washed in PBS and resuspended to 10^7 cells/ml. Typically $0.5–1.0\times10^6$ cells were injected in the tail vein of male mice.

Blood analysis

Every week after the transfer 3–4 drops of blood were collected from the tail vein into an Eppendorf tube containing 3 µl of 0.5 M EDTA. Then 50 µl of blood was transferred into a fresh tube containing anti-Thy-1.1–FITC (HO-22.1.1) (6) and anti-CD4–PE in a total volume of 3–5 µl PBS. Blood was incubated for 30 min at room temperature with the antibodies before it was transferred into 0.5 ml FACS lysing solution (Becton Dickinson). After 2–6 min incubation the cells were pelleted and washed in 1 ml PBS with 5% FCS. Cells were resuspended in 200 µl PBS/FCS and analyzed using a FACScan (Becton Dickinson). For each sample 40,000 events in a FSC/SSC lymphocyte gate were acquired. Data were analyzed using the program WinMDI (7).

Spleen and lymph node (LN) analysis

Spleen and LN (mesenteric LN or a pool of two inguinal and two submaxillary LN) were homogenized through a steel mesh. Red blood cells were removed from the splenocytes by osmotic shock. Viable cells were counted using a hemocytometer and ~10^6 cells were used for each sample of antibody labeling. For the determination of reconstitution cells were incubated with anti-Thy-1.1–FITC (HO-22.1.1) and anti-CD4–PE for 30 min on ice, washed and analyzed on a FACScan. For the analysis of V_β repertoire and CD44 expression, splenocytes were incubated with biotin conjugates of the following antibodies: anti-V_β5 (MR9-4), anti-V_β6 (44-22.1), anti-V_β8 (F23.1), anti-V_β10 (B21.5) and anti-CD44 (IM7.8.1). Samples were then washed and incubated with anti-Thy-1.1–FITC, streptavidin–PE (Caltag, San Francisco, CA) and anti-CD4–Red613 (Gibco/BRL, Basel, Switzerland). If not otherwise indicated, antibodies were prepared and conjugated at the Ludwig Institute. Three-color analysis was performed on a FACScan (Becton Dickinson) and data were analyzed using WinMDI (7).

Results

T cell-deficient but not MHC class II-deficient mice can be reconstituted with CD4 T cells

Many previous reports have described the reconstitution of nude or SCID mice with peripheral T cells or total splenocytes (8–11). These immunodeficient mice were derived from spontaneously occurring mutations leading to the loss of peripheral T cells in the case of the nude mice, and the lack of B and T cells in the SCID mouse. However, both nude and SCID mice are considered 'leaky' as small numbers of lymphocytes can still be generated, a fact which potentially complicates reconstitution experiments (12). Therefore, we chose T cell-deficient (TCR$\alpha^{-/-}$) mice generated by a gene-targeted mutation of the TCRα constant region (5) for our reconstitution experiments. No $\alpha\beta$ TCR can be expressed in these mice leading to the absence of conventional CD4 and CD8 T cells, while $\gamma\delta$ T cells and B cells are still present.

TCR$\alpha^{-/-}$ mice were reconstituted by i.v. injection of $0.5–1.0\times10^6$ FACS sorted CD4$^+$ splenocytes from B6.PL-Thy-1^a mice. The TCR$\alpha^{-/-}$ mice as well as all other recipient mice used in the following experiments express the Thy-1.2 allele on T cells. The donor CD4 T cells, however, express the Thy-1.1 allele, allowing easy detection of donor-derived cells.

Already 1 week after injection reconstitution of CD4$^+$ T cells in the blood could be observed, reaching a plateau at ~4–5 weeks (Figs 1 and 2). The percentage of CD4$^+$ T cells in the blood of the reconstituted mice never reached values observed in normal C57BL/6 mice; however, similar observations have been made in several previous reports using nude or SCID mice as recipients (8–11). Similar to these earlier reports we also found reconstitution of other lymphatic organs (Fig. 3). Although also reported earlier it is worth mentioning that reconstitution of 'peripheral' (inguinal, submaxillary) LN was much less efficient than of mesenteric LN. (Fig. 3). Thus, TCR$\alpha^{-/-}$ mice can efficiently be reconstituted with peripheral CD4 T cells and will serve as a positive control for the following experiments.

