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Krüppel-Like Factor 2 Controls T Cell Trafficking by Activating L-Selectin (CD62L) and Sphingosine-1-Phosphate Receptor 1 Transcription

Ailin Bai, Hui Hu, Mandy Yeung, and Jianzhu Chen

Krüppel-like factor 2 (KLF2) is a member of zinc-finger transcription factors. Based on its expression in naive and memory T cells and the activated phenotype of few T cells in mice lacking KLF2 in the lymphoid lineage, KLF2 is postulated to regulate T cell homeostasis by promoting cell quiescence. In this study, we show that in reporter gene assays KLF2 directly activates the promoters of both CD62L and sphingosine-1-phosphate receptor 1 (S1P1), whose expression is critical for T cell egress from the thymus and homing to the lymph nodes. Correspondingly, exogenous KLF2 expression in primary T cells significantly up-regulates both CD62L and S1P1. Following adoptive transfer, KLF2-transduced T cells are much more efficient in homing to lymphoid organs than nontransduced T cells. These findings suggest that KLF2 regulates T cell homeostasis at least partly by controlling CD62L and S1P1 expression, and therefore T cell egress from the thymus and circulation in the periphery. The Journal of Immunology, 2007, 178: 7632–7639.

In an adult animal, the number of T cells in the peripheral lymphoid organs is maintained at a stable level despite intermit-tent clonal expansion following Ag stimulation (1, 2). Studies have shown that competition for limiting resources is one of the key mechanisms that regulate the size of the peripheral T cell pool. Thus, naive T cells compete for self-peptide/MHC complexes and IL-7 for survival, whereas memory T cells compete for IL-15 for stable maintenance (3–8). An underexplored, but potentially important mechanism that could affect T cell homeostasis is lymphocyte trafficking (9, 10). To enter the naive T cell pool, newly generated T cells must exit from the thymus and subsequently circulate in the peripheral lymphoid organs. Besides circulating in the lymphoid organs, memory T cells must also acquire the ability to migrate into nonlymphoid tissues (11). Dysregulation of lymphocyte trafficking would be expected to have a significant effect on T cell homeostasis.

The zinc-finger transcription factor Krüppel-like factor 2 (KLF2) (12) has been suggested to play a critical role in regulating T cell homeostasis (12). It is highly expressed in both naive and memory T cells, but down-regulated in activated T cells (13, 14). Due to the embryonic lethality of complete KLF2 deficiency (KLF2−/−), the function of KLF2 in lymphocytes has been studied with chimeric mice, in which lymphoid lineage cells were all derived from KLF2−/− precursors (15). These mice display a relatively normal T cell development in the thymus, but a severe T cell deficiency in the periphery. The few T cells that are found in the secondary lymphoid organs exhibit a surface profile similar to activated T cells, although without any obvious sign of proliferation. These data have been interpreted as evidence for supporting a role of KLF2 in regulating T cell quiescence (12, 16).

Consistent with this notion, exogenous expression of KLF2 in Jurkat T cells dramatically reduced the rate of cell proliferation (17). Two molecules, c-myc and P21WAF1/CIP1, have been suggested as the direct downstream mediators of this effect (17, 18). In addition, exogenous KLF2 expression in Jurkat cells also induced certain phenotypes, such as reduced cell size, which are characteristic of quiescent cells, further supporting a role of KLF2 in regulating T cell quiescence (17). However, because the severe T cell deficiency in KLF2−/− chimeric mice excluded a detailed analysis of KLF2 function in mature T cells, the mechanisms by which the loss of KLF2 leads to T cell deficiency in the periphery have not been fully examined.

