Competition controls the rate of transition between the peripheral pools of CD4$^+$CD25$^-$ and CD4$^+$CD25$^+$ T cells

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Abstract

Recent reports have hinted that it is possible to regenerate CD4$^+$CD25$^+$ regulatory T cells (Treg) from CD4$^+$CD25$^-$ cells, a phenomenon termed conversion. We evaluated the relative contribution of this process to the Treg pool by transferring purified populations of CD4$^+$ T cells into T cell-deficient mice. We report that conversion of CD25$^-$ cells into the CD4$^+$CD25$^+$Treg pool is minor if other bona fide CD25$^+$ Tregs are present. Moreover, in the same hosts, the loss of CD25 expression by a population of Tregs also decreases in the presence of co-injected CD4$^+$CD25$^-$ cells. Thus, the rate of exchange between CD25$^-$ and CD25$^+$ T-cell populations is determined by the presence or absence of T-cell competitors. Our results attest for the role of competition in the contribution of different T-cell subsets for the regeneration of the peripheral CD4$^+$ T-cell pool during lymphopenia.

Introduction

CD4$^+$CD25$^+$ regulatory T cells (Tregs) represent 5–10% of the peripheral T-cell pool in both mice and humans and are essential for the maintenance of the peripheral CD4$^+$ T-cell homeostasis (1). Their development occurs in the thymus and is dependent on the expression of the transcription factor *foxp3* (2–4). In animal models where Tregs are absent (CD25$^{-/-}$, IL-2Rβ$^{-/-}$ and *foxp3*$^{-/-}$ mice), T-cell homeostasis is disrupted and activated T cells accumulate in a non-controlled fashion, leading to the development of autoimmune diseases and subsequent death (4–6). The re-introduction of limited numbers of CD25$^+$ T cells in these animals is sufficient to rescue not only the autoimmune syndrome but also the normal composition of the peripheral T-cell pool, allowing for the presence of normal numbers and proportions of naive and activated T cells and, no less important, of CD4$^+$CD25$^+$ Tregs (1, 4, 7). Thus, the CD4$^+$CD25$^+$ sub-population that plays an essential role in lymphocyte homeostasis is itself also under strict homeostatic control (8).

The initial reports on the functional role of CD4$^+$CD25$^+$ T cells were based on strategies using adoptive T-cell transfers into lymphopenic mice (9). The transfer of naive CD25$^-$ T cells caused the development of autoimmune diseases, which was prevented by the co-transfer of the CD4$^+$CD25$^+$ cells. This

outcome was proof of the existence of both potentially auto-reactive CD4$^+$ T cells and cells with a regulatory role in the CD4$^+$CD25$^+$ T-cell fraction (10, 11). It has been suggested that lymphopenia-driven proliferation (LDP) is required for the development of autoimmune diseases, as the depletion of CD25 T cells from normal adult mice is not sufficient to provoke their development (12). Moreover, the transfer of large numbers of naive T cells into lymphopenic mice also does not result in the development of autoimmune syndromes (13). These observations highlight the relevance of CD4$^+$CD25$^+$ Tregs during lymphopenia and the need to understand the mechanisms responsible for their homeostasis in response to lymphopenic conditions.

Recent reports have shown that during LDP, it is possible to regenerate the CD4$^+$CD25$^+$ Treg compartment from CD4$^+$CD25$^-$ T cells (14–16), a phenomenon termed 'conversion' (15). In contrast, other recent findings argue against the peripheral origin of CD4$^+$CD25$^+$*foxp3*$^+$ T cells during both immune responses (17) and LDP (18). These observations bear interest for two very important issues in CD4$^+$CD25$^+$ Treg biology: the possibility to develop CD4$^+$CD25$^+$*foxp3*$^+$ Tregs from naive CD4$^+$CD25$^-$ precursors (14) and the contribution of such pathway of differentiation in the homeostasis

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of CD4$^+$CD25$^+$ Treg in either steady-state or during LDP. In the present study we evaluated the contribution of the CD25$^-$ to CD25$^+$ conversion during LDP in different situations. Our results demonstrate that in the presence of natural CD4$^+$CD25$^+$ cells, this conversion pathway can only account for a minor fraction of the regeneration of the peripheral Treg pool. Conversely, we also found that loss of CD25 expression by the Treg population was abrogated in presence of co-injected naive CD4$^+$ T cells. We discuss here the role of competition for a limited niche in CD4$^+$CD25$^+$ Treg homeostasis. Our findings are of particular interest for the establishment of cell-therapy techniques, directed toward either the *de novo* generation of specific CD4$^+$CD25$^+$ Tregs or the *ex vivo* expansion and re-transfer of CD4$^+$CD25$^+$ Tregs.

