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Letter to the Editor

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CD69$^+$ memory T lymphocytes of the bone marrow and spleen express the signature transcripts of tissue-resident memory T lymphocytes

It is a matter of current debate, whether the bone marrow is a hub for circulating memory T lymphocytes, and/or the home of tissue-resident memory T (T_{RM}) lymphocytes. While several groups could not find evidence for tissue-residency of bone marrow memory T cells [1], we have previously demonstrated the exclusive maintenance of T-cell memory for distinct (systemic) antigens in the human and murine bone marrow, arguing that at least memory T cells maintaining those specificities were bone marrow-resident [2–4]. We have also shown that bone marrow memory T cells are resting in terms of proliferation [2, 3, 5] and are maintained independent of the circulation, i.e. their numbers are unaffected by treatment with FTY720 [6], a drug that blocks egress of lymphocytes from secondary lymphoid organs by antagonizing of S1PR1. Moreover, in mice and humans approximately 30% to 60% of memory T cells of the bone marrow express the residency marker CD69, and for human bone marrow memory CD4$^+$ T lymphocytes and murine bone marrow memory CD4$^+$ and CD8$^+$ T lymphocytes, we have shown that these cells do not express S1pr1 [5, 7], reported to enable lymphocyte egress into the blood.

Here, we show that CD69 expressing murine memory CD4$^+$ and CD8$^+$ T cells, and human memory CD4$^+$ T cells of the bone marrow also express a set of genes reported to be signature genes of T_{RM} from other tissues [8, 9]. We isolated human memory CD4$^+$ T cells and murine CD8$^+$ memory T cells from bone marrow in steady-state situations and murine CD69$^+$ and CD69$^-$ memory CD4 T cells from spleen and bone marrow 60 days after a secondary immunization with a defined antigen (LCMV $GP_{61–80}$) [7]. The isolation of the murine CD4$^+$ memory T cells was carried out according to published guidelines [10] and is documented in Supporting Information Fig. 1. The isolation of murine memory CD8 and human memory CD4 has been documented earlier [3, 5]. Transcriptomes of murine CD8$^+$ and human CD4$^+$ memory T cells were analyzed by Affymetrix microarrays, while transcriptomes of murine CD4 memory T cells were analyzed by next generation RNA-Seq. Both CD4 (Fig. 1A) and CD8 (Fig. 1B) murine CD69$^+$, but not CD69$^-$ memory T cells of the bone marrow, show the "universal" transcriptional signature of T_{RM} cells reported by Mackay and colleagues for CD8 T_{RM} cells of different tissues (not bone marrow) [9]. It should be noted that the Affymetrix microarrays lacked probes for the genes Zfp683 (Hobit), Sidt1, and A430078G23Rik (Fig. 1B). Interestingly, the murine T_{RM} signature genes were also expressed by the CD69$^+$ CD4 memory T cells from the spleen (Fig. 1A). Human CD69$^+$ but not CD69$^-$ bone marrow memory CD4$^+$ T cells show the T_{RM} signature previously reported for human CD4$^+$ and CD8$^+$ T_{RM} by Kumar and colleagues [8] (Fig. 1C). These differences in relative gene expression are highly significant ($p < 0.0001$ to $p < 0.0072$), as shown by gene set enrichment analysis (Supporting Information Fig. 2). We validated the expression of several T_{RM} signature genes by immunofluorescence and quantitative PCR. We had previously shown by qPCR that CD69$^+$ memory T lymphocytes of murine bone marrow do not express S1pr1 [5, 7], and show here that they also do not express Klf2 (Supporting Information Fig. 3A). In addition, human and murine CD69$^+$ memory CD4$^+$ T cells express CXCR6, but little CD62L (Supporting Information Fig. 3B and C). We also compared the expression of the human homologues from the murine T_{RM} signature genes by human bone marrow memory CD4$^+$ T cells by principal-component analysis. The predominant principal component 1 (PC1, 63%)) separates CD69$^-$CD4$^+$ cells of peripheral blood, when activated, from both, CD69$^-$CD4$^+$ cells of blood and bone marrow and CD69$^+$CD4$^+$ resting cells from bone marrow. PC2 (14%) then separates bone marrow CD69$^+$CD4$^+$ cells from CD69$^-$CD4$^+$ cells from both blood and bone marrow (Fig. 1D). Taken together, murine and human CD69$^+$ memory T cells of the bone marrow express the T_{RM} signature genes reported for T_{RM} of other tissues [8, 9], suggesting that memory T cells are indeed resident in the bone marrow. Whether or not the CD69$^-$ memory T cells of the bone marrow are also resident remains to be shown, e.g. by comparing their repertoire to that of circulating memory