Compared with wild-type mice, KLF2−/− chimeric mice accumulate a higher proportion of single-positive (SP) thymocytes with an aberrant cell surface profile (15). This phenotype, in combination with peripheral T cell paucity, shares a striking similarity to recently reported chimeric mice that are deficient with sphingosine-1-phosphate (S1P) receptor 1 (S1P1) (19). In the latter case, it was demonstrated that the lack of T cells in the periphery is primarily due to a defect in T cell egress from the thymus. Furthermore, interference of cell surface S1P1 expression by a low m.w. molecule, FTY720, results in T cell retention in the lymph nodes (20, 21), indicating that the mechanism that controls T cell egress from thymus also affects mature T cell trafficking in the periphery. The similarity in T cell phenotype between KLF2−/− and S1P1−/− chimeric mice raises the intriguing possibility that a similar mechanism underlies the observed T cell deficiency in KLF2−/− chimeric mice. While this manuscript was in preparation, a study...
published by Carlson et al. (22) showed that KLF2−/− thymocytes are indeed defective in S1P1 expression as well as thymic egress. In this study, we show that KLF2 directly regulates CD62L and S1P1 expression in mature peripheral T cells and modulation of KLF2 expression alters the T cell trafficking pattern in mice, suggesting that T cell deficiency in KLF2−/− chimeric mice at least partly results from a defect in T cell migration.

Materials and Methods

Animals, cell lines, Abs, and flow cytometry

The 2C TCR transgenic mice were on RAG-1−/− and C57BL/6 (B6) background (2C/RAG mice) and were maintained in a specific pathogen-free facility at Massachusetts Institute of Technology. B6, B6.PL-Thy1.1(CyJ (B6-Thy1.1)), and STAT6−/− mice were purchased from The Jackson Laboratory. Animal studies have been approved by the Committee on Animal Care at Massachusetts Institute of Technology. HeLa cells were a gift from P. Sharp (Massachusetts Institute of Technology, Cambridge, MA). Abs to CD8, CD62L, Thy1.1, and Thy1.2 were purchased as conjugates from BD Biosciences. The 2C TCR clonotypic Ab 1B2 was used as a biotin-conjugated form. Cells stained with fluorescent Abs were analyzed on a FACSCalibur (BD Biosciences). Dead cells were excluded from analysis by propidium iodide. Cell sorting was performed on a BD FACSAria (BD Biosciences) with a resulting purity of >96%.

T cell activation and cytokine treatment

Over 90% of lymph node cells in 2C/RAG mice were naive (2C TCR CD44−/low), and they were used without further purification. For activation with anti-CD3, naïve 2C cells were cultured in 24-well plates precoated with an anti-CD3 Ab (10 μg/ml in PBS). For peptide activation, splenocytes from 2C/RAG mice were cultured in 24-well plates in complete RPMI 1640 medium with 100 nM SIY peptide (SIYRYYGL). After 48 h, cells were harvested, washed three times, and resuspended in fresh medium supplemented with IL-2 (10 ng/ml), IL-4 (25 ng/ml), IL-7 (10 ng/ml), or IL-15 (20 ng/ml). IL-4 was purchased from R&D Systems, whereas the rest of cytokines were purchased from PeproTech.

After 4 days, cells were harvested, extensively washed, and resuspended in fresh medium and resuspended in RPMI 1640 medium containing 5% charcoal-stripped FCS. Ten million cells were transferred into B6.Thy1.1 recipient mice by tail vein injection. For CD62L blocking, 10 million cells were incubated with 100 μg of functional grade purified anti-mouse CD62L Ab (Mel14; eBioscience) in 200 μl of PBS for 30 min at room temperature and then injected together with the Ab into recipients through tail veins. At different times after transfer, mice were sacrificed, and lymphocytes from blood, spleen, lymph nodes, lungs, and liver were analyzed by flow cytometry.

