Bcl-10 may be involved in TCR-induced cellular adhesion. Thus, MALT1 protease activity may have a dichotomous role in T cell activation: for Bcl-10, MALT1-dependent cleavage seems to activate or 'unmask' a function, whereas for A20, cleavage brings about a loss of function (**Fig. 1a**).

Alignment of the specific cleavage sites in human Bcl-10 and A20 shows that in both cases, a serine residue precedes the P1 arginine residue (**Fig. 1c**). This pattern is present in several substrate cleavage sites of metacaspases, the related family of plant proteases that specifically cleave after either lysine or arginine. Unexpectedly, the MALT1 cleavage site in human A20 is not conserved in the mouse[4]. Instead, experimental data indicate that MALT1 cleaves mouse A20 at a site in the linker region between zinc fingers 3 and 4. Analysis of this region shows the presence of two lysine residues, each preceded by serine residues (**Fig. 1c**), as well as several arginine residues, any of which might represent the actual cleavage site. Obviously, further studies are needed to define the substrate site specificity of the MALT1 protease. It seems likely that the publication of these two landmark manuscripts will lead to a search for additional physiological substrates of MALT1 and much more will be learned about the nature of this proteolytic activity.

Identification of MALT1 proteolytic activity represents a major step in understanding the molecular events that regulate the normal immune response to antigen and the molecular pathogenesis of lymphoma. The work of Coornaert *et al.* indicates that this proteolytic activity serves to inactivate an inhibitor of NF-κB-dependent gene expression, and the studies by Rebeaud *et al.* suggest involvement of MALT1-mediated proteolysis in promoting integrin-mediated cellular adhesion. Both NF-κB signaling and integrin action are critical to normal immune function, and dysregulation of these activities is associated with immunological and inflammatory diseases as well as malignancy. Thus, both groups wisely point out that the proteolytic activity of MALT1 could represent a promising new target for the development of immunomodulatory and antineoplastic agents. These exciting new discoveries were clearly worth waiting for.

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Innocents abroad: regulating where naive T cells go

Barrett J Rollins

Kruppel-like factor 2 is now shown to regulate chemokine receptor expression in lymphocytes, which leads to their homing to nonlymphoid organs after they leave the thymus.

In one sense, adaptive immunity is all about cell migration. Although specific antigen-recognition structures and the signal-transduction cascades they activate are essential for adaptive immunity, the cells that have those structures and signaling pathways must be at 'the right place at the right time' for immunity to occur. For example, after T lymphocytes mature, they leave the thymus and, as naive cells, circulate through blood, secondary lymphoid organs, lymph and back to blood with regulated 'dwell times' in each environment. When activated, T lymphocytes either travel to peripheral organs as effector cells or become memory cells with 'central' or 'peripheral' patterns of migration. Elegant experimentation has identified many of the molecular controls that govern these movements. In particular, several papers have provided an apparently complete picture of T cell egress from the thymus and secondary lymphoid organs. However, a report in this issue of *Nature Immunology* by Kahn and colleagues puts an unexpected twist on the model and provides an important insight into how naive T cells are kept out of peripheral organs until they are needed[1].

