

NEWS AND COMMENTARY

T-cell migration

Kruppelled T cells move again

Kristin A Hogquist, Michael A Weinreich and Stephen C Jameson

Immunology and Cell Biology (2008) 86, 297–298; doi:10.1038/icb.2008.20; published online 1 April 2008

Kruppel-like transcription factors are a large family of proteins that can both activate and repress genes and regulate a wide variety of biological processes in multiple organ systems. In T lymphocytes, Kruppel-like factor 2 (KLF2) is an essential gene, as very few T cells are found in the spleen and lymph nodes of mice with targeted deficiency of KLF2.¹ This was initially thought to reflect a requirement for KLF2 in T-cell survival and homeostasis. However, subsequent studies showed that KLF2 was required for thymic emigration and lymph node homing by regulating cell surface receptors required for these processes, namely S1P₁ and CD62L.² Now Mark Kahn's group³ adds a new twist to the story. They show that KLF2 also represses chemokine-receptor gene expression. Thus KLF2-deficient T cells aberrantly express multiple chemokine receptors that can cause T cells to home to various tissues in the body. Altogether, these studies establish KLF2 as a 'master regulator' that coordinates expression of multiple different types of cell surface receptors to control T-cell trafficking during an immune response.

Sedbzda *et al.*³ employed a VAV-Cre transgene to eliminate KLF2 in lymphocytes. Their results confirmed that there is not a substantial survival defect in KLF2-null (KLF⁰) T cells due to spontaneous apoptosis. They further confirmed that two genes critical for T-cell trafficking, S1P₁ and CD62L, are downregulated in T cells lacking KLF2, and that these mice have increased numbers of mature thymocytes and reduced numbers of peripheral T cells in the blood, lymph node and spleen. Surprisingly, however, they report a three to fivefold increase in the number of KLF2-deficient T cells outside of lymphoid organs. This was inferred by assessing CD4

mRNA by real-time PCR in various tissues, such as bone marrow, liver, kidney, muscle and brain. Indeed, they used flow cytometry to confirm a threefold increase in the number of KLF2⁰ CD4 T cells in the liver. To explain this altered migration pattern, they examined chemokine receptor expression and found an increase in multiple chemokine receptors that generally control cellular movement to various inflamed and noninflamed tissues, suggesting that KLF2 is a general repressor of certain chemokine receptors.

Together, these data clearly point to KLF2 as a critical regulator of T-cell circulation patterns (Figure 1). KLF2 activates two genes that control access to, and emigration from, lymph nodes: CD62L and S1P₁. As such, naive T cells, which express KLF2, hold the keys to the front and back door of the lymph node—and primarily circulate through secondary lymphoid organs (red arrows). When this mode of trafficking is 'on', it would seem important that the expression of chemokine receptors that direct T cells to peripheral sites be 'off'. Thus it makes intuitive sense that the same transcriptional regulator would also repress certain chemokine receptors. Indeed, when T cells become activated, they rapidly extinguish expression of KLF2, losing S1P₁ and CD62L, and inducing chemokine receptors that direct the T cell to peripheral tissues and sites of inflammation (shaded blue). If the mature T cell becomes activated in the thymus, it is retained there.⁴ These data thus solidify the notion that the critical role of KLF2 is to coordinate multiple molecules (selectins, chemokine receptors and sphingolipid receptors) that need to act together to for appropriate cellular trafficking (Figure 1).

It is tempting to speculate that KLF2 controls these multiple genes by directly binding to the promoters and recruiting cofactors for activation or repression. Indeed, KLF2 was shown to directly bind the S1P₁ promoter

and could transactivate expression of a reporter gene with an isolated S1P₁ promoter.² Similar transactivation studies were performed with CD62L⁵ and both of these genes have a KLF2 consensus binding site in their promoters. Sedbzda *et al.* showed that KLF2 could transactivate reporter constructs with CCR3 and CCR5 promoters, suggesting that KLF2 directly represses these genes. It will be important to establish whether this is the case for other chemokine receptors.