Luciferase reporter assay

The BAC clones, RP23-184B10 and RP23-41G21, which contain the promoter regions of CD62L and S1P1, were obtained from the BACPAC Resource Center. Both p3.7-Luc and p1.4-Luc were constructed by inserting a 3.7-kb Mid-S1P1 fragment or a 1.4-kb SpI-Stud fragment of L-selectin (from RP23-184B10) into pGL3-basic (Promega). The p5.4-Luc was constructed by inserting an Mid-Acul fragment of S1P1 (from RP23-41G21) into pGL3-basic. The 1.3- and 1.0-kb of S1P1 promoter fragments were amplified by PCR with the following primers (5′ to 3′); GCCGTA CCGTCAATGAGTGTCCAGCCGGTGATAGCAAGCAGCACGCAAGCAAGGCACG ACAAAGCCA, and GGCTCGAGCAAGCGAGAAAAGTTGTCCGTGTGAGTCCAC. The amplified fragments were inserted into pGL3-basic to generate p3.3-Luc and p1.0-Luc constructs. HeLa cells were cotransfected with reporter plasmids and either empty pcDNA vector or pcDNA-HA-KLF2 (a gift from J. Leiden, Abbott Laboratories, Abbott Park, IL) using LipofectAMINE 2000 (Invitrogen Life Technologies). Luciferase activity was assayed with a luciferase assay system kit (Promega).

Chemotaxis assay

Retrovirus-transduced 2C T cells were extensively washed with serum-free medium and resuspended in RPMI 1640 medium containing 5% charcoal-extracted FCS (HyClone). A total of 8 × 10^5 cells was added in a Transwell insert (Corning Glass-Costar) and allowed to migrate across the filter with a pore size of 5 μm to various concentrations of S1P (Cayman Chemical) or stromal cell-derived factor-1 (SDF-1) (3) (PeproTech). After 3 h, cells migrated to the bottom compartment were counted and analyzed by FACS.
**Results**

**IL-4 inhibits KLF2 re-expression in activated CD8\(^+\) T cells**

KLF2 is expressed in naive T cells, down-regulated in activated T cells, and re-expressed in memory T cells (13, 14). The re-expression of KLF2 coincides temporally with the phenotypical transition that is attributed to the action of several common \(\gamma\)-chain (\(\gamma_{c}\)) cytokines. To determine the effect of the \(\gamma_{c}\) cytokines on KLF2 expression, we measured KLF2 transcript levels in naive CD8 T cells expressing the 2C TCR, activated 2C T cells, and activated 2C T cells that were treated with IL-2, IL-4, IL-7, or IL-15. As expected, abundant KLF2 was detected in naive T cells by Northern blotting, but very little was detected in T cells that have been activated by anti-CD3 Ab (or agonist SIY peptide) for 48 h (Fig. 1A and data not shown). Consistent with a previous report (13), if activated T cells were treated with IL-2, IL-7, or IL-15 for 24 h, significant levels of KLF2 transcript were detected (Fig. 1A and data not shown), indicating that these \(\gamma_{c}\) cytokines promote KLF2 re-expression following T cell activation.

Surprisingly, very little KLF2 transcript was detected when activated T cells were cultured with IL-4 for 24 h (Fig. 1A). Although IL-4-treated cells expressed very little KLF2, they survived and proliferated for an extended period of time (data not shown), excluding cell death being an issue. To confirm this observation, we measured the levels of KLF2 transcript in STAT6\(^{-/-}\) T cells because STAT6 is required for mediating IL-4R signaling (23). As shown in Fig. 1B, KLF2 was present in STAT6\(^{-/-}\) naive T cells, but down-regulated when T cells were activated by anti-CD3. In the absence of STAT6, KLF2 re-expression was readily induced even in the presence of IL-4, suggesting that IL-4 can suppress the re-expression of KLF2 in activated T cells via a STAT6-dependent pathway.

**Exogenous KLF2 expression promotes T cells homing to lymphoid organs**

Because endogenous KLF2 expression remains low in T cells cultured with IL-4, we used this culture condition to study the effect of exogenous KLF2 expression on T cell function. A retroviral vector encoding a bicistronic HA-tagged KLF2 and Thy1.1 was used to infect activated 2C T cells, which express Thy1.2. Western blotting of cell lysates with an anti-HA Ab detected a band at the expected 40-kDa position in KLF2-transduced T cells, but not in control virus-infected cells (data not shown). Cytokines that affect endogenous KLF2 expression had no significant effect on the protein level of exogenously expressed KLF2. Although exogenous KLF2 expression resulted in inhibition of proliferation (data not shown), no difference in apoptosis was detected between nontransduced (Thy1.1\(^{-}\)) and KLF2-transduced (Thy1.1\(^{+/−}\)) T cells (data not shown).

