Chapter 5

Multiple Players in the Mechanical Control of T Cell Quiescence

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http://dx.doi.org/10.5772/intechopen.68869

Abstract

Naive T cells are kept in a quiescence state, characterized by small cell size, with low proliferative and metabolic activities, until antigen engagement. T lymphocyte quiescence is a tightly controlled mechanism regulated by multiple quiescence-associated factors. Loss or impaired functions of these factors regularly result in spontaneous activation of T cells that is ensured by fatal autoimmune diseases. Elucidating the mechanism to facilitate the switch on or off of T cells could be beneficial to ameliorate pathology triggered by T cell hyperactivation or dysfunction. In this chapter, we discuss multiple quiescence-associated factors along with the mechanisms utilized to promote lymphocyte quiescence and longevity.

Keywords: T cell quiescence, Foxo, KLF2

1. Introduction

T lymphocytes are important players in adaptive immune responses to invading pathogens. In an individual, T lymphocyte repertoires are principally generated through a somatic recombinant process named as VDJ rearrangement in the thymus. Upon maturation, these T cells exit thymus to reside in the secondary lymph organs and patrol in the circulation. It is crucial to maintain T cell repertoires as standby for future assault by diverse types of antigens that might come from the massive array of microbes. Eventually, most of the lymphocyte reservoirs do not encounter their cognate (or specific) antigens throughout their lifetime. These virgin or naïve T cells must be kept in a state known as quiescence to prevent immune activation that is often ensued by activation-induced cell exhaustion or death. At quiescence state, cells are

sustained through homeostatic cell renewal without activation or expansion to maintain the size of the peripheral lymphocyte pool. Loss control of T cell quiescence has been associated with autoimmune diseases; hence, this stage is imperative to render an immune tolerance. In this chapter, we discuss the intricate transcriptional mechanism of T lymphocytes at quiescence stage to attain a long-term standby status until they encounter the cognate antigens.

Lymphocyte quiescence refers to a state of inaction of cells characterized by small cell size with limited cytoplasmic region, low rates of cell metabolism, proliferation, transcription, and translation activities. Quiescence suppresses cell activation and prevents the unnecessary use of energy resources that will be consumed by the huge T lymphocyte repertoires in an individual. In addition, cells at quiescence state can reduce genetic damage due to repetitive replication therefore preventing development of malignancy [1] because constant replication of lymphocytes may increase the risk of leukemia and lymphoma [2]. Upon identification of a specific antigen, activation of a T cell beyond the cell signaling threshold triggers cells to exit quiescence state in a non-reversible manner, thus, the T cell undergoes robust clonal expansion followed by cell cytotoxicity and cytokine-secreting activities.

A quiescence state of lymphocytes was previously regarded as a default stage of cells in the absence of antigen recognition activation [3]. However, recent evidences accumulated suggest that quiescence is a steadily regulated stage by functionally diverse mechanisms [4], which include intrinsic control by gene expression programs as well as extrinsic suppression by regulatory T cells (Treg). Evidence derived from the microarray study demonstrates strikingly distinct expression patterns of diverse molecules in the quiescence versus stimulated T lymphocytes [5, 6]. Following T cell activation, the major change in gene expression profile is not limited to only increased expression of genes that promote growth and differentiation but also suppression of a group of genes that is linked to the quiescence program [6, 7]. Transcription factors and components in cell cycle control play a central role in the quiescence regulation. Recently, ubiquitination degradation pathway has also been added into the growing list of the quiescence-associated factors [8].

A common characteristic shared by most of these quiescence-associated molecules is their high abundance in the naïve T cell but the expression has rapidly vanished upon cell activation. Quiescence molecules are different from other negative regulators of T cells such as cytotoxic T lymphocyte antigen 4 (CTLA4) and program cell death protein 1 (PD-1). Although both impose inhibitory signals on T cell proliferation and effector activity, it is important to note that the expression of quiescence molecules is usually high in naïve T cells but reduce upon cell activation. In contrast, CTLA4 and PD1-1 are expressed only after T cell activation.

Deficiency or dysfunction of the quiescence molecules often results in loss of the quiescence control marked by the cell's semi-activation to hyperactivation with robust proliferation, accompanied by exert activities such as cytokines secretion and predisposition to apoptotic cell death. The direct physical consequence to loss of T cells quiescence is impaired immune tolerance and development of autoimmune diseases in an individual. In this chapter, we will discuss several quiescence factors along with the mechanisms utilized to promote lymphocyte quiescence and longevity.