The story begins 10 years ago with work on how transcription factors determine T cell phenotypes. One set of studies focused on Kruppel-like factor 2 (KLF2), a member of a family with homology to the drosophila Kruppel transcription factor. KLF2 was originally known as 'Lklf' because of its expression in lung, but it is also present in lymphoid tissue, erythroid cells and endothelial cells. Its influence on T cell activity was inferred from its higher expression in thymocytes after positive selection and lower expression in T cells after activation[2]. Study of KLF2 *in vivo* is complicated by the fact that *Klf2*$^{-/-}$ embryos die *in utero*. Therefore, chimeric mice were developed by injection of *Klf2*$^{-/-}$ cells into blastocysts deficient in recombination-activating gene 2, so that mature B cells and T cells in surviving mice were KLF2 deficient[2]. Notably, these mice had almost no detectable circulating T cells and many fewer T cells in their spleens and lymph nodes, and because the few surviving peripheral T cells had a 'partially activated' phenotype (CD44^{hi}CD62L^{lo}CD69^{hi} but CD25^{-}), their near absence was thought to be a consequence of upregulation of the cytokine FasL, leading to apoptosis. Thus KLF2 was identified as a 'master regulator' of mature T lymphocyte quiescence and, perhaps, survival[3]. One curious property of these initial mice with cell-specific KLF2 deficiency[2] was that despite lacking normal numbers of peripheral T cells, they had more single-positive (SP) cells in the thymus; to explain these phenotypes, the authors mentioned in passing that they might be due to a defect in the export of SP cells[2]. However, it would be several more years before a detailed molecular explanation was forthcoming.

Thymic egress had been shown to be dependent on G proteins, which suggested the involvement of a chemoattractant receptor[4]. Its eventual identification was facilitated by elucidation of the mechanism of action of FTY720, an immune suppressant with an interesting mechanism of action: FTY720 'depletes' animals of circulating T cells by causing the cells to accumulate in secondary

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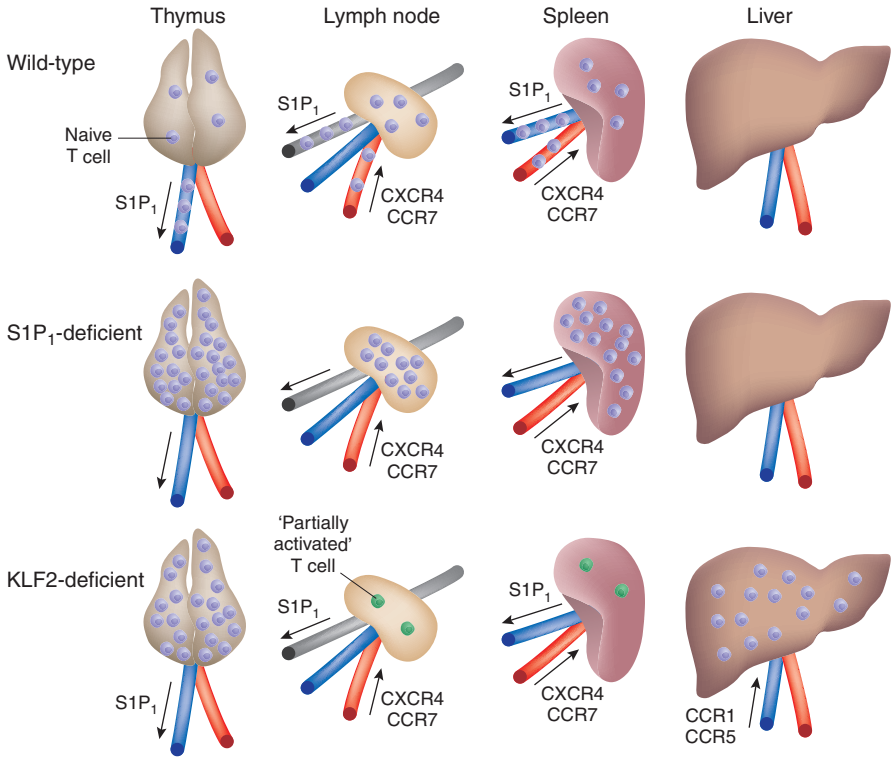