To examine the effect of exogenous KLF2 expression on T cell homeostasis, we performed adoptive transfer study. The mixture of transduced (Thy1.1\(^{+/−}\)) and nontransduced (Thy1.1\(^{-}\)) cells was injected i.v. into normal Thy1.1\(^{+}\) (Thy1.2\(^{-}\)) recipient mice. Seven
days after transfer, the donor-derived Thy1.2 cells that were either transduced (Thy1.1) or nontransduced (Thy1.1) cells was analyzed by flow cytometry. CD62L transcript was measured by semiquantitative RT-PCR using total cells from A. RNA was serially diluted every 3-fold. Some of the transduced T cells were cultured in the presence of IL-4 for 4 days and then adoptively transferred into B6-Thy1.1 recipients. Splenocytes were recovered from recipients 7 days after transfer and assayed for CD62L expression gating on donor-derived (Thy1.2) T cells. CD62L is required for lymph node homing by KLF2-transduced T cells. Four days after retroviral transduction, 10 million cells were incubated with 100 μg of purified anti-CD62L (Mel-14) or isotype control Abs in 200 μl of PBS for 30 min at room temperature. The cells together with the Abs were then adoptively transferred into B6-Thy1.1 recipients. After 24 h, Thy1.1 donor lymphocytes in the indicated organs were analyzed by cell counting and flow cytometry. PLN, peripheral lymph nodes; MLN, mesenteric lymph nodes. The retroviral transduction efficiencies were 58.8% (vector) and 49.6% (KLF2) for the experiment shown in D.

To examine the contribution of proliferation in the observed T cell recovery, we labeled 2C cells with CFSE and analyzed the CFSE profiles of Thy1.1 and Thy1.1 donor cells 5 days following the transfer. As shown in Fig. 2D, nontransduced and

FIGURE 3. Induction of CD62L expression by KLF2 in T cells. Activated 2C T cells were transduced with either control or KLF2-expressing retroviruses and cultured for additional 48 h in the presence of either IL-4 or IL-7. A, Expression of CD62L by transduced (Thy1.1) and nontransduced (Thy1.1) cells was analyzed by flow cytometry. B, CD62L transcript was measured by semiquantitative RT-PCR using total cells from A. RNA was serially diluted every 3-fold. C, Some of the transduced T cells were cultured in the presence of IL-4 for 4 days and then adoptively transferred into B6-Thy1.1 recipients. Splenocytes were recovered from recipients 7 days after transfer and assayed for CD62L expression gating on donor-derived (Thy1.2) T cells. D, CD62L is required for lymph node homing by KLF2-transduced T cells. Four days after retroviral transduction, 10 million cells were incubated with 100 μg of purified anti-CD62L (Mel-14) or isotype control Abs in 200 μl of PBS for 30 min at room temperature. The cells together with the Abs were then adoptively transferred into B6-Thy1.1 recipients. After 24 h, Thy1.1 donor lymphocytes in the indicated organs were analyzed by cell counting and flow cytometry. PLN, peripheral lymph nodes; MLN, mesenteric lymph nodes. The retroviral transduction efficiencies were 58.8% (vector) and 49.6% (KLF2) for the experiment shown in D.

