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Short communication

Significant expression of Foxp3 in murine extrathymic CD4$^+$CD8$^+$ double positive T cells

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Abstract

Forkhead box protein 3 (Foxp3) is a specific marker and the key factor in the development and function of regulatory T (Treg) cells. The present study investigates Foxp3 expression in murine head and neck lymph node (HNLN) and peripheral blood (PB) CD4$^+$CD8$^+$ double positive (DP) T cells and compares it with that in CD4$^+$ or CD8$^+$ single positive (SP) T cells. Here we provide evidence that murine extrathymic DP cells express Foxp3. We determined that the mean percentage of Foxp3-expressing cells within HNLN and PB DP cells was, respectively, 22.4 and 16.2. The obtained results clearly indicate that DP cells are very similar to CD4$^+$ SP cells in respect of Foxp3 expression, and thus considerably deviate from CD8$^+$ SP cells. It was found that Foxp3 expression in DP cells is positively correleted with CD25 expression. These results suggest that murine extrathymic Foxp3$^+$ DP T cells may represent a unique regulatory T cell subset.

Key words: CD4$^+$CD8$^+$ double-positive (DP) T cells, CD25, Foxp3, Treg cells, mouse

Introduction

Double-positive CD4$^+$CD8$^+$ T cells (hereinafter referred to as DP cells) normally represent a thymic population that is developed in the thymus as a precursor of CD4$^+$ or CD8$^+$ single-positive T cells (hereinafter referred to as CD4$^+$ or CD8$^+$ SP cells) (Perez et al. 2012). However, T cells bearing the DP phenotype exist in the peripheral blood and secondary lymphoid organs of healthy humans and animals, and their proportion is increased in several pathological conditions (Parel and Chizzolini 2004). In contrast to immature DP thymocytes, they display an effector memory phenotype but no markers of recent thymic emigrants (Parel and Chizzolini 2004). However, the function of extrathymic DP T cells is a conflicting matter. Regulatory T cells (Treg) comprise numerous subsets including, among others, naturally-occurring Forkhead box protein 3 (Foxp3)-expressing CD25$^+$CD4$^+$ and CD25$^+$CD8$^+$ Treg cells. Foxp3 is a unique marker and a "master" regulator of the development and suppressive function of these cells in humans and mice (Curotto de Lafaille and Lafaille 2009). In this study, we hypothesized that – similarly to CD4$^+$ or CD8$^+$ SP cells – separate subsets of effector and regulatory cells co-exist within the peripheral DP cell pool. Therefore, the aim of the study was to determine whether murine extrathymic DP cells express CD25 and Foxp3 and, if they do, whether they resemble CD4$^+$ or CD8$^+$ SP cells in this respect.