Materials and methods

Mice

C57Bl/6.Ly5^b mice from Iffa-Credo (L'Arbresle, France), and B6.CD3ε$^{-/-}$ and C57Bl/6.Ly5^a mice from the CDTA-CNRS (Orléans, France) were kept on our own facilities at the Pasteur Institute and were matched for age (6–12 weeks) and sex.

Cell sorting and cell transfers

Lymph node (LN) cells from the Ly5^b and Ly5^a donor mice were prepared for sorting as described (1). Intact non-irradiated B6.CD3ε$^{-/-}$ hosts were injected intravenously with the purified CD4 T-cell populations alone or mixed at different cell ratios. By using mice differing by Ly5 allotypes, we were able to discriminate the cells originating from the different donor mice. Host mice were sacrificed at different time intervals after cell transfer. Spleen, inguinal LN and mesenteric LN cell suspensions were prepared and the number and phenotype of the cells from each donor population evaluated. The total peripheral T cells shown in the results represent the number of cells recovered in the host's spleen added to twice the number of cells recovered from the host's inguinal and mesenteric LNs.

Flow cytometry analysis

The following monoclonal antibodies were used: anti-CD45.1, anti-CD45.2, anti-CD3ε (145-2C11), anti-CD4 (L3T4/RM4-5), anti-CD25 (7D4), anti-CD45RB and anti-TCRβ (H57) from Pharmingen (San Diego, CA, USA); anti-CD25 (Southern Biotechnologies, Birmingham, AL, USA) and anti-*foxp3* from Biosciences (San Diego, CA, USA). Four-color staining was preformed with the appropriate combinations of FITC, PE, TRI-Color, PerCP, biotin and APC-coupled antibodies. Biotin-coupled antibodies were labeled with APC-, TRI-Color- (Caltag, San Francisco, CA, USA) or PerCP-coupled (Becton Dickinson, San Jose, CA, USA) streptavidin. Dead cells were excluded during analysis according to their light-scattering characteristics. All acquisitions and data were performed with a FACScalibur (Becton Dickinson) interfaced to the Macintosh CellQuest software; analysis was performed also with FloJo software.

Statistical analysis

Sample means were compared using the unpaired Students' *t* test. Sample means were considered significantly different at $p < 0.05$.

Results

### Origin of the CD4$^+$CD25$^+$ T cells during LDP

Recent reports have shown that peripheral CD4$^+$CD25$^-$ T cells can differentiate into CD25$^+$ Tregs in lymphopenic conditions. Recovered CD4$^+$CD25$^+$ Tregs were shown to express the lineage-specific transcription factor *foxp3* and to be able to suppress naive T-cell expansion *in vitro* (14, 15). In order to assert the reconstitution ability of this pathway, we evaluated the recovery of CD25$^+$ T cells after the transfer of 10^4 (Fig. 1A) or 2×10^5 (Fig. 1B) purified CD4$^+$CD25$^-$CD45RB^{high} or CD4$^+$CD25$^-$CD45RB^{low} T cells into lymphopenic hosts. We found that a sizeable CD4$^+$CD25$^+$ compartment including CD4$^+$CD25^{bright}*foxp3*$^+$ T cells was only observed in the mice injected with the higher number of activated CD4$^+$CD25$^-$CD45RB^{low} T cells (Fig. 1A and B). We have observed a good correlation between CD25^{bright} conversion and *foxp3* expression (Fig. 1B, bottom). In mice injected with CD25$^-$CD45RB^{high} cells, we always recovered a lower fraction of CD25$^+$ or *foxp3*$^+$ cells (Fig. 1A and B, left). Thus, the ability to generate a peripheral CD4$^+$CD25$^+$ T-cell pool upon expansion of purified CD25$^-$ cells is dependent on the initial number of CD25$^-$ cells transferred and of their activated CD45RB phenotype. We have also found that in spite of the recovery of CD4$^+$CD25$^+$ T cells after transfer of CD25$^-$ T cells into T cell-deficient hosts, the mice receiving CD25$^-$ cells did not fully reconstitute the peripheral CD4$^+$CD25$^+$ T-cell pool (Fig. 1B, left panel). We investigated therefore whether during LDP the generation of a complete CD4$^+$CD25$^+$ Treg compartment was dependent on the presence of CD4$^+$CD25$^+$ cells in the injected population. We transferred a fixed number of purified CD25$^-$CD45RB^{high} cells (10^5) alone, with CD25$^-$CD45RB^{low} cells (depleting CD25$^+$) or including 10% CD25$^+$ cells in total non-separated CD4$^+$ T cells. We observed that only in the group containing CD25$^+$ cells was the fraction of CD25$^+$ cells recovered in the LN similar to normal steady-state values (Fig. 2, right panel), suggesting that after transfer into immunodeficient hosts the regeneration of a complete peripheral CD4$^+$CD25$^+$ Treg pool requires the presence of CD25$^+$ T cells in the initial cell transfer.