FIGURE 4. KLF2 activates the CD62L promoter. A, A schematic diagram of intergenic region between 3′ of E-selectin and the 5′ of L-selectin. B, Schematic diagrams of promoter reporter constructs. p3.7-Luc: pGL-luciferase vector with the 3.7-kb MluI-Stul fragment that contains all the intergenic sequences between E-selectin and L-selectin. pL.4-Luc: pGL-luciferase vector with the 1.4-kb SpeI-Stul fragment that contains the most 5′ proximal sequences. C, Comparison of luciferase activities among various reporter constructs. HeLa cells were cotransfected with the indicated reporter constructs plus either pcDNA vector or pcDNA-KLF2. IL-7R indicates pGL control construct in which luciferase is driven by the murine IL-7R promoter. The luciferase activity was analyzed 24 h after transfection. Representative data from one of three independent experiments are shown.
vector-transduced 2C cells shared the same pattern of proliferation, as follows: almost all cells proliferated in the liver; some in the lungs, blood, and spleen; but very few in the lymph nodes. In contrast, almost none of the KLF2-transduced cells proliferated regardless of their localization. These results further strengthen that the accumulation of KLF2-expressing 2C cells in the lymph nodes is due to preferential migration. As shown in vitro (17, 18), exogenous KLF2 expression also results in inhibition of T cell proliferation in vivo.

KLF2-transduced T cells express a uniformly high level of CD62L

To investigate the molecular mechanisms underlying the observed T cell homing into the lymph node, we assayed expression of several molecules, including selectins, integrins, and chemokine receptors, by KLF2-transduced T cells. Flow cytometry analysis revealed that the levels of LFA-1, α4, and β7 integrins were very similar between KLF2-transduced and nontransduced cells (data not shown). Although the level of CCR7 was significantly lower on transduced cells, there was no difference in chemotactic response to CCL21 between KLF2-transduced and nontransduced 2C cells (data not shown). In contrast, KLF2-transduced T cells expressed a uniformly high level of CD62L (Fig. 3A). Correlating with cell surface expression, semiquantitative RT-PCR analysis revealed that the level of CD62L transcript was significantly increased in KLF2-transduced 2C cells as compared with vector-transduced 2C cells (Fig. 3B). Although protein shedding is known to regulate cell surface level of CD62L (24–26), neither activation-induced acute proteolytic cleavage nor constitutive shedding was diminished by KLF2 expression as compared with naive T cells (data not shown), indicating that the elevated CD62L expression was predominantly due to transcriptional regulation. Consistently, KLF2-transduced 2C cells that were recovered from the spleen 7 days posttransfer largely maintained the initial high level of CD62L expression (Fig. 3C). Furthermore, CD62L expression is required for the preferential homing of KLF2-transduced T cells to the lymph nodes because Ab blocking of CD62L reduced the accumulation of KLF2-transduced cells in the lymph nodes by ~100-fold (Fig. 3D). Together, these results suggest that KLF2 induces CD62L expression, resulting in the preferential homing of transduced T cells to the lymph nodes.

KLF2 activates the CD62L promoter

We next tested whether KLF2 activates CD62L transcription directly. The gene encoding L-selectin, selk, is closely linked with the E-selectin gene (sele). Between these two genes, there are only 3.7-kb sequences (Fig. 4A). The 3.7-kb region, residing in the MluI-Stul fragment, was inserted upstream of the firefly luciferase gene in the pGL reporter construct (Fig. 4B). The promoter activity

FIGURE 5. KLF2 activates S1P1 transcription and enhances chemotaxis to S1P. A, Real-time RT-PCR analysis of KLF2 and S1P1 transcript levels in 2C T cells that were activated for 48 h and cultured in the presence of IL-4 or IL-7 for additional 24 or 48 h. B, Real-time RT-PCR analysis of total KLF2 and S1P1 transcript levels in activated 2C cells that were transduced with either control or KLF2-expressing retrovirus. Transduced 2C cells were purified to >96% (Thy1.1+) before the PCR assay. RNA from naive 2C T cells was used as a control. C, Chemotactic responses of retrovirus-transduced 2C cells to S1P and SDF-1. D, A schematic diagram of the S1P1 locus. E, Schematic diagrams of luciferase reporter constructs with 5.4-, 1.3-, or 1.0-kb promoter sequences. F, Indicate the conserved region containing three KLF2 binding sites. F, Comparison of luciferase activities among various reporter constructs. HeLa cells were cotransfected with the indicated reporter constructs plus either pDNA vector or pDNA-KLF2. The luciferase activity was analyzed 24 h after transfection. Representative data from one of three independent experiments are shown. The retroviral transduction efficiencies were 66.2% (vector) and 75% (KLF2) for the experiment shown in B, and 69% (vector) and 46.4% (KLF2) for the experiment shown in C.
of the 3.7-kb fragment was monitored by assaying luciferase activity in transient transfection of HeLa cells. By itself, the 3.7-kb fragment exhibited little promoter activity (Fig. 4C). However, cotransfection of a KLF2-expressing plasmid enhanced the promoter activity by ~80-fold. A 1.4-kb fragment derived from the most 5′ proximal region of S1P was sufficient to retain KLF2 responsiveness in the reporter assay. In addition, exogenous expression of KLF2 in immortalized T cell lines was able to activate endogenous CD62L expression (data not shown). These results suggest that KLF2 can directly activate CD62L transcription.

