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The Krüppel-like factor family of transcription factors plays an important role in differentiation, function, and homeostasis of many cell types. While their role in lymphocytes is still being determined, it is clear that these factors influence processes as varied as lymphocyte quiescence, trafficking, differentiation, and function. This review will present an overview of how these factors operate and coordinate with each other in lymphocyte regulation. *The Journal of Immunology*, 2012, 188: 521–526.

The founding member of the Krüppel-like factor (KLF) family was found to regulate development in *Drosophila melanogaster*, mutants in this gene leading to embryonic deformity (1, 2), hence the name Krüppel (which means cripple in German). All KLFs, which are related to the sp1 family of transcription factors, share a highly homologous set of three DNA-binding zinc fingers at the C terminus that imparts specificity for CACCC boxes and related GC-rich DNA regions (3, 4). Individual factors are distinguished by regions that dictate regulation and binding partner specificity. There are at least 17 KLFs in mammals, and they have been implicated in numerous biological processes, especially in the context of cell differentiation and quiescence. For example, KLF1 (also called EKLF) is critical for the switch to adult hemoglobin expression in developing erythrocytes, whereas KLF4 (GKLF) is well studied as a factor involved in reprogramming mature cells to become induced pluripotent stem cells (3, 4).

Interest in the KLF family among immunologists was sparked by a report from Leiden’s group (5), which showed that deficiency for KLF2 (LKLF) caused upregulation of T cell activation markers and a dramatic loss of peripheral T cells. This led to the hypothesis that KLF2 enforced naive T cell quiescence (5–7). However, as we discuss next, further study on the function of KLF2 have lead to reinterpretation of its function. This serves as a good example of how the varied activities of KLFs in lymphocyte biology can confound simple characterization of their role.

While the function of individual KLFs is still being deciphered, some general principles are emerging. One principle is that KLFs participate in multiple aspects of lymphocyte differentiation, trafficking, and function, especially in the context of regulating late stages of maturation. Another principle is the appreciation that distinct KLFs may balance each other in control of certain differentiation steps; this is illustrated by the reciprocal effects of KLF2 and KLF13 deficiency in NKT cell differentiation, and by examples of both cooperation and antagonism in the control of B cell subset differentiation by KLF2 and KLF3. The well-characterized functions of KLFs are illustrated in Fig. 1, but it is likely that we have only scratched the surface of regulation by this versatile family of transcription factors.

**Lymphocyte quiescence**

T cells. KLF2 is induced late during maturation of thymocytes and is maintained in peripheral naive T cells (5, 8–11). Upon T cell activation, KLF2 expression is lost, a process that is thought to initiate with KLF2 protein degradation (probably involving ubiquitination) and subsequent loss of KLF2 mRNA (5, 7, 12–15). Re-expression of KLF2 occurs late in the effector phase of the CD8+ T cell response, as memory cells begin to differentiate (a process that can be directed by appropriate cytokines) (5, 7, 12–15). These expression patterns, together with data showing that KLF2-deficient thymocytes display activation markers suggested that KLF2 was important for maintaining naive T cell quiescence. In this model, the loss of KLF2 induced inappropriate activation of mature thymocytes and their subsequent cell death (5, 6). This idea was reinforced by studies indicating that KLF2 inhibits cell cycle progression, as dramatically shown by the capacity of KLF2 overexpression to halt tumor cell line proliferation and by evidence that KLF2 inhibits expression of c-myc while promoting transcription of p21WAF1/CIP1 (7, 16–19). KLF4, a close relative of KLF2, is also downregulated upon T cell activation (20), and it is interesting to note that KLF4-deficient memory CD8+ T cells show a gradual increase in representation over time, in keeping with a role for KLF4 in restraining cell cycle progression (20).

Subsequent data have led to the re-evaluation of the role of KLFs in quiescence. For example, the deficit of naive KLF2−/− T cells in peripheral lymphoid sites is explained by the role of KLF2 in lymphocyte trafficking rather than spontaneous cell

**Abbreviations used in this article:** FO, follicular; iNKT, invariant NKT; KLF, Krüppel-like factor; MZ, marginal zone; S1PR1, sphingosine 1-phosphate receptor 1; Treg, regulatory T cell.