MHC class II-deficient (MHC II$^{-/-}$) mice have very few CD4$^+$ T cells due to a selective block in positive selection (4,13,14). The small number of CD4$^+$ cells that can still be found in these mice are selected on MHC class I or CD1 and express a lower level of CD4 than conventional CD4$^+$ T cells (Fig. 1) (15). Thus the CD4$^+$ T cell compartment in these mice is nearly empty and one should be able to reconstitute MHC

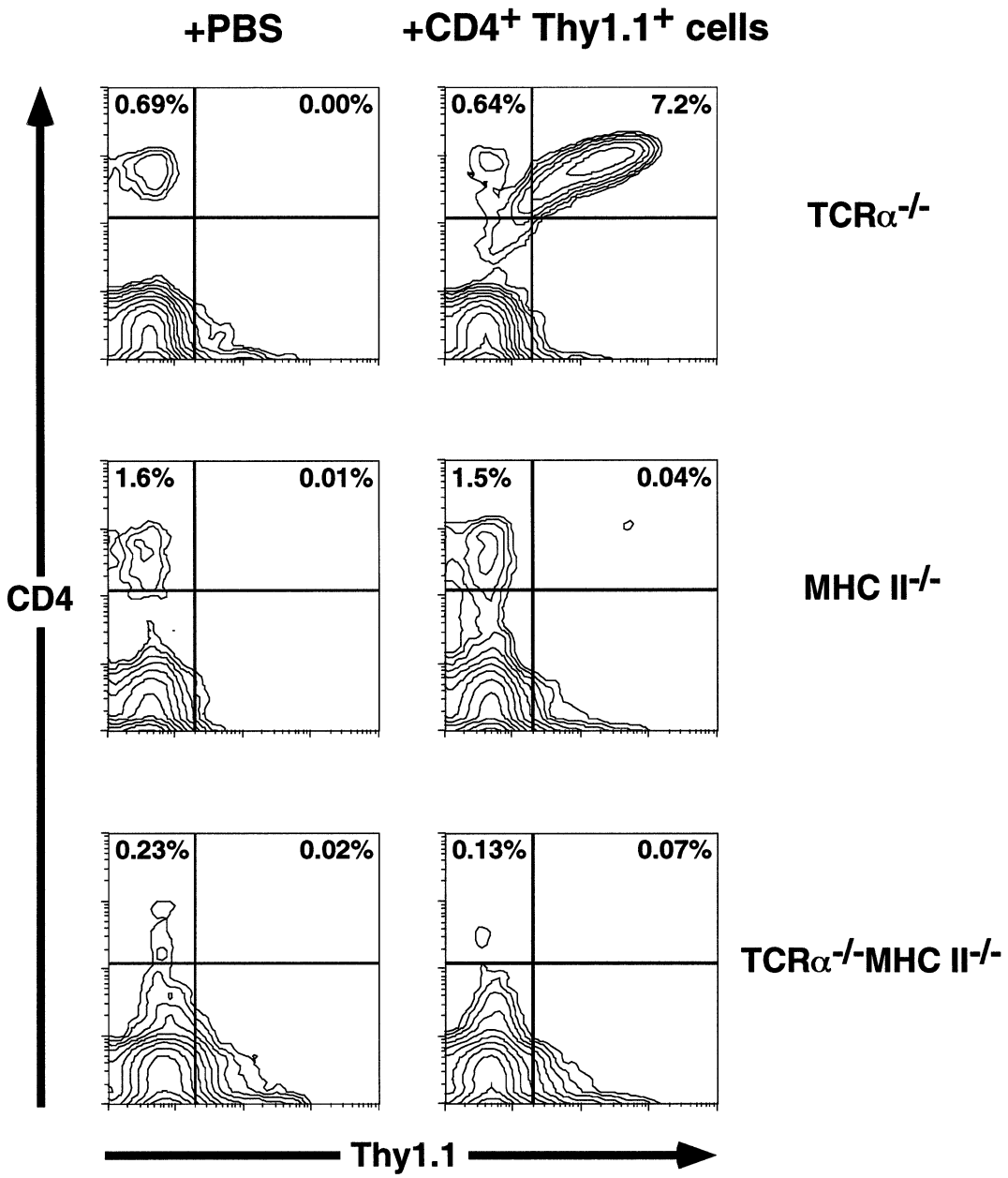

Fig. 1. CD4$^+$ T cell reconstitution of TCR$\alpha^{-/-}$ mice is MHC class II dependent. Shown are FACS contour plots of PBL (labeled with anti-Thy-1.1–FITC and anti-CD4–PE) from mice 4 weeks after injection of Thy-1.1$^+$ CD4$^+$ T cells (right panels) or PBS (left panels). The type of recipient is indicated on the right-hand side. Threshold for the contour plots was 0%.

II$^{-/-}$ mice with CD4 $^+$ T cells if this expansion is independent of MHC class II expression.

However, only trace numbers of CD4$^+$ Thy-1.1$^+$ donor-derived T cells (0.04%) could be found in the blood of reconstituted MHC II$^{-/-}$ mice in the first few weeks after reconstitution (Fig. 1). These numbers were slightly, but consistently, above the background staining values of PBS injected littermates (0.01%). At later time points (>6 weeks) donor-derived T cells were virtually undetectable. In the spleen and LN the number of donor-derived CD4$^+$ T cells