Katie Ris-Vicari

Figure 1 Several systems control the migration of naive T lymphocytes. Top, the function of $S1P_1$ in controlling the egress of thymocytes and naive T cells from the thymus, lymph node and spleen and the function of chemokine receptors such as CXCR4 and CCR7 in the extravasation of such cells into secondary lymphoid organs. Middle, the consequences of $S1P_1$ deletion or blockade (such as use of the $S1P_1$ agonist FTY720), which leads to loss of T cells in blood or lymph because they cannot exit the thymus and secondary lymphoid organs. Bottom, the findings of Kahn and colleagues obtained with mice lacking the transcription factor KLF2 in T lymphocytes[1]. Normally, KLF2 drives $S1P_1$ expression; thus, in the absence of KLF2, thymocytes accumulate in the thymus. But because loss of $S1P_1$ expression in this setting is incomplete[1], some thymocytes can exit the thymus and populate peripheral tissues. Similar to earlier reports, here KLF-deficient T cells show a 'partially activated' phenotype in secondary lymphoid organs[1]. In addition, Kahn and colleagues find a large population of naive KLF-deficient T cells with upregulated expression of inflammatory chemokine receptors (such as CCR1 and CCR5) in peripheral nonlymphoid organs. The authors conclude that one of KLF2's functions is to prevent naive T lymphocytes from trafficking to nonlymphoid organs.

lymphoid organs and, notably, the thymus. Phosphorylated FTY720 shares structural features with sphingosine 1-phosphate (S1P) and is a potent agonist for several S1P receptors[5], but the relevant receptor in this setting is $S1P_1$, a heptahelical G protein–coupled receptor also known as 'Edg1'. The identification of $S1P_1$ as the relevant receptor by which FTY720 'depletes' animals of T cells was demonstrated by transplantation of fetal liver hematopoietic precursors from $S1P_1$-deficient ($Edg1^{-/-}$) mice into irradiated recipient mice to form fetal liver chimeras. (Like $Kfl2^{-/-}$ mice, $Edg1^{-/-}$ mice also die *in utero*.) These mice had no detectable T cells in blood, spleen, lymph nodes or liver, but their thymi had more SP cells[6].

The general similarity in the phenotypes of the two gene-deficient chimeras raised the possibility that KLF2 might regulate $S1P_1$ expression. Indeed, evidence has been published showing that this is the case[7]. In those studies, the authors took the fetal liver chimera approach with recipient mice deficient in recombination-activating gene 2 to produce mice lacking KLF2 in their T cells and B cells[7]. Again, peripheral T cells were absent, whereas mature SP cells accumulated in the thymus. No evidence for peripheral T cell apoptosis was found, which indicated that KLF2 does not promote survival itself. Furthermore, when a mixture of $Kfl2^{-/-}$ and $Kfl2^{+/-}$ cells was injected into the thymus, the $Kfl2^{+/-}$ cells were detected in the periphery, which indicated that KLF2 was required for thymic emigration. The most parsimonious model of thymic egress is that KLF2 upregulates $S1P_1$ in SP thymocytes after positive selection, thereby promoting emigration from the thymus. The authors showed, in fact, that KLF2 directly binds to the $S1P_1$ promoter and activates transcription.

Although that model has some explanatory power, Kahn and colleagues note that it fails to explain why KLF2-deficient and $S1P_1$-deficient T cells act differently after adoptive transfer into wild-type mice: KLF2-deficient cells cannot be detected in lymph nodes after transfer[7], whereas $S1P_1$-deficient cells actually accumulate there[6]. To investigate the reason for this difference, Kahn and colleagues use conditional gene disruption to develop mice lacking KLF2 only in their hematopoietic cells or only in their T cells (with the same results for each). Like the KLF2-deficient chimeras, these mice have more thymic SP cells and extremely low numbers of T cells in blood, spleen and lymph nodes. And, consistent with earlier reports, the few cells in the periphery have a partially activated phenotype and are not undergoing apoptosis. No naive T cells are found in the periphery at all.

As expected, the KLF2-deficient SP thymocytes in this model have less $S1P_1$, but careful analysis shows that they still have low expression of functional $S1P_1$. Furthermore, *in vivo* administration of FTY720 leads to even more thymic accumulation of SP cells, which suggests that KLF2-deficient thymocytes still emigrate from the thymus in an $S1P_1$-dependent way, although less efficiently than wild-type cells. This means that the absence of KLF2-deficient T cells in blood and secondary lymphoid organs is not solely a consequence of a failure of thymocyte emigration.