Competition and conversion: CD25$^-$ to CD25$^+$ conversion is a poor contributor for the CD4$^+$CD25$^+$ Treg pool during lymphopenia

To determine the exact contribution of the CD25$^-$ and CD25$^+$ cells to the CD25$^+$ T-cell pool recovered after expansion in lymphopenic conditions, we co-transferred the CD25$^-$ (CD45RB^{high} alone or including CD45RB^{low}) cells mixed with 10% CD25$^+$ cells, distinguishable by the Ly5 allotype (Fig. 3). We observed that the vast majority of the CD25$^+$ cells recovered carried the allotype of the original CD25$^+$ cells, demonstrating that the contribution of the CD25$^-$ cells to the Treg pool is minor (less than 10%) when CD25$^+$ cells are available at the onset of expansion. Based on these findings we investigated whether the presence of CD4$^+$CD25$^+$ Tregs would modify the conversion of CD25$^-$ to CD25$^+$ T cells. We transferred peripheral CD4$^+$CD25$^-$ T cells with or without the physiological proportion (10%) of CD25$^+$ T cells into CD3ε$^{-/-}$ mice. Ten weeks after transfer we counted the number of
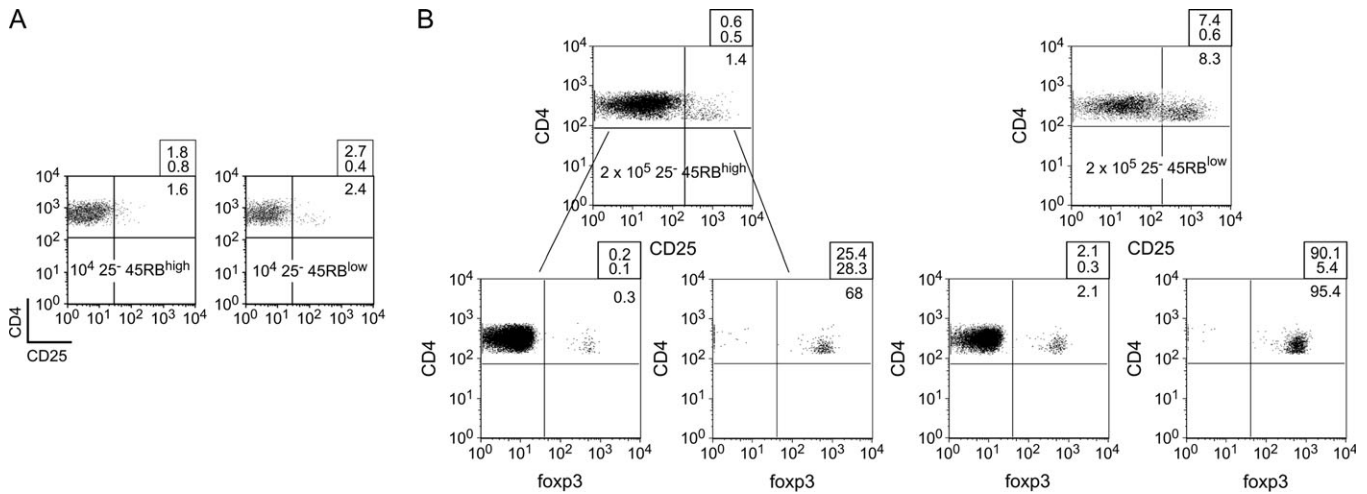
Fig. 1. Regeneration of CD4⁺CD25⁺ T cells from CD25⁻ precursors (A) 10⁴ CD4⁺CD25⁻CD45RB^high T cells or 10⁴ CD4⁺CD25⁻CD45RB^low T cells were transferred into CD3ε^−/− hosts. Eight weeks after transfer, the proportion of CD4⁺CD25⁺ cells recovered was evaluated, and LN populations are depicted. Boxed values represent values found in the two groups of host mice (mean + SE) (left panel, $n = 3$; right panel $n = 4$) and values in quadrant are from the host shown. Dot plots are gated for CD3⁺CD4⁺ T cells. (B) 2 × 10⁵ CD4⁺CD25⁻CD45RB^high naive T cells or 2 × 10⁵ CD4⁺CD25⁻CD45RB^low T cells were transferred into CD3ε^−/− hosts. Eight weeks after transfer, the proportion of CD4⁺CD25⁺ recovered among CD3⁺CD4⁺ T cells (top panels) and *foxp3*⁺ (bottom left gated on CD25⁻, bottom right gated on CD25⁺) was evaluated and is shown. Boxed values represent values found in the two groups of host mice (mean + SE) ($n = 3$ in each group) and values inside quadrant are from the depicted host LNs.
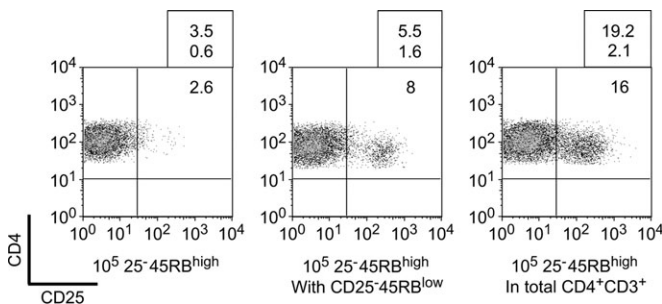