was also barely over the background values found in littermates injected with PBS (Fig. 3). Furthermore, donor-derived CD4$^+$ T cells could not be detected in the thymus, bone marrow, gut epithelium, lamina propria or liver of MHC II$^{-/-}$ mice (data not shown).

CD4$^+$ T cells in reconstituted TCR$\alpha^{-/-}$ mice have a random TCR V$_\beta$ repertoire and are CD44^{hi}

Previous studies of CD4 T cell reconstitution of nude and SCID mice have shown that reconstitution does not significantly bias the TCR V$_\beta$ repertoire (16,17). As shown in Fig. 4, donor-derived splenic CD4 T cells in reconstituted TCR$\alpha^{-/-}$ mice likewise exhibited an apparently random V$_\beta$ repertoire, although variations in V$_\beta$ expression between individual mice

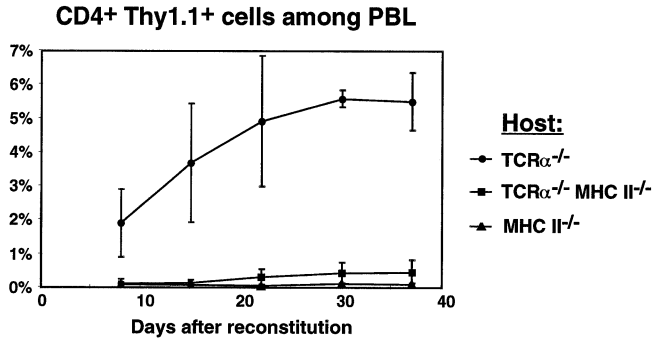

Fig. 2. Kinetics of CD4$^+$ T cell reconstitution. The percentage of donor-derived CD4$^+$ T cells was determined by flow cytometry in the blood of immunodeficient mice at various times after reconstitution (see Fig. 1). In all cases background values (usually $<0.03\%$) of PBS injected littermates were subtracted. Data points represent the averages of nine TCR$\alpha^{-/-}$, seven TCR$\alpha^{-/-}$ MHC II$^{-/-}$ and five MHC II$^{-/-}$ mice (except for the last two data points of the TCR$\alpha^{-/-}$ mice, which are the averages of three mice) from two independent experiments. Error bars indicate SD.