KLF2 activates S1P1 expression

More abundant KLF2-transduced cells were also found in the peripheral blood in the short-term adoptive transfer experiments (Fig. 2B). Their presence in the blood was not affected by CD62L blocking (Fig. 3D), suggesting that additional molecules that are involved in lymphocyte emigration into the circulation may also be regulated by KLF2. Recent studies indicate that S1P1−/− and KLF2−/− chimeras share a striking similarity in T cell lymphopenia in the periphery (15, 19, 27), raising the possibility that KLF2 may control T cell trafficking by regulating S1P1 expression. To test this hypothesis, we investigated whether KLF2 and S1P1 expression is correlated. In agreement with previous studies (13, 15, 19, 28), both KLF2 and S1P1 are transcribed in naive T cells, and the transcription is down-regulated upon T cell activation (Fig. 5A). Correlating with KLF2 expression, IL-7 induced S1P1 expression, whereas IL-4 suppressed S1P1 expression in activated T cells (Fig. 5A). Strikingly, ~35-fold more S1P1 transcripts were detected in purified T cells that were transduced with KLF2-expressing retrovirus (Fig. 5B). Although we were unable to directly assay the cell surface S1P1 level due to a lack of Abs specific for mouse S1P1, we examined the effect of KLF2 expression on chemotactic response to S1P1 ligand S1P. Nontransduced and vector-transduced cells migrated poorly in response to various concentrations of S1P. In contrast, the migration was dramatically enhanced by KLF2 expression (Fig. 5C). The observed difference is unlikely due to changes in cells’ intrinsic ability to migrate, because both control cells and KLF2-transfected cell migrated equally toward SDF-1. These results indicate that the high level of S1P1 transcript most likely results in a high level of cell surface S1P1 expression, and therefore enhanced functional response to S1P.

We next assessed whether KLF2 directly activates the S1P1 promoter by luciferase reporter assay. The S1P1 gene has only two exons. The entire coding region is located in exon 2. To include all potential transcriptional regulatory elements, a 5.4-kb DNA fragment, consisting of a 3.5-kb promoter region, exon 1, and the intron (Fig. 5, D and E), was subcloned into the pGL-luciferase reporter construct. As shown in Fig. 5F, the 5.4-kb fragment by itself displayed significant promoter activity in HeLa cells. However, coexpression of KLF2 further enhanced this fragment’s promoter activity by 20-fold. Sequence analysis revealed that the region between −1050 and −1300 is highly conserved between mice and humans. Three consensus KLF2 binding sites are present in this region. However, promoter reporter constructs with or without this region exhibited similar levels of promoter activity both in the presence and absence of cotransfected KLF2 (Fig. 5F). Because the 1-kb promoter region was as active as the 5.4-kb promoter region in the reporter gene assay, the KLF2-responding elements in the S1P1 promoter most likely reside in the proximal 1-kb region. Together, these results show that KLF2 can also activate S1P1 transcription.