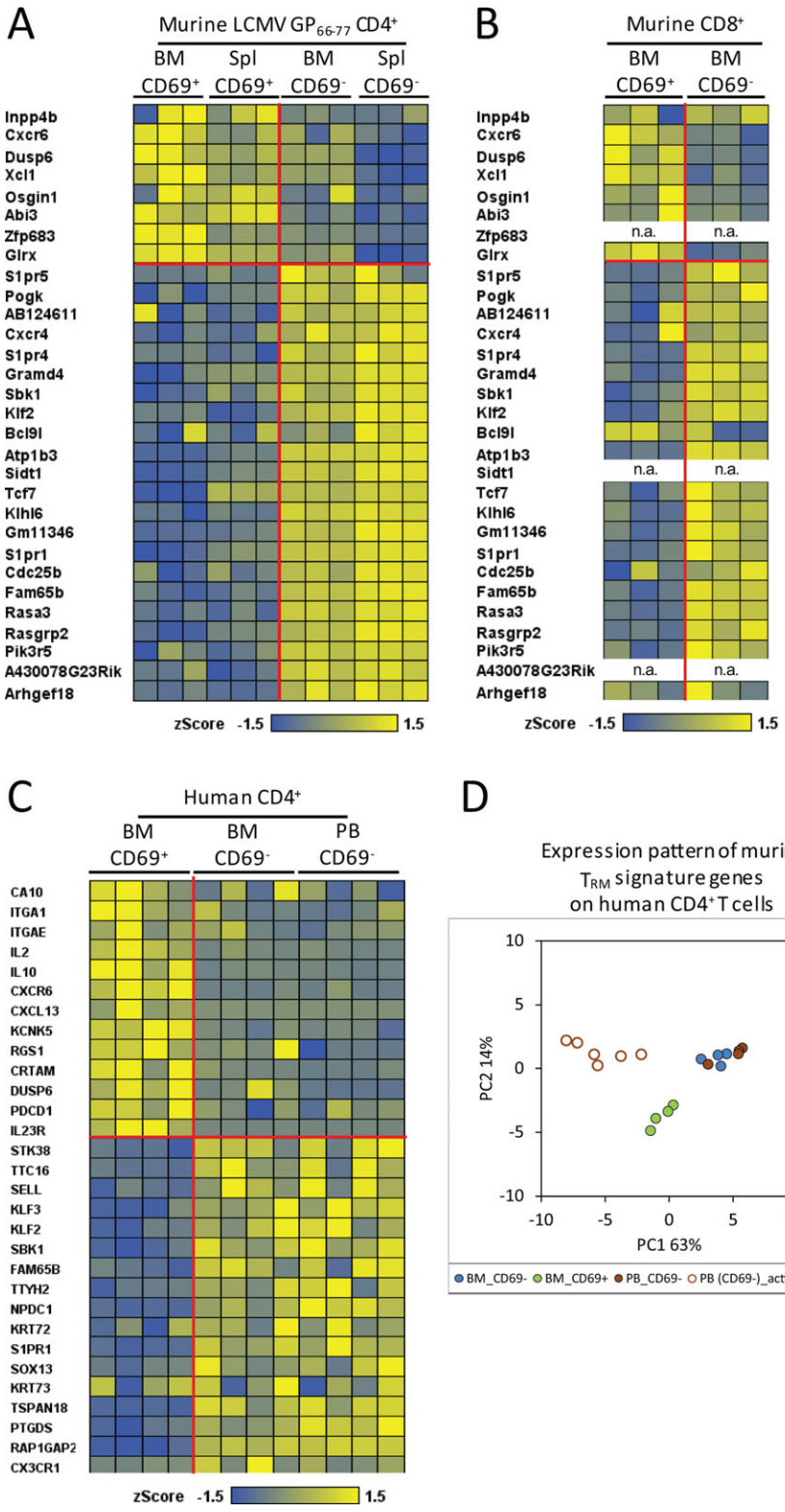

Figure 1. CD69 expression of bone marrow and spleen memory T cells defines a transcriptionally distinct population as reported earlier for T_{RM} cells of other tissues. (A and B) Heatmaps showing reported murine T_{RM} signature genes of other tissues for CD8 T cells [9], on bone marrow and spleen LCMV-specific memory $CD4^+$ T cells (A) and steady-state bone marrow memory $CD8^+$ T cells (B) isolated from C57BL/6 mice (Supporting Information Fig. 1; [5, 7]). n.a., (probes) not present on the arrays, and thus gene expression not analyzed. (C) Heatmap of reported human T_{RM} signature genes for both $CD4^+$ and $CD8^+$ T cells of other tissues [8] on human bone marrow and paired peripheral blood (>98% being $CD69^-$) memory $CD4^+$ T cells according to CD69 expression [3]. (D) Principal-component analysis (PCA) of described murine T_{RM} signature genes [9] on the cells analyzed in C in comparison with recently activated blood effector/memory $CD4^+$ T cells. In A–C, the horizontal and vertical red lines separate up- or downregulated genes as described for murine and human T_{RM} signature genes of other tissues [8, 9]. (A) Data shown are from individual mice with a total of three mice from one experiment; (B) data shown are pooled from three independent experiments with cells pooled from 8 to 10 mice per experiment; C/D, data shown were taken from our previously published data GSE50677 [3].

T cells from blood. The difference in gene expression between $CD69^+$ and $CD69^-$ memory T cells probably also explains why Baliu-Pique and colleagues were not able to detect any clear T_{RM} signatures in total memory T cells isolated from goat bone marrow [1]. Similarly, a lack of discrimination between resting memory T cells and their (proliferating) precursors may have impacted on their lifetime analysis by deuterium labeling. An alternative explanation would be that indeed the maintenance of T-cell memory is differently organized in goats versus mice and humans.

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Data deposition: The data about resting murine memory CD4$^+$ and CD8$^+$ T lymphocytes discussed in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE124796).

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