Fig. 2. CD4⁺CD25⁺ T cells are required to reconstitute the CD4⁺CD25⁺ T cell pool. Peripheral LN cells from normal WT C57Bl/6 donors were stained for CD4, CD3, CD25 and CD45RB. These cells were either non-sorted (bottom right) or sorted excluding the CD25⁺ cells (bottom middle) or both the CD25⁺ and the CD45RB^low cells (bottom left) and transferred into CD3ε^−/− lymphopenic hosts ($n = 3$, $n = 3$ and $n = 8$, respectively). The number of CD25⁻CD45RB^high cells transferred was fixed at 10⁵. Host mice were sacrificed 8 weeks after transfer and the phenotype of the resulting populations was analyzed for the expression of CD4 and CD25. Values shown represent the percentage in the respective quadrant in the LN of the host mice and boxed values represent values found in each group (mean + SE) in the CD25⁺ quadrant. Dot plots are gated for CD3⁺CD4⁺ T cells.

converted CD25⁺ cells recovered in the presence or in the absence of CD4⁺CD25⁺ Tregs (Table 1). The presence of CD4⁺CD25⁺ cells reduced the fraction and the number of cells that convert from CD25⁻ to CD25⁺, suggesting that the extent of conversion is limited by the presence of competitors of CD4⁺CD25⁺ origin. Interestingly, in the course of these experiments we observed that upon co-transfer of naive CD25⁻CD45RB^high and CD25⁺ cells into lymphopenic hosts, the fraction of the CD25⁺ cells that retained CD25 expression

was inversely correlated to the fraction of CD25⁺ cells present in the inoculum. Indeed, after transfer of a population containing 10% naive and 90% CD25⁺ cells, 22% of the progeny of the latter cells retained CD25 expression, while upon transfer of a 50/50% mix, 43% of the progeny of CD25⁺ cells retained CD25 expression (Fig. 4). As the total number of recovered cells expressing CD25 was the same, this observation suggests that the number of cells of CD25⁺ origin which 'lose' CD25 expression is conditioned by the presence or absence of other CD4⁺CD25⁻ cells (Fig. 4).