So, if KLF2-deficient thymocytes can leave the thymus and if they do not die after they leave, then where are the peripheral T cells? The unexpected answer is provided by the effects of FTY720 on mice with conditional knockout of *Klf2*: their lymph nodes are filled with naive T lymphocytes. This means that a population of naive KLF2-deficient T cells is present in the periphery and can be 'trapped' in nodes by interference with the $S1P_1$ pathway by, in this case, treatment with FTY720. So if they are not in blood or secondary lymphoid organs, where had those naive T cells been hiding? Kahn and colleagues discover that KLF2-deficient naive T cells accumulate in peripheral nonlymphoid organs.

The explanation for how KLF2-deficient naive T cells go to nonlymphoid organs is provided by the finding that the cells express chemokine receptors that are more characteristic of activated T cells; such 'inflammatory' chemokine receptors respond to small amounts of chemokines secreted by nonlymphoid organs. For example, hepatic cells secrete ligands for the chemokine receptors CCR1 and CCR5, which are among those that are upregulated in KLF2-deficient T cells. Kahn and colleagues establish the relevance of these receptors to nonlymphoid tissue trafficking by treating the mice with conditional deletion of KLF2 with

met-RANTES, a peptide antagonist of CCR1 and CCR5; they find that this treatment prevents the accumulation of KLF2-deficient T cells in the liver while having no effect on their migration to lymph nodes or spleen.

These findings are important for a few reasons. First, they help to reconcile the disparate activities of KLF2- and $S1P_1$-deficient T cells. The key to understanding the difference seems to be the quantitative effects of these genetic disruptions. In adoptive transfer experiments, the number of *Klf2*$^{-/-}$ cells in the nodes is 90% lower and the number of cells in the spleen is almost unchanged relative to that of wild-type mice; the number of $S1P_1$-deficient cells in nodes is about the same and the number of cells in the spleen is actually more than that in wild-type mice. When cells enter nodes (or other lymphoid organs), under the influence of CXCR4 and CCR7, complete absence of $S1P_1$ efficiently 'traps' them there, whereas the partial deficiency of $S1P_1$ in *Klf2*$^{-/-}$ cells allows them to exit, although inefficiently (**Fig. 1**). The effect of smaller than normal amounts of $S1P_1$ is also reflected in the thymic accumulation of SP cells in KLF2-deficient mice.

But more important is what these findings indicate about the tight coordination between T cell activation state and patterns of migration. Naive T cells generally must be kept from peripheral, nonlymphoid organs where they would serve no practical purpose or might be rendered anergic if they encountered antigen. In contrast, activated T cells should be sent to the periphery to accomplish their effector functions. Ten years of work on KLF2 and $S1P_1$, now capped by that of Kahn and colleagues, suggests that KLF2's transcriptional program includes activation of $S1P_1$ and suppression of 'inflammatory' chemokine receptors. This keeps naive cells circulating through secondary lymphoid organs, blood and lymph and prevents their entry into nonlymphoid organs. After activation, the amount of KLF2 falls. $S1P_1$ expression also falls, but not so low as to prevent egress from lymphoid organs. In addition, however, chemokine receptor expression rises and allows activated T cells to enter nonlymphoid organs.

Although this model provides some satisfactions, it also raises questions. Does the decrease in $S1P_1$ expression that accompanies the increase in chemokine receptor expression have functional importance? Is this also required for removal of the constraints on naive T cell trafficking? What about the adhesion molecules essential for these extravasation events? Are they also coordinately regulated, and does the regulation involve KLF2? Finally, what do these molecular events suggest about adaptive immune function and autoimmunity? The mice with conditional knockout described here, along with reagents such as FTY720 and specific chemokine receptor antagonists, should provide answers.

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