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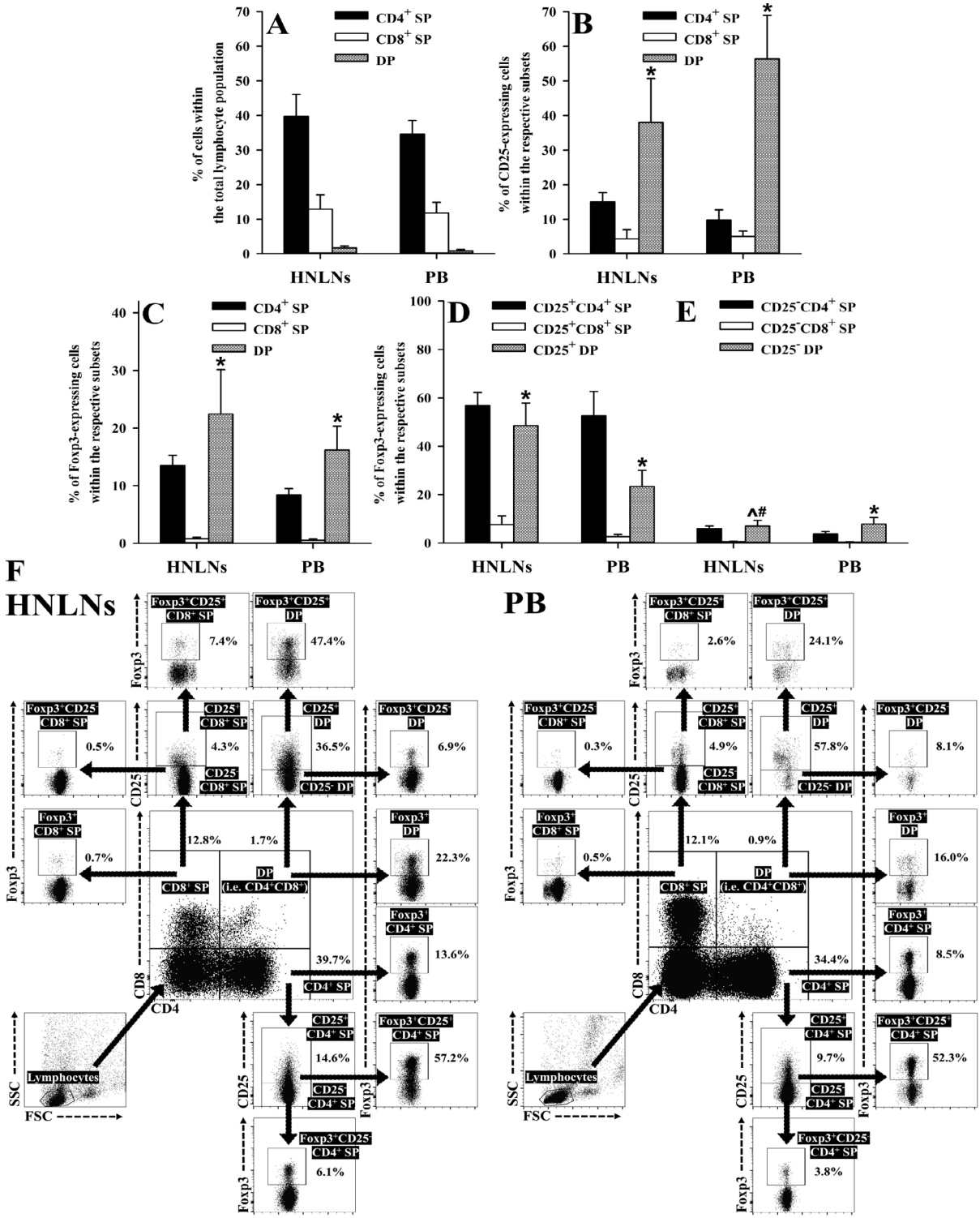

Fig. 1. Comparative phenotypic analysis of single (SP)- and double-positive (DP) $CD4^+$ and $CD8^+$ T cells in mouse head and neck lymph nodes (HNLNs) and peripheral blood (PB) with respect to CD25 and Foxp3 expression. (A) The distribution of $CD4^+$ SP, $CD8^+$ SP and DP cells within the total lymphocyte population. (B) Expression of CD25 on $CD4^+$ SP, $CD8^+$ SP and DP cells. (C-E) Foxp3 expression in the entire $CD4^+$ SP, $CD8^+$ SP and DP T cell populations (C) and in their $CD25^+$ (D) and $CD25^-$ (E) subsets. (F) Gating strategy for flow cytometric data analysis. Lymphocytes were identified based on forward and side scatter (FSC/SSC) properties and, among them, individual $CD4^+$ SP, $CD8^+$ SP and DP T cell populations were gated. Relative to the presence of CD25 expression, they were subdivided into $CD25^+$ and $CD25^-$ T cell subsets. Foxp3 expression was analyzed comparing two gating strategies. Foxp3-expressing cells were gated within the entire $CD4^+$ SP, $CD8^+$ SP and DP T cell populations and also among their $CD25^+$ and $CD25^-$ T cell subsets. Results are expressed as the mean (± SD) of three independent experiments with 10 mice per individual experiment (overall n = 30; *$p<0.001$ DP versus $CD4^+$ SP and $CD8^+$ SP; ^$p=0.025$ DP versus $CD4^+$ SP; #$p<0.001$ DP versus $CD8^+$ SP).