An interesting suggestion arising from the study by Sedbzda *et al.* is that KLF2 may not be required for thymic emigration *per se*, but rather that deficient T cells leave the thymus normally and are immediately sequestered in non-lymphoid tissues. Consistent with this, the authors showed that recent thymic emigrants could be recovered from the liver of KLF2 deficient mice. Even presuming recent thymic emigrants are also present in other tissues, it is difficult to know if the increased number of recent thymic emigrants in tissues could numerically represent 'normal' thymic emigration ($1-2 \times 10^6 \text{ day}^{-1}$). The idea that thymic emigration is not impaired in KLF2⁰ mice is difficult to reconcile with other data, as well. For example, KLF2⁰ SP thymocytes are over-represented in the thymus. Such retention would not be predicted if emigration occurred normally in KLF2⁰ mice. In fact, using a RAG2GFP reporter as a 'molecular timer', we found that KLF2⁰ mature thymocytes are retained for a striking length of time (at least five times longer than wild-type cells; unpublished data). Normal thymic emigration is also inconsistent with the profound reduction of S1P₁ mRNA and protein in KLF2⁰ T cells.^{2,3} The authors suggest that KLF2⁰ T cells, despite a dramatic reduction of message and cell-surface protein, may have enough residual S1P₁ to emigrate normally. This is based on their finding that an S1P agonist, FTY720, could influence migration of KLF2⁰ T cells. However, it should be noted

KA Hogquist is at the Center for Immunology at the University of Minnesota, Minneapolis, MN, USA.
E-mail: hogqu001@umn.edu

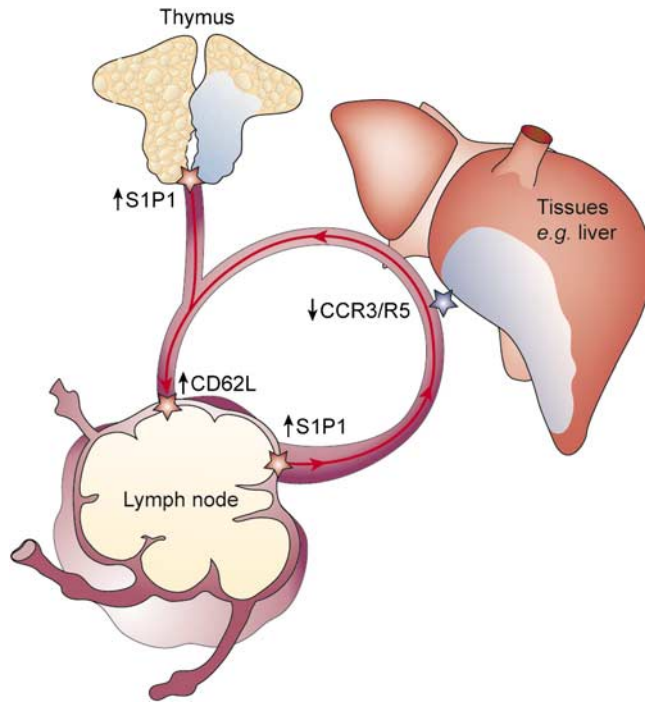


Figure 1 Kruppel-like factor 2 (KLF2) controls T-lymphocyte trafficking. The transcription factor KLF2 controls key molecules (starred) that are involved in T-lymphocyte trafficking. Mature T cells in the thymus require the sphingosine 1 phosphate receptor 1 (S1P₁) for egress into circulation. Once circulating, T cells require L-selectin (CD62L) for entry into lymph nodes through high endothelial vessels. Egress from the lymph node into lymphatic vessels again requires S1P₁. As KLF2 controls these ‘keys’ to the front and back door on the lymph node, when naïve T cells express KLF2 (and subsequently S1P₁ and CD62L) they circulate through secondary lymphoid tissue (red arrows). When T cells are activated, they downregulate KLF2, causing a loss of S1P₁ and CD62L, and express chemokine receptors. This results in thymic retention (if the T cell is in the thymus) or movement from circulation to tissues (if the T cell is in the periphery) (shaded blue).