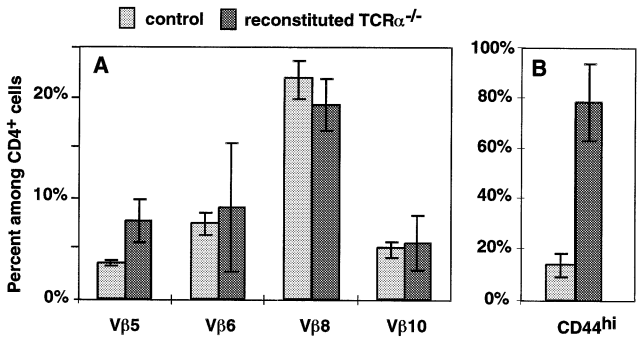

Fig. 4. TCR V_β repertoire and CD44 phenotype of donor-derived T cells in reconstituted TCR$\alpha^{-/-}$ mice. (A) TCR V_β usage was analyzed among CD4$^+$ splenocytes in control mice and among CD4$^+$ Thy-1.1$^+$ splenocytes of CD4 T cell reconstituted TCR$\alpha^{-/-}$ mice. Histograms represent the average percentage of cells positive for a given TCR V_β among CD4$^+$ T cells; error bars indicate SD. The control group consisted of one C57BL/6, one B6.PL and three (MHC II$^{-/-}\times$ TCR$\alpha^{-/-}$)F_1 mice. (B) The percentage of CD44^{hi} CD4$^+$ T cells was determined as in (A).

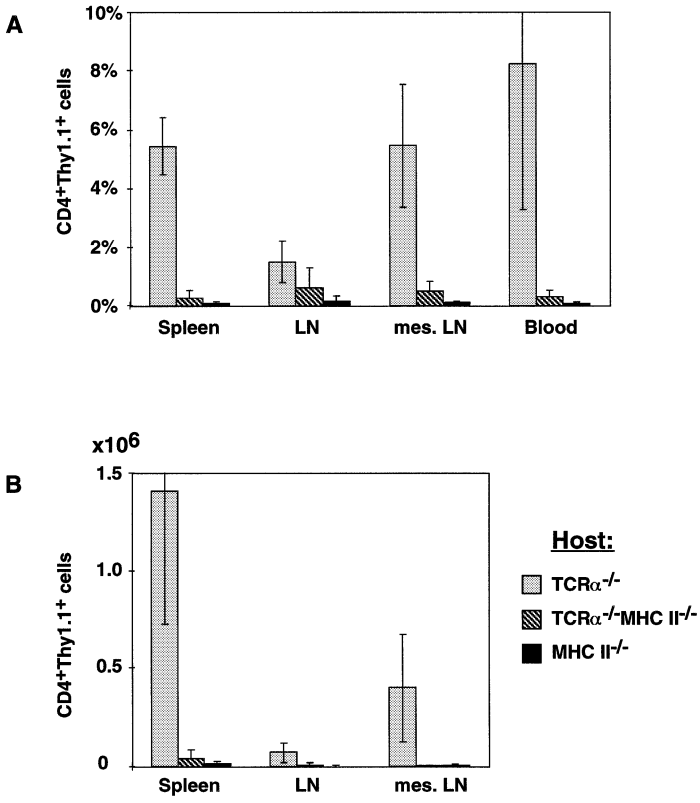

Fig. 3. CD4$^+$ T cell reconstitution of peripheral lymphoid organs. The percentage of donor-derived T cells in peripheral lymphatic organs was determined by flow cytometry 3–5 weeks after CD4$^+$ T cell reconstitution. All values are corrected for the background values found in PBS injected control animals. Histograms represent the averages of six TCR$\alpha^{-/-}$, five TCR$\alpha^{-/-}$ MHC II$^{-/-}$ and three MHC II$^{-/-}$ mice; error bars indicate SD. LN represents a pool of two inguinal and two submaxillary LN, mes. LN represents the mesenteric LN. (A) The percentage of donor-derived CD4$^+$ T cells among lymphocytes (determined by a FSC/SSC gate). (B) The absolute number of donor-derived T cells in these organs.