Materials and Methods

The mice (n = 30) were housed and treated as described previously (Zuśka-Prot and Maślanka, 2017). The mice were asphyxiated with CO_2 and a whole set of head and neck lymph nodes (HNLNs) and peripheral blood (PB) samples (100 µl) were collected. HNLN and PB samples were prepared and stained as previously described (Zuśka-Prot and Maślanka 2017). Flow cytometry analysis was performed as previously described (Zuśka-Prot and Maślanka 2017). All data are presented as the mean ± SD. Student's t test was used to compare the results between DP and SP cells.

Results and Discussion

It has been demonstrated that as many as 37.9% and 56.4% of HNLN and PB of DP cells, respecively, expressed (most likely constitutively) CD25, i.e. IL-2 receptor α chain (Fig. 1B). The percentage of CD25-expressing within PB DP cells was approximately 6- and 11-fold higher than that of PB $CD4^+$ i $CD8^+$ SP cells, respectively. Thus, relative to CD25 expression, peripheral DP cells deviate considerably from both $CD4^+$ and $CD8^+$ SP cells.

To the authors' best knowledge, this is the first report attesting to the fact that extrathymic DP cells express Foxp3. We determined that the mean percentage of Foxp3-expressing cells within HNLN and PB DP cells was, respectively, 22.4 and 16.2 (Fig. 1C). The mean percentages of $Foxp3^+$ cells among HNLN $CD4^+$, PB $CD4^+$, HNLN $CD8^+$ and PB $CD8^+$ SP cell subsets were, respectively, 13.5, 8.4, 0.8 and 0.5 (Fig. 1C). These results clearly indicate that DP cells are very similar to $CD4^+$ SP cells in respect of Foxp3 expression, and thus considerably deviate from $CD8^+$ SP cells. Interestingly, a substantially higher percentage of DP cells express Foxp3 as compared with that in $CD4^+$ SP cells. The percentage of Foxp3-expressing cells within HNLN and PB $CD25^+$ DP cells was approximately 7- and 3-fold higher, respectively, than in HNLN $CD25^-$DP and PB $CD25^-$DP cells (Fig. 1C). In turn, the percentage of $Foxp3^+$ cells among HNLN $CD25^+CD4^+$, PB $CD25^+CD4^+$, HNLN $CD25^+CD8^+$ and PB $CD25^+CD8^+$ SP cells was, respectively, about 10-, 14-, 14- and 8-fold higher than in the corresponding CD25 non-expressing cell subsets (Fig. 1C). These results suggest that Foxp3 expression in extrathymic DP cells is positively correleted with CD25 expression, although not as strongly as in the case of $CD4^+$ and $CD8^+$ SP cells.

It is well known that a significant induction of Foxp3 occurs at the DP stage, prior to $CD4^+$ or $CD8^+$ lineage commitment, in the human thymus (Nunes-Cabaço et al. 2011). It is believed that $Foxp3^+$ DP thymocytes differentiate into mature $Foxp3^+CD4^+$ and $Foxp3^+CD8^+$ SP cells, which subsequently exit the thymus to populate the periphery as functional SP Treg cells (Nunes-Cabaço et al. 2011). In the light of this, $Foxp3^+$ DP cells present in the thymus represent progenitors for SP Treg cells. Our study provides evidence that $Foxp3^+$ DP cells also occur in extrathymic sites. Assuming that peripheral DP T cells have the properties of mature T cells (Parel and Chizzolini 2004) and that Foxp3 confers suppressive properties and is confined to regulatory T cells (Curotto de Lafaille and Lafaille 2009), the results obtained strongly suggest that these lymphocytes may represent a unique Treg cell subset. It is unknown whether these cells co-express CD4 and CD8 because they did not differentiate into SP cells in the thymus, or whether this is the result of re-expression of CD8 or CD4 in $CD4^+$ and $CD8^+$ SP cells outside the thymus. In the light of current knowledge, it is unlikely that these cells constitute immature DP thymocytes (i.e. progenitors for SP Treg cells) which have prematurely escaped from the thymus. Determination of the origin of extrathymic $Foxp3^+$ DP cells and confirmation of their regulatory properties requires further study.

References

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