These results strongly suggest that CD4⁺CD25⁻ and CD4⁺CD25⁺ T cells represent different populations competing for different niches. However, the expression of CD25⁺ by itself does not identify a specific lineage of cells, and the presence of competitors might influence the expression of the marker without affecting the numbers of cells from *foxp3*⁺ or *foxp3*⁻ lineages. Thus, we transferred 1.2 × 10⁴ CD4⁺CD25⁻CD45RB^low cells in the absence or in the presence of competitor CD4⁺CD25⁺CD45RB^low T cells, distinguishable by the expression of the Ly5.1 allotypic marker (Fig. 5A). Under these conditions, it is expected that the presence of CD4⁺CD25⁺ Tregs provide strong competition and limit the expansion of converted CD25⁺ and *foxp3*⁺ lineage cells. We found that the percentage of both CD25⁺ and *foxp3*⁺ cells of CD25⁻CD45RB^low origin recovered 8 weeks after transfer was reduced in mice co-injected with the two cell populations. Indeed, we only observed relevant conversion in the group where CD25⁻CD45RB^low T cells were transferred in the absence of competitors (in two out of four hosts) and in none of the co-transferred hosts (out of three hosts). We also observed that among the recovered cells the percentage of CD25⁺ cells correlated to the fraction of *foxp3*⁺ cells (although the percentage of *foxp3*⁺ was higher, confirming
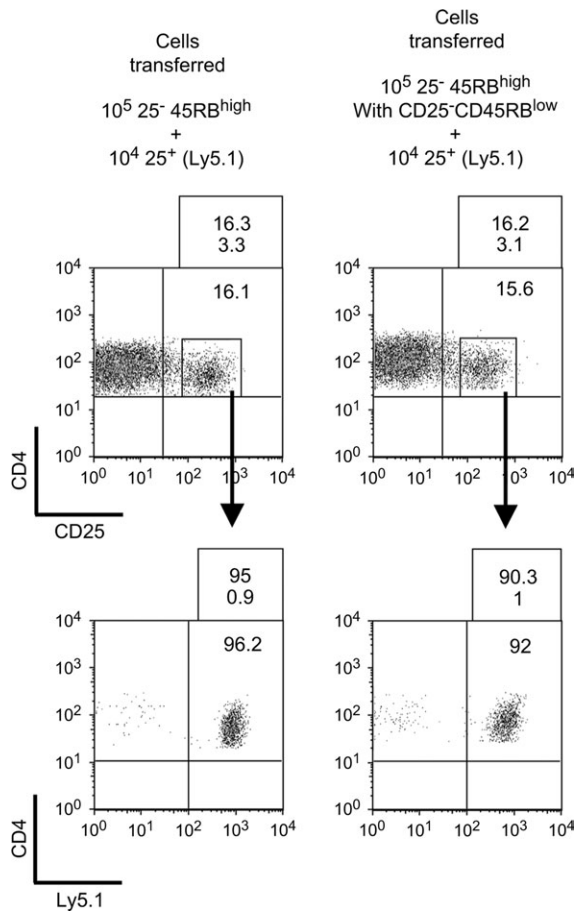
Fig. 3. Minor contribution of conversion for the CD4$^+$CD25$^+$ T-cell pool. CD25$^-$CD45RB^{high}CD4$^+$ cells (Ly5^b) including (right, $n = 3$) or not including (left, $n = 4$) CD25$^-$CD45RB^{low}CD4$^+$ T cells were transferred with 10^4 CD4$^+$CD25$^+$ Ly5^a T cells (10%) into lymphopenic CD3ε$^{-/-}$ hosts. Host mice were sacrificed 8 weeks after transfer and the proportion of CD25-expressing cells was evaluated (upper panels). CD25$^{+tohigh}$-expressing LN T cells were gated and the percentage of Ly5^a cells was evaluated (lower panels), demonstrating that the large majority is derived from the original CD4$^+$CD25$^+$ T cells. Values shown represent the percentage in the respective quadrant and boxed values represent values in the groups of transferred mice (mean + SE).

Table 1. Reduced numbers of converted cells in the presence of CD25$^+$ competitors

Transferred cells	CD25$^+$ cells of CD25$^-$ origin recovered ($\times 10^5$)
10^5 CD25$^-$CD45RB^{high} with CD25$^-$CD45RB^{low}	7.35 ± 0.4
10^5 CD25$^-$CD45RB^{high} with CD25$^-$CD45RB^{low} + 10^4 CD25$^+$	1.89 ± 0.03

CD25$^-$CD4$^+$T cells including 10^5 CD25$^-$CD45RB^{high}CD4$^+$ T cells (Ly5^b) were transferred into CD3ε$^{-/-}$ lymphopenic hosts with or without 10^4 CD4$^+$CD25$^+$ Tregs (Ly5^a). Ten weeks after transfer the host mice were sacrificed and the absolute number of CD4$^+$CD25$^+$ of CD25$^-$ origin recovered was evaluated and is shown. The results show that in the presence of competitor CD25$^+$ Tregs the absolute number of converted cells is strongly reduced.
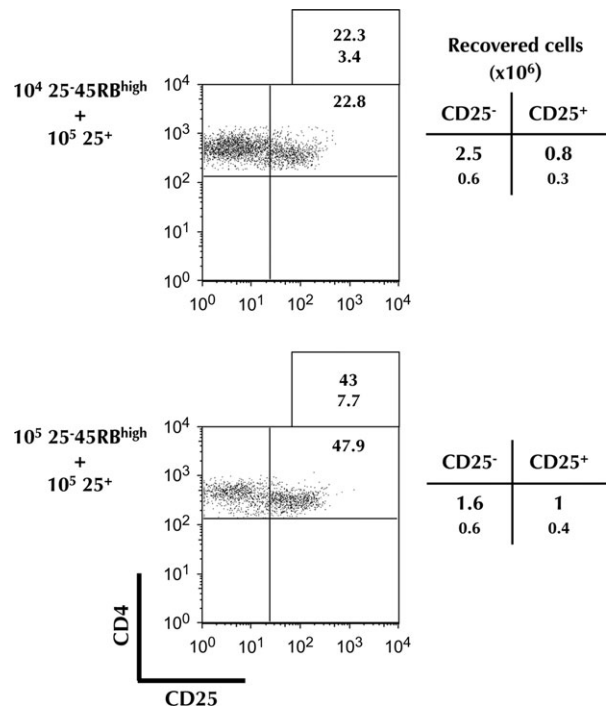