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Studies on activated CD8+ T cells provides a useful opportunity to test how normal KLF2 reinduction influences expression of cell cycle regulatory factors. Interestingly, expression of c-myc and p21mRNA were found to be independent of KLF2 expression in postactivated CD8+ T cells, and KLF2 deficiency did not lead to dysregulated T cell expansion in vivo (13). Such findings do not rule out redundancy between distinct KLFs for control of cell cycle progression in T cells, or that KLF2 (and/or related KLFs) enforce T cell quiescence under certain circumstances. However, in contrast with more clear-cut examples of factors regulating naïve T cell quiescence, such as recent studies on Foxp1 (24, 25), the role of KLFs is currently unclear.

B cells. KLFs have also been proposed to control B cell quiescence. As for T cells, KLF2 and KLF4 are expressed in naive B cells and are downregulated with B cell activation (26–28). KLF4 overexpression was shown to induce cell cycle arrest of transformed mouse pro/pre-B cell lines (29), whereas forced expression of KLF4 or KLF9 caused reduced proliferation of naive and memory human B cells after stimulation through the BCR or CD40 (28). Because expression of KLF4 and KLF9 were found to be lower in memory versus naïve B cells, these data offer a potential explanation for the superior proliferation of memory B cells (28). In contrast, Klaewsongkram et al. (26) reported that KLF4 deficiency also causes a block in B cell proliferation. KLF4−/− B cells (activated by BCR/CD40) exhibited decreased progression through the cell cycle; this correlated with reduced expression of cyclin D2 and the finding that KLF4 directly binds the cyclin D2 promoter (26). The apparent contradictions between these reports reinforces the emerging concept that the function of KLF4 (and perhaps other KLFs) depends on the context of other cell-cycle-regulatory factors (30). This concept adds to the difficulty in determining the significance of KLFs for enforcing lymphocyte quiescence. Although several KLFs have been shown to have the potential to strongly limit lymphocyte cycle entry, their physiologic role in controlling quiescence is not well defined.

**Lymphocyte trafficking**

**T cells.** To exit the thymus and peripheral lymphoid tissues, mature CD4+ and CD8+ T cells need to express sphingosine 1-phosphate receptor 1 (S1PR1), which detects the high levels of S1P maintained in blood and lymph (31, 32). We and others found that KLF2 is required for thymocyte expression of S1PR1 (8, 15), suggesting that the lack of peripheral T cells in KLF2-deficient mice can arise as a consequence of thymic retention (8). Indeed, the accumulation of mature KLF2-deficient T cells in the thymus is prevented by overexpression of S1PR1 (33), suggesting that this factor is the key target of KLF2 for thymic emigration. Incidentally, the upregulation of CD69 on KLF2−/− thymocytes can also be attributed to impaired S1PR1 expression, because basal cell surface expression of CD69 is restrained by a protein–protein interaction with S1PR1 (34, 35). In addition, KLF2 directly promotes expression of λ-selectin (CD62L), which is relevant for access of naive T cells to lymph nodes (8, 15), indicating that this KLF coordinates distinct aspects of lymphocyte trafficking. Whereas those studies focused on TCRβ T cells, similar changes in trafficking molecule expression were observed for TCRγδ T cells (36).

KLF2-deficient thymocytes also show depressed levels of β7 integrin and elevated expression of the CXCR3 chemokine receptor (8, 21); however, subsequent studies suggest that these changes are induced by IL-4 (produced by KLF2-deficient NKT cells), rather than a consequence of direct gene control by KLF2 (22, 23).

KLF2 is constitutively expressed in naive T cells, but is rapidly extinguished after T cell stimulation, a situation that may help to retain activated T cells in lymphoid sites through...
transcriptional downregulation of S1PR1 (12–15). Likewise, KLF2 re-expression in postactivated CD8+ T cells is necessary for S1PR1 and CD62L re-expression (13, 15), and this may be key to allow late effector CD8+ T cells to begin recirculation again.