2. KLF2

The Sp/Kruppel-like factor (KLF) family of zinc-finger transcription factors contains at least 20 identified members, which include numerous Kruppel-like factors that have different roles across the mammalian system [9]. Kruppel-like family 2 (KLF2, also known as lung Kruppel-like factor or LKLF) has been implicated in programming T cell quiescence [10]. KLF2 is highly expressed in mature $CD4^+$ and $CD8^+$ T cells. Similar to many other key players in quiescence control, its expression is rapidly switched off after T cell activation [11]. KLF2 can also be detected in thymocytes at single-positive (SP) stage but not during earlier double-negative (DN) or double-positive (DP) stages [11]. KLF2 controls mature T cell egress from thymus [12] and recirculation through secondary lymphoid tissues [13] by regulating transcription of sphingosine-1-phosphate receptor 1 (S1Pr1). KLF2-deficient thymocytes show impaired thymocyte emigration, whereas KLF2-transduced T cells are prone to homing in lymphoid organs following adoptive transfer [14].

The conventional KLF2-deficient mice died between embryonic days, from 12.5 to 14.5, due to severe intra-embryonic and intra-amniotic hemorrhage resulting from defects in the smooth muscle cells migration during blood vessel maturation [15, 16]. To study KLF2-deficient T cells, a Rag-$2^{-/-}$KLF$2^{-/-}$ chimeric mouse system was applied, in which KLF$2^{-/-}$ embryonic stem cells were injected into RAG$2^{-/-}$ blastocyst to populate the T cell pool [11]. This model provides strong evidence supporting the role of KLF2 in the quiescence control. First, a massive loss (up to 90%) of the peripheral T cell is observed. Intriguingly, these KLF2-deficient T cells displayed stigmata of activated phenotype, that is, an increased cell size and surface expression of activation markers ($CD69^{hi}$ $CD44^{hi}$ $CD62L^{lo}$); however, these cells are non-proliferative. A large number of KLF2-deficient T cells are apoptotic, attributable to high surface expression of Fas ligand (FasL) [11].

In a different experiment using an overexpression model, in vitro forced expression of KLF2 in Jurkat T cells using doxycycline inducible system programs the cells into a quiescent phenotype [17]. KLF2 overexpression dramatically inhibits proliferation of Jurkat T cells and prevents synthesis of surface molecules such as CD30 and CD71, by which this effect can be reversible when the KLF2 expression is removed. KLF2-mediated regulation of quiescent T cells is partially achieved through its suppression of c-Myc. Conversely, transient expression of MadMyc, a dominant negative form of c-Myc, recapitulates the phenotype produced by KLF2 overexpression [17]. On the other hand, expression of neurotransmitter dopamine D4 receptor on resting T cells promotes T cell quiescence by upregulating KLF2 expression and an administration of U101958; a D4 antagonist could diminish the effect [18]. KLF2-deficient B cells showed increased apoptosis and impaired proliferation after B-cell receptor cross-linking [19]. B cell distribution and trafficking are disturbed due to low surface expression of CD62L and β7-integrin expression. Percentages of B cell subsets is also disturbed as B1 cells are almost disminished accompanied by increased in the number of MZ and transitional B cells. [19, 20]. These suggest a potential role of KLF2 in control of B-cell quiescence.

Another KLF family member, KLF4, also known as gut-enriched Kruppel-like factor or GKLF, is also important in T cell biology by regulating thymocyte development and IL-17 expression

during Th17 differentiation [21]. Both thymocytes and mature T cells express high level of KLF4. In KLF4 knock-out mice, the proliferation of thymocytes at double-negative stage was significantly reduced, attributed to loss of KLF4 control on Cdkn1b, a cell cycle molecule. KLF4 is also involved in Th17 differentiation and IL-17 expression by which its deficiency contributes to reduced IL-17 production and thus ameliorates the severity of in vivo experimental autoimmune encephalomyelitis [21]. KLF4 is able to exert a global inhibitory effect on macromolecular biosynthesis, including protein biosynthesis, transcription, and cholesterol biosynthesis [22]. The expression pattern of KLF4 in B cells highly resembles those in T cells, whereby the expression is abundant in mature resting cells but rapidly decreased upon cell activation. In KLF4-deficient mice, a modest decrease in the numbers of pre-B cells in the bone marrow and mature B cells in the spleen can be observed [23]. Fewer B cells enter S phase of the cell cycle and complete cell division in response to BCR and/or CD40L engagement, in vitro, in the absence of KLF4, suggesting its role in maintaining quiescence in B cells. This could be a result of decreased expression of cyclin D2 in B cells because KLF4 regulates cyclin B2 through a direct binding to its promoter [23]. Thus, we can also postulate a potential role of KLF4 in controlling T or B lymphocytes quiescence.