Fig. 4. The fraction of the sorted CD25$^+$ cells that maintains CD25 expression is inversely correlated with their own fraction in the inoculum. 10^5 CD25$^+$CD45RB^{low}CD4$^+$ T cells (Ly5^a) were transferred with variable numbers of CD25$^-$CD45RB^{high}CD4$^+$ naive T cells (Ly5^b) into CD3ε$^{-/-}$ lymphopenic mice. Host mice were sacrificed 8 weeks after and cells recovered were analyzed for Ly5, CD3, CD4 and CD25 expression. Shown are the CD4 versus CD25 expression profiles in Ly5^a-gated cells (CD25$^+$ origin) recovered in the LN of the two groups of mice. Values shown represent the percentage in the respective quadrant and boxed values represent values in all mice from each group (mean + SE) ($n = 4$ in both groups). The absolute numbers of CD25$^+$ and CD25$^-$ cells from the original CD25$^+$ cells transferred (Ly5^a) were also quantified and are depicted on the further right column. The increase in the CD25$^+$ proportion is due to a reduction in the number of Ly5^a CD25$^-$ T cells recovered.

that not all *foxp3*$^+$ lineage cells express CD25 at all times) (Fig. 5A). These findings also exclude that CD4$^+$CD25$^-$ may preferentially give rise to CD4$^+$CD25$^-$ *foxp3*$^+$ T cells. In order to confirm that the conclusions drawn for CD25$^+$-expressing cells could be further extended to *foxp3*$^+$ and *foxp3*$^-$ lineages, we transferred CD4$^+$CD25$^+$ Tregs (3.6×10^4) with or without naive CD4$^+$CD25$^-$CD45RB^{high} *foxp3*$^-$ lineage cells (1.2×10^4) and evaluated recovery of *foxp3*$^+$ lineage cells of CD25$^+$ origin (Fig. 5B). We found that the fraction of CD25$^+$ or *foxp3*$^+$ progeny of the transferred CD25$^+$ population was equally higher in the presence of competitor populations, excluding the possibility that the CD25$^+$-derived CD25$^-$ cells are *foxp3*$^+$ cells that down-regulate CD25 expression.

Discussion

The existing cooperation between different cell types during immune responses suggests that the immune system requires
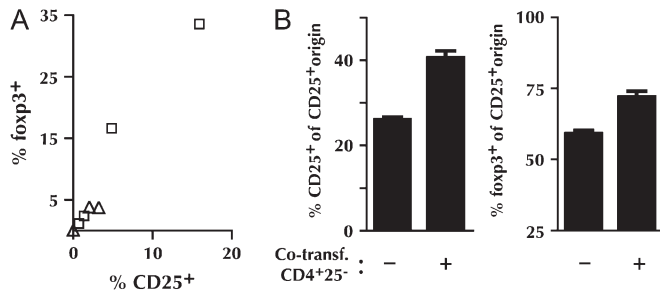
Fig. 5. CD25-expressing cells reflect *foxp3* expression in the presence of competitors. (A) 1.2 × 10⁴ CD4⁺CD25⁻CD45RB^low (Ly5^b) were transferred alone (triangles; *n* = 3) or with (squares; *n* = 4) 3.6 × 10⁴ CD4⁺CD25⁺ (Ly5^a) T cells. The figure shows the percentage of CD25 and *foxp3*-expressing cells recovered 8 weeks after transfer among the CD4⁺CD25⁻CD45RB^low progeny (Ly5^b). The expression of CD25 and *foxp3* correlates and decreases in the presence of competitors. (B) 3.6 × 10⁴ CD4CD25+CD45RBlow (Ly5^a) were transferred alone (*n* = 3) or with (*n* = 3) 1.2 × 10⁴ CD4⁺CD25⁻CD45RB^high naive T cells. The graphs show the percentage of CD25⁺ (left) or *foxp3*⁺ (right) among the CD25⁺ progeny (Ly5^a) recovered 8 weeks after transfer in the absence or presence of co-transferred naive CD4⁺ T cells. The expression of CD25 and *foxp3* correlates also in the presence of the competitor cells of CD25⁺ origin and the percentage of CD25⁺ and *foxp3*⁺ cells is higher (*P* = 0.01 for CD25 and *P* = 0.03 for *foxp3*) in presence of the co-transferred cells.