Such studies on the trafficking role of KLF2 have helped define signaling pathways that control its expression. A key mechanism in regulation of KLF2 expression involves PI3K signaling. Strong induction of this pathway leads to activation of AKT, which in turn induces sequestration of the transcription factor Foxo1 outside the nucleus (37). Foxo1 promotes expression of KLF2 in mature T cells (38–40), and additional Foxo factors may also contribute to this regulation (37). As has been discussed in a recent review, this pathway provides a mechanism by which the strength of PI3K signaling can directly regulate T cell trafficking mediated, at least in part, by control of KLF2 (41). Cytokines have a central role in dictating KLF2 regulation. For activated mouse CD8+ T cells, high-dose IL-2 promotes efficient PI3K activation, leading to low KLF2 expression, whereas exposure to IL-7, IL-15, or weaker IL-2R signals permits KLF2 re-expression (13–15, 42). In addition, cytokines involved in active effector differentiation, such as IL-12 and IL-4, act to acutely repress KLF2 re-expression (14, 15, 43, 44). The signaling pathways involved in these processes are not well defined, although JAK/STAT signaling appears to be critical (15, 43, 44). An emerging picture is that changes in the cytokine milieu that an activated T cell encounters has a dramatic effect on whether the T cell is equipped to traffic (via KLF2 re-expression and subsequent induction of S1PR1) or whether the cell is retained in its local tissue. Indeed, recent studies have indicated that Th2 cells use a specialized mechanism to initiate trafficking from lymph nodes. Th2 cells produce the protein ECM1, which attenuates IL-2 signaling allowing for expression of KLF2 and S1PR1 and subsequent egress of Th2 cells from peripheral lymphoid tissues (45).

B cells. Because S1PR1 is also required for recirculation of B cells and, like T cells, B cells downregulate KLF2 after activation, it would be reasonable to predict that KLF2 induces S1PR1 expression in the B cell pool. However, three recent studies describing B cell-specific KLF2-deficient mice reached the surprising conclusion that S1PR1 expression and function on follicular B cells was minimally altered (46, 47), mirroring the phenotypic changes observed in KFL2-deficient mice (23). In addition, KLF2-deficient mice have an exaggerated representation of an unusual subset of TCRγδ T cells that use Vγ6,3/2 and bear the CD4 coreceptor (36). Like iNKT cells, these nonconventional TCRγδ T cells express the transcription factor PLZF and have been termed γδ-iNKT. The altered representation of these populations is not simply a consequence of impaired S1PR1 expression by KLF2−/− T cells, because the frequency of these subsets is not enhanced in S1PR1−/− animals (23, 36).

A surprising consequence of the enhanced production of thymic iNKT cells is the appearance of memory-phenotype T cells (especially CD8+ T cells) in the KLF2-deficient thymus (22, 23). Through a series of studies, this effect was found to result from PLZF+ NKT cell production of IL-4, which acted directly on mature thymocytes, leading to up-regulation of the transcription factor Eomes and acquisition of memory markers (22, 23). Through a series of studies, this effect was found to result from PLZF+ NKT cell production of IL-4, which acted directly on mature thymocytes, leading to up-regulation of the transcription factor Eomes and acquisition of memory markers (22, 23). As a result, this striking change in thymocyte differentiation is not a direct effect of KLF2 deficiency, but reflects a nonautonomous effect. Such findings are useful to underscore the difficulty in dissecting direct and indirect effects of KLF manipulation.

Interestingly, expansion of PLZF+ NKT cells (and attendant IL-4–mediated bystander effect) is evident in some normal mouse strains (e.g., BALB/c) but not others (e.g., C57BL/6) (23). Recent studies show that a different KLF, KLF13, is also involved in this phenomena (53). KLF13 deficiency has reciprocal effects on PLZF+ iNKT cells (23, 52).

Lymphocyte development

Studies on KLF factors have suggested a minimal role in early lymphocyte development—exceptions being a proposed role for KLF5 in germline transcription from the Dβ1 promoter in thymocytes (51) and data showing that KLF4 deficiency leads to slightly decreased pre-B cell numbers (26). However, recent work has revealed significant roles for KLFs in differentiation of mature T and B cell subsets.