(assessed by the magnitude of the standard derivation) were somewhat greater than in normal controls. These data argue against any major selective expansion of the transferred CD4 T cells driven by environmental antigens or pathogens, which might be expected to significantly alter the TCR repertoire.

CD44 is a phenotypic marker that is up-regulated on activated and/or memory T cells (18). Reconstitution of nude or SCID mice with low numbers of CD4 T cells leads to accumulation of cells with an activated/memory phenotype in lymphoid organs (reviewed in 19). Figure 4 shows that, in contrast to the original donor cell inoculum, most splenic CD4 T cells present in reconstituted TCR$\alpha^{-/-}$ mice have a CD44^{hi} phenotype, consistent with the fact that considerable CD4 T cell expansion has occurred.

TCRα and MHC class II double-deficient mice cannot be reconstituted with CD4 T cells

The failure to reconstitute MHC II$^{-/-}$ mice with peripheral CD4 T cells suggests that a TCR–MHC class II interaction is required for their expansion. However, it remains formally possible that MHC II$^{-/-}$ mice could not be reconstituted because the large number of CD8$^+$ T cells found in these mice compete out the small number of transferred CD4$^+$ T cells or even directly suppress their expansion. To exclude this possibility we generated TCRα and MHC class II double-deficient (TCR$\alpha^{-/-}$ MHC II$^{-/-}$) recipient mice which have no CD8$^+$ T cells. TCR$\alpha^{-/-}$ MHC II$^{-/-}$ as well as control TCR$\alpha^{-/-}$ and MHC II$^{-/-}$ mice were injected with the same preparation of Thy-1.1$^+$ CD4$^+$ splenocytes. Four weeks after reconstitution only extremely small numbers (0.07%) of donor-derived T cells could be found in the blood of TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice, barely above the background level of PBS-injected control mice (Fig. 1) and similar to the MHC II$^{-/-}$ mice. On the other hand, the blood of MHC class II$^+$, TCR$\alpha^{-/-}$ mice contained 4–10% of donor-derived, Thy-1.1$^+$ cells (Fig. 1). Thus, reconstitution of TCR$\alpha^{-/-}$ mice with peripheral CD4$^+$ T cells is at least 100-fold less efficient in the absence of MHC class II expression.

When we analyzed the reconstituted mice at various time points the reconstitution kinetics of the TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice were essentially similar to that of MHC II$^{-/-}$ mice, even though reconstitution of the TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice was marginally better (Fig. 2). Still at any given time point the reconstitution in the TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice was at least 10-fold less efficient than in the MHC class II$^+$,TCR$\alpha^{-/-}$ mice. Only after extended periods of time (>60 days) could some significant reconstitution (>1%) of CD4$^+$ T cells in the TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice but not of MHC II$^{-/-}$ mice be observed (data not shown).

Similar to our results obtained from blood, we could not detect significant numbers of donor-derived CD4$^+$ T cells in the spleen, peripheral LN or mesenteric LN of the TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice 3–5 weeks after reconstitution (Fig. 3). While the difference in the percentage of donor-derived cells between TCR$\alpha^{-/-}$ mice and TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice seems to be less in LN and spleen than in blood, the difference in total numbers is actually much greater (Fig. 3). LN of TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice were extremely small, while the LN of reconstituted TCR$\alpha^{-/-}$ mice had approximately normal sizes. The size of the spleen in the various mice did not show any obvious differences, but the cell number in the spleen of the TCR$\alpha^{-/-}$ MHC II$^{-/-}$ mice was much lower than in MHC class II$^+$ TCR$\alpha^{-/-}$ mice.