both a diverse repertoire and a complete set of different lymphocyte subpopulations. This adds a qualitative dimension to lymphocyte homeostasis: mechanisms that act in the control of T-cell numbers must also keep the essential subpopulations (8). However, lymphocyte homeostasis is a dynamic process and the equilibriums attained will differ depending on both T-cell properties and environmental cues (8). Within the different T-cell subsets, CD4⁺CD25⁺ Tregs represent a specific lineage, which originates in the thymus, depends on the expression of transcription factor *foxp3* (2–4) and is essential for peripheral T-cell homeostasis (1). Since *foxp3* is an intracellular marker and cannot be used to purify these cells, we must rely on other cell-surface markers to isolate and study this cellular subset. The most commonly used is CD25. The caveats of using this marker include the fact that CD25 is expressed by activated T cells and therefore CD25⁺ cells may include a contaminant population of non-Tregs (19, 20) and it is yet unknown whether a fraction of Tregs may not express CD25 (20). A recent report (17) using a Gfp-*Foxp3* reporter knock-in confirmed these assumptions: although the large majority of CD25⁺ cells are Tregs (*foxp3*⁺), non-Treg CD25⁺ cells exist; moreover, CD4⁺*foxp3*⁺ Treg lineage cells include a small proportion of CD25⁻ cells. These observations are extremely important for the interpretation of several recent studies on CD4⁺CD25⁺ Treg biology (14–17, 21).

Generation of CD4⁺CD25⁺*foxp3*⁺ Tregs from naive CD4⁺ CD25⁻ T cells is possible either *in vitro* after infection with retroviral vectors containing *foxp3* (2) or *in vivo* via the administration of low doses of antigen (22). It has also been suggested that during LDP, CD4⁺CD25⁻ T cells can convert into CD4⁺CD25⁺ Tregs (14–16) probably through the expan-

sion of the CD25⁻*foxp3*⁺ Treg lineage cells (16). However, recent reports using knock-in *foxp3* mice (17, 18) contradict this possibility and state that these events do not occur *in vivo* during either immune responses (17) or LDP (18). Our findings indicate that the CD4⁺CD25⁻ T-cell pool contains indeed cells that are able to give rise to CD4⁺CD25⁺ T cells, but the frequency of these Treg precursors is very low and that only the transfer of considerably high numbers of CD25⁻ cells allows significant cell conversion in LDP.

We evaluated the relevance of the CD25⁻-derived CD25⁺ Treg pool. We found that the presence or absence of CD25⁺ competitors directs the outcome and expansion of CD25⁻ cells: the contribution of CD25⁻ cell conversion to the peripheral CD4⁺CD25⁺ Treg pool is minor if other CD25⁺ cells are present. We also observed that the number of CD4⁺CD25⁻ cells derived from a transferred population of purified CD4⁺CD25⁺ cells was influenced by the presence of other co-injected CD4⁺CD25⁻ competitors; in the presence of co-injected naive cells, few of the final population of CD25⁻ cells obtained after LDP are of donor CD25⁺ T-cell origin. These results attest the important role of competition in the final representation of the different T-cell subpopulations (Fig. 6). Indeed, when CD4⁺CD25⁺ T cells are used to rescue CD25^−/− BM chimeras (1), IL-2Rβ^−/− neonatal mice (7) or *sfy* mice (4), most of the cells of CD4⁺CD25⁺ origin recovered late after transfer retained CD25 expression. This reflects the competition of the endogenous cells with the CD25⁻ cells of CD25⁺ origin and the ability of the CD25⁺ cells to occupy a vacant specific niche. The expression of high levels of the high-affinity IL-2Rα by the CD4⁺CD25⁺ Tregs specializes these cells in the exploitation of IL-2, promoting their survival and expansion in the peripheral pools (1, 14, 21, 23). In conclusion, the expression of the potential CD4⁺CD25⁻ T cells to generate CD25⁺ T cells will depend on the specific context at the onset of expansion, namely the presence of competitor cells and probably of IL-2 availability. Thus, upon depletion of CD25⁺ T cells or when naive CD4⁺CD25⁻ T cells are transferred into lymphopenic mice, we are in presence of the most favorable conditions for the expansion of residual numbers of CD25⁻*foxp3* lineage cells. IL-2 is abundant and competitor CD25⁺ cells are absent, allowing CD25⁻*foxp3*⁺ Treg lineage cells to expand, express CD25 and fill-up the available niche. Conversely, when CD4⁺CD25⁺ cells are used as a source of Tregs, contaminant CD4⁺CD25⁺*foxp3*⁻ T cells provide a potential for the reconstitution of an activated pool of cells that are indistinguishable from the activated cells generated by the expansion of CD4⁺CD25⁻ naive T cells. As a direct consequence, these cells will also have to compete for the occupancy of the same niche (Fig. 6).