NKT and TCRγδ T cells. It has become clear that distinct KLFs promote and oppose differentiation of NKT cells during thymic differentiation. Loss of KLF2 leads to an increase in thymic CD1d-restricted invariant NKT (iNKT) cells (23). In addition, KLF2-deficient mice have an exaggerated representation of an unusual subset of TCRγδ T cells that use Vγ6,3/2 and bear the CD4 coreceptor (36). Like iNKT cells, these nonconventional TCRγδ T cells express the transcription factor PLZF and have been termed γδ-iNKT. The altered representation of these subsets is not simply a consequence of impaired S1PR1 expression by KLF2−/− T cells, because the frequency of these subsets is not enhanced in S1PR1−/− animals (23, 36). However, other genetic defects (including loss of Ilk and CBP and forced expression of class II MHC molecules on mouse thymocytes) lead to a similar enhancement of PLZF+ iNKT and/or γδ-NKT cells (23, 52).

A surprising consequence of the enhanced production of thymic iNKT cells is the appearance of memory-phenotype T cells (especially CD8+ T cells) in the KLF2-deficient thymus (22, 23). Through a series of studies, this effect was found to result from PLZF+ NKT cell production of IL-4, which acted directly on mature thymocytes, leading to up-regulation of the transcription factor Eomes and acquisition of memory markers (22, 23, 52). As a result, this striking change in thymocyte differentiation is not a direct effect of KLF2 deficiency, but reflects a nonautonomous effect. Such findings are useful to underscore the difficulty in dissecting direct and indirect effects of KLF manipulation.
factors intersect. Loss of KLF13 leads to reduced PLZF expression levels in thymic iNKT cells, and this could influence the efficiency of their development (53). However, loss of KLF2 does not appear to change PLZF expression levels (23), and it is possible that the capacity of KLF2 to restrain proliferation acts to contain the size of the PLZF+ thymocyte pool. Regardless, these findings serve as another example of how different KLF family members intervene in lymphocyte differentiation programs.

**B cells subsets.** Recent studies suggest that KLF2 and KLF3 have opposing roles in the development of mature B cell subsets. The three major mature B cell subsets—FO, MZ, and B1 B cells—show distinct phenotypic, functional, and tissue localization characteristics. Whereas the signals that determine B cell subset differentiation are incompletely defined, some transcription factors have been implicated in dictating FO, MZ, or B1 generation. For example, Notch2 and Aiolos are required for differentiation of MZ B cells (56). KLF2 shows an intriguing pattern of gene expression in B cell lineage cells, being uniformly expressed at the large pre-B cell stage (46, 57), but then showing differential expression in mature B cells, following the order MZ < FO < B1 (46, 47). This heterogeneity in expression correlates with a profound impact on B cell subset generation in KLF2-deficient animals (46–48). Using B cell specific conditional knockout approaches, loss of KLF2 was shown to have a relatively modest effect on the FO pool, but it lead to an increase in the size of the MZ subset while the peritoneal B1 population was severely depleted (46–48). Changes in the splenic transitional and marginal zone precursor populations of B cells suggest these effects reflect a role of KLF2 in B cell subset differentiation, although an impact on homeostasis has not been ruled out. Furthermore, it is currently unclear whether KLF2 deficiency causes a loss of B1 cells or alterations in their trafficking (46–48).

KLF3 may play a reciprocal role to KLF2 in controlling the differentiation and homeostasis of certain B cell subsets. KLF3 overexpression leads to substantial enhancement of MZ B cell numbers (49), whereas the opposite phenotype has been observed in KLF3 knockout mice (50). The capacity of KLF3 overexpression to promote MZ B cell generation was sufficient strong to overcome CD19 deficiency and blockade of the BAFF-family factor TACI (both of which are required for differentiation of MZ B cells, suggesting that the regulation of KLF2 and KLF3 is important for dictating FO B cell identity (46, 47, 49). It remains to be seen whether these differentiation effects relate to alterations in BCR signaling (as has been proposed previously) (58, 59) or altered trafficking. Alternatively, development or maintenance of B1 B cells evidently involves distinct regulation, because deficiency for either KLF2 or KLF3 caused a reduction in the size of the peritoneal B1 pool, whereas KLF3 overexpression leads to enhanced B1 B cell frequencies (46–50). Regulation between KLFs has been proposed to operate in complex networks (4, 50, 60), and the activity of KLF2 and KLF3 in control of B cell subset differentiation appears a good example of how factor may have cooperative or opposite roles even within the same lymphocyte lineage (Fig. 1).