3. Foxo

Forkhead box (Foxo) family genes are the orthologs of DAF-16 gene identified in nematode worm *Caenorhabditis elegans,* which programs cells for resistance to oxidative stress and cell cycle control to maintain cells at a dauer (non-action) state [24]. In mammals, there are three members in Foxo family, Foxo1, Foxo3, and Foxo4. The Foxo family of transcription factors triggers the induction or suppression of multiple target genes dependent on context molecules [25] and hence plays multiple functions in cell quiescence control [3, 26–29], including maintenance of stem cells pluripotency [30], oxidative stress control [31], cell cycle, cancer progress [32], and others. In T cells, Foxo molecules have multiple roles by controlling cell-surface molecules, signaling proteins, and nuclear factors that control gene expression [33]. Foxo1 is also detected in thymocyte subsets, dominant negative inhibition of Foxo1 causes increased proliferation capacity of thymocytes, thus interferes with central tolerance control [34].

In the animal model, Foxo1 deletion causes spontaneous T cell activation that leads to development of colitis [35]. Higher percentages of activated/memory T phenotypes have been reported in T cell-specific Foxo1 knock-out mice model [35, 36]. Besides, inflammatory bowel disease is also observed in the wildtype mice after receiving Foxo1-deficient T cells. In a mouse model with CD4 promoter-driven T cell-specific deletion of Foxo1, mice develop exocrine pancreatitis, hind limb paralysis, and multiorgan lymphocyte infiltration. Anti-nuclear antibodies and formation of germinal centers are detected in mice, suggesting Foxo1 suppresses cells from differentiating into follicular helper (T_{FH}) subtypes [37]. Foxo1-deficient T cells demonstrate a highly defective ability for cell homing to lymph nodes, due to impaired L-selectin [38] and CCR7 expression [36]. Although some argue that Foxo is not a true quiescence factor stating the activated phenotype may be due to its functions like cell trafficking, these distinct roles of Foxo may directly or indirectly impose lymphocyte quiescence.

In contrast to Foxo1's specific expression in lymphocytes, Foxo3 is ubiquitously expressed in many tissues in the body. It mediates cell death in many cells including T and B lymphocytes. The role of Foxo3 in the regulation of cell quiescence remains controversial. An earlier report using Fox3TRAP mice (created by retroviral gene-trap technique) demonstrates typical autoimmune characteristics including spontaneous lymphoproliferation, hyperactivation, and lymphocyte infiltration into multiple organs [39]. However, another two mice generated, using gene-trap, show no such symptoms except for abnormal ovary development [40, 41]. Different mice generated with targeted recombinant techniques demonstrated only some decrease in the number of pre-B and circulating B cells [42, 43]. Foxo3 protects quiescent cells from oxidative stress through regulation of antioxidant manganese superoxide dismutase (MnSOD) [44] and growth arrest, and damage response gene (Gadd45a) is a direct target of Foxo3a [45].

In the last decade, increasing numbers of target genes regulated by Foxo transcription factor have been identified. These include KLF2 [36], GTPase of immunity-associated protein 5 (Gimap5) [46], IL-7Ra, homing molecules (L-selectin, CCR7, and Fam65b), CTLA4 [37], and shingosine-1 phosphate receptor [38], among others. A major target of Foxo is KLF2, an essential transcription factor for quiescence control. Introduction of Foxo1 into T cells causes induction of KLF2 transcription factor while T cell's specific deletion of Foxo1 showed lower expression of KLF2 [36]. Foxo1 binds directly to promoter of KLF2 gene to induce its expression [47].