Discussion

Based on the stage-specific expression and the phenotype observed in KLF2−/−/H11011 chimeric mice, KLF2 has been postulated to regulate T cell homeostasis by programming T cell quiescence and promoting T cell survival in the periphery (12, 15–17). In this study, we provide evidence that KLF2 plays a critical role in controlling T cell trafficking by directly regulating CD62L and S1P1 expression at the transcriptional level. Our findings provide an explanation not only for the pattern of T cell trafficking following the adoptive transfer reported in this study, but also for the T cell deficiency observed in KLF2−/−/H11011 chimeric mice.

KLF2 regulates CD62L transcription in T cells

There is a general correlation between KLF2 and CD62L expression throughout T cell development. In the thymus, both KLF2 and CD62L are absent in CD4+CD8+ thymocytes, but expressed in SP thymocytes (15, 29, 30). In the periphery, both KLF2 and CD62L are expressed in naive T cells, but down-regulated upon T cell activation (15, 24, 31). As activated T cells acquire memory phenotype, both KLF2 and CD62L are re-expressed (13, 14, 32). The re-expression in activated T cells is significantly affected by the cytokine milieu. We show that IL-4 suppresses the re-expression of both KLF2 and CD62L in activated T cells. In the absence of STAT6, which mediates IL-4R signaling, KLF2 expression is no longer inhibited by IL-4 (Fig. 1).

Multiple lines of evidence also support a direct regulation of CD62L by KLF2. In KLF2−/−/H11011 chimeric mice, CD62L fails to be up-regulated in SP thymocytes, and the few T cells present in the periphery are CD62Llow, in contrast to high levels of CD62L expression by naive T cells in normal mice (15). Conversely, we show that exogenous expression of KLF2 in primary T cells under the condition that endogenous KLF2 expression is suppressed leads to up-regulation of the endogenous CD62L (Fig. 3). Most directly, we show that CD62L promoter can be activated by KLF2 in a reporter gene assay and the KLF2-responsive elements reside in the proximal 1.4-kb promoter region (Fig. 4). Three GGGTG sites, which are antiparallel to the conventional KLF2 core binding sequence (CACCC) (33), are present in this region, although whether KLF2 exerts its activity by directly binding to any of these sites remains to be determined. Together, the current evidence strongly suggests that KLF2 directly activates CD62L transcription in T cells.

KLF2 regulates S1P1 transcription in T cells

Multiple lines of evidence support that KLF2 also controls S1P1 expression. First, there is a general correlation between KLF2 and S1P1 expression during T cell development. As with KLF2, S1P1 is absent in CD4+CD8+ thymocytes, but expressed in SP thymocytes (19). The expression of S1P1 also parallels that of CD62L, i.e., it is low in CD62Llow SP thymocytes and high in CD62Lhigh SP thymocytes. In the periphery, S1P1 is expressed in naive T cells, down-regulated in activated T cells, and re-expressed as early as 3 days postactivation in vivo (19, 28). By directly comparing the expression of KLF2 and S1P1 in a homogeneous T cell population under defined cytokine conditions, we show that the expression pattern of S1P1 completely mirrors that of KLF2 during both initial T cell activation and subsequent differentiation under different cytokine milieu (Fig. 5A). In fact, evidence suggests that the KLF2-S1P1 correlation extends beyond the immune system. During embryogenesis, expression of both KLF2 and S1P1 is initiated at a similar stage, as indicated by observations that both KLF2−/− and S1P1−/− mice die from intraembryonic hemorrhage between embryonic day 12.5 and 14.5 (34, 35). Second, overexpression studies suggest that there is a direct causal relationship in
gene expression between KLF2 and S1P1. Haaland et al. (36) reported that S1P1 was one of the most highly up-regulated genes upon KLF2 induction in Jurkat T cells. Our retroviral transduction studies show that this regulation also occurs in primary T cells. Finally, by using a S1P1 promoter reporter assay, we provide the most direct evidence that KLF2 regulates S1P1 expression by activating its promoter (Fig. 5). These findings suggest that KLF2 regulates S1P1 at the transcription level.