**Activated lymphocyte differentiation.** In addition to alterations in lymphocyte development, it has become clear that KLFs may also influence expansion and differentiation of activated T and B cells.

**Regulatory T cells.** Foxp3-expressing regulatory CD4+ T cells (Tregs) are vital for protecting against autoimmune responses, and TGF-β is crucial for differentiation of a least some Treg populations (61). KLF10 is rapidly induced by TGF-β (hence the alternative name of KLF10 as TIEG1) and is important for maturation and function of Tregs. KLF10 deficiency lead to reduced differentiation of Foxp3+ CD4+ T cells and impaired capacity of these cells to suppress airway inflammation in vivo (62). Complementary studies showed that KLF10 overexpression induces CD4+, CD25+ cells to express TGF-β1 and FOXP3 (63). KLF10 activity hinges on differential ubiquitination, with monoubiquitination (involving the E3 ligase Itch) being necessary for KLF10 to induce Foxp3 transcription, whereas polyubiquitination of KLF10 (as occurs downstream of IL-6 signals) leads to KLF10 exclusion from the nucleus (62, 64). Th17. Another family member, KLF4, has recently been implicated in differentiation of Th17 CD4+ T cells, which are significant for their role in control of certain extracellular bacteria as well as autoimmune diseases (65, 66). KLF4 was found to be necessary for Th17 cell differentiation in vitro, and in an adoptive transfer EAE model, and chromatin immunoprecipitation assays revealed that KLF4 occupies the promoter of the IL-17 gene (67, 68). It is not clear how KLF4 integrates with other transcription factors that dictate Th17 differentiation, including RORγt, RORαt, and STAT3 (65, 66); nor is it known whether KLF4 plays a similar role in control of CD8+ Tc17 cells.

Recent studies have reported that HIF-1α is important for the differentiation of Th17 and Treg cells (69). Interestingly, studies in myeloid cells indicate that KLF2 can act as a negative regulator for HIF-1α expression (70), and it will be important to determine whether KLF2 has any similar activity in lymphocytes.

**B cell activation.** Studies of KLF2 deficient B cells indicated that they were compromised in the proliferation, survival, and maintenance of responding follicular B cells (46–48). After in vitro anti-BCR stimulation, KLF2-deficient follicular B cells exhibited impaired proliferation and a substantial survival defect, a situation that could be corrected by simultaneous stimulation of BCR and CD40 (46). Because CD40 engagement is potent at activation of NF-kB signals (71), this pathway may be promising for additional investigations into the role of KLF2 in B cell priming. Following in vivo priming for T dependent responses, Wilkelmann et al. (47) reported a reduction in Ag specific Ig, a result that may be partially explained by fewer KLF2−/− plasma cells/Ab secreting cells in the bone marrow. It is unclear whether this relates to compromised survival of activated B cells, or relates to the proposed relevance of KLF2 in migration of plasma cells to the bone marrow (72).
Conclusions
It is becoming clear that KLFs play multiple and varied roles in lymphocyte development and function, and that individual KLF family members can reinforce or antagonize each others’ functions. However, substantial questions remain, based partly on the complexity of this family. Whereas mRNA expression of individual KLFs within lymphocyte subsets can be readily determined, posttranscriptional regulation of KLFs has been observed in several systems, making it harder to predict functional KLF protein expression. The fact that many KLFs share a similar DNA binding site motif, together with the shortage observed in several systems, making it harder to predict function of individual KLFs within lymphocyte subsets can be readily evidenced by the burst of information over the last few years, we are approaching a renaissance in understanding the function and significance of KLFs in lymphocyte biology.

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