4. Foxp1

A member in the subfamily P of the large Fox family, Forkhead box protein P1 (Foxp1), has an essential role in B lymphopoiesis to control the expression of recombination-activating genes 1 and 2 and transition from pro B to pre B cells [48]. Foxp1 has been implicated in the quiescence control of naïve T cells by inhibiting IL-7Ra expression and diminishing signaling by the kinase Erk [49, 50]. Acute deletion of Foxp1 induces naïve T cells to gain effector phenotype. Homeostatic proliferation of quiescence cells is regulated by IL7 signaling pathway, which can be negatively regulated by autocrine feedback control of IL7Ra expression [51]. Transcription factor Foxp1 helps maintain the quiescence of naïve T cells by binding to 3.5 kb upstream of IL7R transcription start site and inhibiting IL-7Ra expression [36]. Foxp1 is a negative regulator of Foxo1 as they compete with each other for the forkhead binding site at IL7R enhancer region [49].

5. Tob

A member of the Tob family shares a highly conserved NH_2 terminal sequence. Tob is expressed in resting T cells as well as in anergic T cells [52]. Its expression is diminished upon T cell activation by anti-CD3/anti-CD28 or mitogen PMA stimulation. Forced expression of exogenous Tob molecule inhibits T cell proliferation. Tob interacts with Smad2 and Smad4 molecules and enhances Smad4, signaling to suppress the transcription of multiple

cytokines including IL-2. Overexpression of Tob also blocks cell cycle progression by promoting p27kip1, a negative regulator of cell cycle. In contrast, the positive regulator of cell cycle molecules including cyclin E, cyclin A, and Cdk2 was suppressed. Elimination of Tob protein synthesis using antisense oligonucleotide reduces the threshold of T cell activation.

6. Tsc1

Tuberous sclerosis 1 (Tsc1) functions as a GTPase-activating protein (GAP) that binds small GTPase RHEB and negatively regulates mTOR1 signaling. Tsc1 is important in T cell biology in memory cell differentiation, effector, and regulatory functions [53, 54]. Tsc1 is also implicated in anergy T cells, and its expression is higher in anergy as compared to activated T cells [55]. $Tsc1^{-/-}$ T cells in mice model loss quiescence as demonstrated by increased cell surface marker ($CD44^{hi}CD122^{-}$) and prominent upregulation of activation markers such as CD69, CD25 and CD71 [56]. Besides, $Tsc1^{-/-}$ T cells demonstrated increased cell size, proliferation, reactive oxygen species (ROS) generation, and susceptibility to apoptosis. Tsc1 deficiency also dampens anti-bacterial immune response in animal model as reduced OVA-reactive tetramer-positive T cells and interferon-producing $CD8^{+}$ T cells. The effect of Tsc1 deficiency is attributed to its ability to inhibit mTORC1 as this effect can be reverted by rapamycin treatment.

7. Slfn2

The word "schlafen" means sleeping in German. Schlafen (Slfn) family of genes, so-called owing to their ability to promote cells into an inactive state, consists of six genes with RNA helicase-like motif in human. Schlafen proteins promote growth inhibitory responses and play roles in thymocytes development [57], effector, and regulatory T cells [58]. Slfn2 was added to the gaining list of quiescence factors coincidentally when scientists investigate the phenotype in elektra mouse, a G_3 mice homozygous for chemically induced random germline mutation [59]. The eureka mice carry a single mutation in Slfn2, which results in isoleucine-to-asparagine substitution of amino acid residue 135, which is induced after exposure to N-ethyl-N-nitrosourea. Eureka mice are defenseless and succumb to lymphocytic choriomeningitis virus and *Listeria monocytogenes* infection due to immunodeficient phenotype. This increased susceptibility to bacterial and viral infections can be reversed with bacteria artificial chromosome (BAC) transgenesis of Sfln2 gene, thus confirming the role of Sfln2. Interestingly, T cells from eureka mice exist in a semi-activated state [59]. The T cells are generally lesser in amount but express higher surface activation marker ($CD44^{hi}$) and proliferate strongly and are more prone to apoptosis in response to anti-CD3/anti-CD28 activation signal. A chronic ER stress under steady-state conditions observed in Eureka T cells could explain the loss of immune cell quiescence [60]. Slfn2 has been suggested as a promising target for treating human T-ALL malignancy [61]. In T-ALL mice model, impaired Slfn2 functions rescue the mice from disease progress as the proliferation potential and survival of leukemic T cells are affected. In addition, the symptoms of severe lymphoproliferative disease in Fas deficient mice can also be rescued by Sfln2 loss-of-function mutation.