Discussion

Taken together our data demonstrate formally that the expansion of normal peripheral CD4 cells transferred to T cell-deficient hosts requires MHC class II expression. This in turn implies that direct TCR–MHC class II interactions are required for expansion and self-renewal of the CD4$^+$ T cell pool. This conclusion confirms and extends an earlier study by Mackall *et al*. (20) showing that CD4 T cell reconstitution of thymectomized irradiated mice (protected by a syngeneic bone marrow graft) could be partially (~ 50%) inhibited by simultaneous treatment with anti-MHC class II mAb. On the other hand, Takeda *et al*. (21) have recently reported that MHC II$^{-/-}$ RAG2$^{-/-}$ mice engrafted with fetal thymus from wild-type mice develop significant numbers of peripheral CD4 T cells that initially proliferate but ultimately disappear over time. These apparently conflicting findings could be related to differences in the experimental models utilized. Alternatively it is possible that CD4$^+$ recent thymus emigrants are less dependent on TCR–MHC class II interactions for their survival and/or expansion than fully mature peripheral CD4 cells.

The MHC class II-expressing cell(s) required for successful peripheral CD4 T cell reconstitution of TCR$\alpha^{-/-}$ mice has not been identified. In this respect MHC class II$^+$ B cells are unlikely to be involved, since CD4 T cell reconstitution of B cell-deficient SCID (2,3) and RAG1$^{-/-}$ mice (data not shown) has been observed. Rather we would speculate that MHC class II$^+$ APC (such as dendritic cells and/or macrophages) are necessary for the expansion of transferred CD4 T cells in T cell-deficient mice.

In contrast to MHC II$^{-/-}$ recipients, TCR$\alpha^{-/-}$ MHC II$^{-/-}$ double-deficient mice could be reconstituted with small but significant numbers of donor CD4$^+$ T cells (~ 1%) at later times. It is possible that these cells are derived from small numbers of MHC class I- or CD1-restricted CD4$^+$ T cells originally described in MHC class II-deficient mice (15). However, these cells did not express NK1.1 (data not shown), a marker expressed by many non-MHC class II-restricted CD4 T cells (22). In the MHC II$^{-/-}$ mice this expansion might not occur as the endogenous MHC class I- or CD1-restricted CD4$^+$ T cells would compete out the small number of such cells found in the sorted donor inoculum.

Finally, an important unresolved issue arising from our study is whether self or foreign peptides are responsible for the MHC class II-dependent expansion of peripheral CD4 T cells in TCR$\alpha^{-/-}$ mice. Although our reconstituted mice have been maintained under specific pathogen-free conditions for the duration of the analysis and show no pronounced V_β bias, some degree of contamination by environmental pathogens cannot be formally excluded. In this context several previous reports in which TCR transgenic CD4 or CD8 T cells were transferred to naive (MHC sufficient) hosts in the presence or absence of nominal antigen have suggested that peripheral T cell expansion is strictly dependent upon TCR recognition of MHC–antigen complexes (20,23). On the other hand, a very recent study by Tanchot *et al*. (24) has demonstrated that monoclonal TCR transgenic CD8 cells with a memory phenotype can actually expand *in vivo* as a consequence of self MHC class I recognition in the absence of nominal antigen. In contrast, naive phenotype TCR transgenic CD8 T cells could only expand upon encountering self MHC class I plus antigen, although they could survive in the presence of self MHC alone. By analogy with these transgenic experiments, it could be speculated that the MHC class II-dependent expansion of normal CD4$^+$ T cells transferred into immunodeficient hosts reflects the selective proliferation of memory cells interacting with self peptides presented by MHC class II molecules. Alternatively exposure to MHC class II-associated 'foreign' peptides derived from gut-associated microflora or food antigens (which would still be present in specific pathogen-free mice) may be necessary to sustain the expansion of the transferred CD4$^+$ T cells.

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Abbreviations

MHC II$^{-/-}$	MHC class II deficient
LN	lymph node
PBL	peripheral blood lymphocyte
PE	phycoerythrin
TCR$\alpha^{-/-}$	TCRα deficient

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