Although obtained in the mouse model, the present findings are relevant when the generation of CD25⁺ Tregs from naive peripheral T cells and the *ex vivo* expansion of CD4⁺CD25⁺ Tregs and subsequent adoptive transfer are being envisaged as potential therapeutic techniques (24). These strategies should take into account that competition from endogenous cells will have to be dealt with in order to successively establish donor cells, and conversely, that replacing the pre-existing pool of CD4⁺CD25⁺ Tregs with donor cells may have as a consequence the failure to control potentially autoimmune clones present in the host.
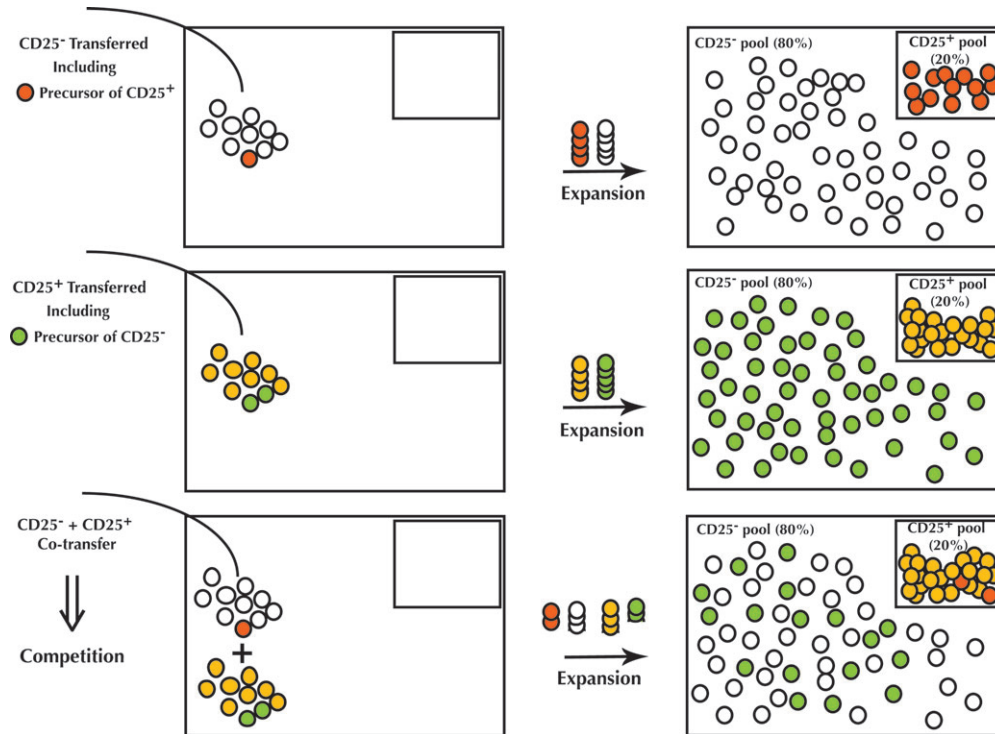
Fig. 6. Competition and the expression of the reconstitution potential of specific sub-populations of CD4$^+$ T lymphocytes. CD4$^+$CD25$^+$ and CD4$^+$CD25$^-$ lymphocyte populations are not homogeneous, but rather include cells from different lineages (16). Thus, upon transfer of CD4$^+$CD25$^-$ T cells (in white) into lymphopenic mice (top panels), a small proportion of *foxp3* lineage CD4$^+$ T cells (in red) is present. During the expansion of these cells in the hosts, it is likely that IL-2 produced allows the *foxp3* lineage cells to express CD25 and use IL-2 in their expansion. As CD25 expression is required for the assembly of the high-affinity IL-2 receptor, these cells will be the best competitors for IL-2 usage and will prosper eventually to fill up the CD4$^+$CD25$^+$ Treg pool until an equilibrium is reached, keeping CD4$^+$CD25$^+$ T cells as a constant fraction of about 20% of the total non-naive CD4+ T-cell pool (23). When CD4$^+$CD25$^+$ T cells (in yellow) are transferred alone (middle panels), a small proportion of *foxp3*$^-$CD4$^+$CD25$^+$ cells (in green) is also present. These cells will expand in the host to eventually reconstitute the activated/memory CD4$^+$ T-cell pool, while *foxp3*$^+$CD4$^+$CD25$^+$ lineage cells will reconstitute their own Treg pool, making up for 20% of the total reconstituted pool. As the two pools are not shared, *foxp3*$^-$ lineage cells will have the possibility to expand to a higher extent. When the two populations (CD25$^+$ and CD25$^-$) are co-transferred (bottom panel) the rare precursors will share the resulting pool with the progeny of the major contributors from the counterpart populations. Their expansion will thus be translated into smaller numbers of descendents and their contribution for the reconstituted pools will reflect the part they make of the initial precursors for the respective final pool. Conversion is thus a less likely event in the presence of competitors when the final reconstituted pool is analyzed.

Acknowledgements

Abbreviations

LDP	lymphopenia-driven proliferation
LN	Lymph node
Tregs	regulatory T cells

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