8. Runx1

Runx family comprise three members: Runx1 and Runx3 play crucial roles in T cell development and differentiation [62], whereas Runx2 is a key player in osteoblast differentiation during bone formation [63]. Runx1 knock-out mice die of impaired fetal hematopoiesis at embryonic day E12.5 [64]. Conditional knock-out mice driven by CD4-Cre promoter lead to low number of CD4+ T cell population attributed to apoptotic cell death. When an anti-apoptotic Bcl-2 transgene is introduced to rescue the T cells, $Runx1^{-/-}$ T cells demonstrate spontaneous hyperactivation ($CD44^{hi}CD62L^{lo}$) phenotype. This mouse model displays a breakdown of immune tolerance as the signs and complications of systemic inflammatory response syndrome (SIRS) such as cytokine storm, monocytosis, blood coagulation, and muscle wasting syndrome can be observed. Infiltration of $Runx1^{-/-}$ cells into the lung causes autoimmune lung disease similar to human pulmonary alveolar proteinosis (PAP) [65]. In addition, increased surface activation markers, CD40L and CD69, were observed along with increased cytokine, chemokines, and other signaling molecules [66]. It is noteworthy that similar to other quiescence factors such as KLF2, Runx1 is abundantly expressed in naïve T cells, and TCR signaling results in rapid reduction of Runx1 expression [67]. One possible mechanism of Runx1 to exert quiescence is through silencing the expression of cytokines IL-2. Besides, Runx1 may control quiescence indirectly through transcriptional control of Foxp1, Foxo1, and KLF2 by binding to their promoter region [66].

9. Peli

Ubiquitination is a post-translational mechanism for protein degradation. During ubiquitination, small ubiquitin proteins attach to the lysine residues of substrate protein catalyzed by sequential action of E1, E2, and E3 ubiquitin-activating enzymes. Ubiquitination process has been shown to involve in immune regulation [8, 68, 69]. Peli family is composed of three members, Peli1, Peli2, and Peli3 [70], among which E3 ligase Peli1 has been implicated in T cell quiescence control. $CD4^+$ and $CD8^+$ T cells from Peli-deficient mice demonstrate hyperactivation and increased proliferation response upon TCR-CD28 signaling [8]. Most of the $Peli1^{-/-}$ T cells turn into memory cells. $Peli^{-/-}$ mice develop autoimmune diseases, as demonstrated by multiorgan inflammation, detection of antinuclear autoantibody, and prominent immune complex deposition in kidney, and more pathogenic potentials are induced in experimental autoimmune encephalitis model [8].

10. Gimap5

Gimap5 stands for GTPase of immunity-associated protein 5. A missense mutation in the Gimap5 results in abrogation of quiescence and reduced number and survival of lymphocytes [46]. Gimap5-deficient $CD4^+$ T cells from the mice are Th1/Th17 polarized and thus promotes colitis and early mortality. Gimap5 also plays a role in regulatory T cells because in its

absence, regulatory T cells become reduced in frequency in the peripheral tissues and their immunosuppressive capacity becomes impaired.

11. Summary

Mature T lymphocytes in our body can remain in a quiescence state for a prolonged duration in the absence of infectious or stimulatory factors. Loss of T cell quiescence control leads to breakdown of immune tolerance and is the main causative factor for various types of autoimmune diseases, lymphoma, and leukemia. The understanding of the interaction among the multiple factors that are involved in intrinsic control of quiescence status is hence crucial to control the balance between immune tolerance and activation.

Acknowledgements

This study is supported by Fundamental Research Grant Scheme (FP030-2014A) from Malaysia Ministry of Higher Education.

Abbreviations

VDJ	variable, diversity and joining
Sp	Specificity protein transcription factors
IL	interleukin
BCR	reakpoint cluster region protein
PMA	phorbol 12-myristate 13-acetate
OVA	ovalbumin
ER	endoplasmic reticulum
CD	cluster of differentiation
Th17	helper T 17
RHEB	Ras homolog enriched in brain
CDKH1b	Cyclin-dependent kinase inhibitor 1B
CCR7	C-C chemokine receptor type 7
Fam65b	Family With Sequence Similarity 65 Member B
T-ALL	T-cell acute lymphoblastic leukaemia
Runx	Runt-related transcription factor
Peli	Pellino E3 Ubiquitin Protein Ligase
BCL-2	B-cell lymphoma 2

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