that FTY720 binds to three other S1P receptors, of which at least one, S1P₄, is known to be expressed in T cells.⁶ Further work will be required to more precisely quantitate the emigration defect and accumulation of KLF2⁰ T cells in tissues. Nonetheless, the current findings clearly reveal an important new set of KLF2 target genes.

Since KLF2 is emerging as a critical regulator of T-cell trafficking, it is imperative to understand what signals regulate KLF2

expression itself. T-cell receptor signaling is clearly important, as KLF2 mRNA is dramatically repressed after T-cell activation. KLF2 protein may also be destabilized by phosphorylation and targeted for degradation.⁷ In endothelial cells, KLF2 is regulated by the map kinase ERK5 and the transcription factor MEF2D.⁸ However, it is not known if this pathway is involved in regulating the expression and function of KLF2 in lymphocytes. Interestingly, in the thymus, KLF2 seems to

be upregulated by T-cell receptor signaling, in the sense that it is not expressed until after positive selection.¹ However, a recent study showed that KLF2 is not upregulated directly after positive selection, but rather at the latest mature single positive stage.⁹ We estimate this is at least 3 days after the onset of positive selection signaling. Thus it seems likely that there are other developmental or microenvironmental signals that direct the cell to express KLF2, subsequently emigrate from the thymus and adopt the circulation pattern of a naïve T cell. It will also be interesting to determine how KLF2 is regulated in memory T cells, and whether it controls the balance of central and effector memory cells. Ultimately, manipulation of KLF2 in peripheral T cells may be a promising way to control lymphocyte migration patterns for therapeutic purposes.

1 Kuo CT, Veselits ML, Leiden JM. LKLF: a transcriptional regulator of single-positive T cell quiescence and survival. *Science* 1997; **277**: 1986–1990.

2 Carlson CM, Endrizzi BT, Wu J, Ding X, Weinreich MA, Walsh ER *et al*. Kruppel-like factor 2 regulates thymocyte and T-cell migration. *Nature* 2006; **442**: 299–302.

3 Sebzda E, Zou A, Lee JS, Wang T, Kahn ML. Transcription factor KLF2 regulates the migration of naïve T cells by restricting chemokine receptor expression patterns. *Nature Immunology* 2008; **9**: 292.

4 Uldrich AP, Berzins SP, Malin MA, Bouillet P, Strasser A, Smyth MJ *et al*. Antigen challenge inhibits thymic emigration. *J Immunol* 2006; **176**: 4553–4561.

5 Bai A, Hu H, Yeung M, Chen J. Kruppel-like factor 2 controls T cell trafficking by activating L-selectin (CD62L) and sphingosine-1-phosphate receptor 1 transcription. *J Immunol* 2007; **178**: 7632–7639.

6 Rosen H, Sanna MG, Cahalan SM, Gonzalez-Cabrera PJ. Tipping the gatekeeper: S1P regulation of endothelial barrier function. *Trends Immunol* 2007; **28**: 102–107.

7 Zhang X, Srinivasan SV, Lingrel JB. WWP1-dependent ubiquitination and degradation of the lung Kruppel-like factor, KLF2. *Biochem Biophys Res Commun* 2004; **316**: 139–148.

8 Sohn SJ, Li D, Lee LK, Winoto A. Transcriptional regulation of tissue-specific genes by the ERK5 mitogen-activated protein kinase. *Mol Cell Biol* 2005; **25**: 8553–8566.

9 McCaughy TM, Wilken MS, Hogquist KA. Thymic emigration revisited. *J Exp Med* 2007; **204**: 2513–2520.