**KLF2 controls T cell trafficking through CD62L and S1P1**

By controlling expression of both CD62L and S1P1, KLF2 is expected to serve as a critical regulator at multiple points of T cell trafficking. Studies have shown that CD62L facilitates lymphocyte homing to lymph nodes through binding to its ligands on high endothelial venules (37). In CD62L−/− mice, although T cell development is normal, the number of lymphocytes in lymph nodes is reduced (38). When normal T cells are treated with the FTY720 reagent or S1P1+/− SP thymocytes are adoptively transferred into recipient mice, T cells are retained in the lymph nodes (19, 20). We found that adoptive transfer of T cells that differ only in levels of KLF2 expression into the same host results in preferential homing of KLF2-transduced T cells into the lymph nodes at both 2 and 24 h posttransfer (Fig. 2, B and C). At these early time points, transferred T cells did not proliferate significantly, indicating proliferation is not a contributing factor to the observed preferential homing. Furthermore, CD62L is induced in KLF2-transduced T cells and Ab blocking of CD62L abolishes preferential homing to the lymph nodes (Fig. 4).

Our findings that KLF2 activates CD62L and S1P1 transcription and thereby regulates T cell trafficking lend an alternative explanation to the T cell phenotype in KLF2−/− chimeric mice (15). The severe peripheral T cell lymphopenia in these mice is consistent with a defective thymocyte egress due to the lack of S1P1 up-regulation at the final stage of T cell maturation. In a recent study, Carlson et al. (22) demonstrated that KLF2−/− SP thymocytes fail to up-regulate S1P1 and fail to egress out of thymus in KLF2−/− chimeric mice. Thus, the function of KLF2 in regulating T cell trafficking is 2-fold. In the thymus, it promotes S1P1 expression, and therefore egress of mature SP thymocytes into the periphery. In the periphery, it promotes both S1P1 and CD62L expression, and therefore T cell migration into and out of lymph nodes.

**The role of KLF2 in proliferation and T cell memory**

Overexpression of KLF2 in Jurkat cells has been shown to profoundly inhibit proliferation (17, 18). However, we found that KLF2 has only a modest antiproliferative effect on Ag-activated primary T cells in vitro (data not shown). By CFSE dilution assay, KLF2-transduced cells underwent similar number of cell divisions compared with nontransduced cells (5.2 vs 5.5 cell divisions in the first 3 days after retroviral transduction). The inhibition of proliferation can only be detected by a gradual decrease in the ratio of Thy1.1+ to Thy1.1− cells over a 5-day culture (data not shown). However, in vivo, nontransduced or vector-transduced T cells proliferated significantly more than KLF2-transduced T cells in the spleen and nonlymphoid organs (Fig. 2D). Therefore, the antiproliferative effect of KLF2 appears to depend on the cell type and their environment.

KLF2 has been implicated in memory T cell development because it is re-expressed in memory T cells (13, 14). Interestingly, CD62L is commonly used as a marker to differentiate central memory T (T_{CM}) and effector memory T (T_{EM}) cells (11, 39). This raises an intriguing question as to whether KLF2 re-expression is involved in the T_{CM} vs T_{EM} differentiation. We found that KLF2-transduced and nontransduced T cells had the same ability to persist in the recipients up to 8 mo (data not shown). Furthermore, purified T_{CM} and T_{EM} cells from influenza virus-infected mice expressed comparable levels of KLF2 (data not shown). Although these results do not support a simple requirement of KLF2 in memory T cell development, they do not exclude the possibility that KLF2 functions at an early stage to direct certain effector T cell populations into lymphoid organs for T_{CM} differentiation. In this regard, it is interesting to note that differential KLF2 expression is reported in T cells in bronchial alveolar lavage and spleen during influenza virus infection (40).

In summary, findings reported in this work provide direct evidence that KLF2 activates CD62L and S1P1 transcription and thereby regulates T cell trafficking. Disruption of this regulatory network can result in severe lymphopenia, demonstrating a critical role of T cell trafficking in T cell homeostasis.

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**References**


