

Chapter 2

Th1/Th2 Cell Differentiation and Molecular Signals

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Abstract The distinctive differentiated states of the CD4+ T helper cells are determined by the set of transcription factors and the genes transcribed by the transcription factors. In vitro induction models, the major determinants of the cytokines present during the T-cell receptor (TCR)-mediated activation process. IL-12 and IFN- γ make Naïve CD4+ T cells highly express T-bet and STAT4 and differentiate to TH1 cells, while IL-4 make Naïve CD4+ T cells highly express STAT6 and GATA3 and differentiated to TH2 cells. Even through T-bet and GATA3 are master regulators for TH1/TH2 cells differentiation. There are many other transcription factors, such as RUNX family proteins, IRF4, Dec2, Gfi1, Hlx, and JunB that can impair TH1/TH2 cells differentiation. In recent years, noncoding RNAs (microRNA and long non-coding RNA) join in the crowd. The leukocytes should migrate to the right place to show their impact. There are some successful strategies, which are revealed to targeting chemokines and their receptors, that have been developed to treat human immune-related diseases.

Keywords Th1/Th2 · Transcription factor · Migration · Epigenetics · MicroRNA · LncRNA

2.1 Introduction

Naïve T cells can differ into different subsets based on the signals they faced. Right and moderate stimulation will lead to precise CD4+ T-cells responses, while incorrect signals cause dysfunctions like auto-immune diseases and allergy.

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Activation of CD4⁺ T cells takes place in secondary lymphoid organ, and chemokine receptor expression patterns on CD4⁺ T cells induces them to ingress, stay and egress, which drive them to the right place, at the right time, and receive right signals. The main frame of how chemokines orchestrate has been established in recent years, and many studies showed some critical CCR molecules drive T cells toward where they were needed, which means same T cells subset with different chemokine expression will lead to divergent disease condition. As CD4⁺ T cells plays the key role in adoptive immune system, chemokine receptors interference by small molecules or antibodies is new approach to disease treatment.

Classic Th cell model tells us every subset develops by producing its unique cytokines and master regulator. But recent researches show that things maybe a little complicated. With new technologies such as ChIP-seq, RNA-seq, and database analysis, potential transcript factors and binding sites were found in different subsets and stage of T cells. These results tell us T cell differentiation is not clear cut from starting point to the end, but struggling from mass of signals and molecules regulation, under subtle controls by master regulator, instead of simply switch on and off. In epigenetic level, the modification of DNA or histone may help to deciding cell fate, by facilitating or blocking transcript factor binding.

2.2 Transcription Factors and Cytokines in Th1/Th2

This Th1/Th2 paradigm was first thought useful for initial categorization of mechanisms involving elimination of microbial pathogens. For instance, Th1 cells are critical for the clearance of many intracellular pathogens, such as *Leishmania major* [136], while Th2 cells were found to be important for elimination of helminthic parasites, such as *Nippostrongylus brasiliensis* and *Schistosoma mansoni* [134].

For several decades, it has been recognized that CD4 T cells specialize in response to antigens like microbial challenges [109]. The first subsets identified were Th1 and Th2 cells based on the selective production of their cytokines, IFN- γ and IL-4 [156], respectively. The details of the Th1/Th2 transcription factors/cytokines/chemokines production and regulation research will be discussed in this chapter.

2.2.1 A Network of Transcription Factors Determines Th1/Th2

(a) T-bet

T-bet is not expressed in naive CD4⁺ T cells, but is readily induced in response to TCR, IFN- γ and IL-12R signaling pathways. T-bet is regarded as the master regulator for Th1 cell differentiation and IFN- γ production. Selective expression of T-bet accounts for TH1 cell development and for the TH1 cell-specific expression of IFN γ [158]. And T-bet deficient (*Tbx21*^{-/-})

cells produce diminished but measurable amounts of IFN- γ during in vitro culture and in vivo responses to *Leishmania major* infection [160]. The IFN γ -STAT1-T-bet pathway serves as a powerful amplification mechanism for in vitro Th1 differentiation [1, 93]. STAT4 activation by IL-12 is critical for Th1 responses both in vitro and in vivo [19, 82, 163] and the expression level of STAT4 is higher in Th1 than that in Th2 cells [165]

(b) **GATA3**

The expression of GATA3 is induced by the TH2-cell-inducing cytokine interleukin-4 (IL-4) in a signal transducer and activator of transcription 6 (STAT6)-dependent manner [122]. The role of GATA3 in Th2 cells is well known as the master regulator to guide the differentiation of Th2. After the ligation of TCR in naïve CD4+ T cell, the mRNA levels of IL-4 and GATA3 are both up-regulated within several hours and 24 h, respectively [60, 178]. GATA3 can facilitate the conversion of the IL4-IL5-IL13 locus to an open conformation, so that the other transcription factors involved in Th2-cell differentiation can access this locus [9, 88, 89, 152]. In addition to the function of promoting in Th2 cells, GATA3 also inhibits the expression of interferon- γ (IFN- γ) and directly transactivates IL5 and IL1. The production of high levels of IL-4 and the autoactivation of GATA3 expression build a positive-feedback loop that further induces the expression of GATA3 and the differentiation of Th2 [121]. Zhu et al. generated mice with a conditional deletion of Gata3 and Gata3-deficient mouse T cell lines and found that both IL-4—dependent and —independent Th2 differentiation was diminished, permitting Th1 differentiation in the absence of Ifng and IL12. They concluded that Gata3 serves as a principal switch in determining Th1-Th2 responses [190].

(c) **RUNX family**

Besides the traditional master regulator T-bet of Th1 [159] with activation of STAT1 by receiving IFN γ [1, 93] together with IL-12 induced activation of STAT4, and GATA3 of Th2 [183] through phosphorylation of STAT6 under IL-4 stimulation [81, 150, 161]. Other transcript factors were found in Th1 and Th2 differentiation, proliferation, and migration. And there results consist of an intact network of how Th1 and Th2 cells are developed and regulated. One example of this is Runx (Runt-related transcript factor) family. There are 3 Runx family members, Runx1, Runx2 and Runx3 [43]. It is showed that binding sites for Runt domain transcription factors are essential for CD4 transcriptional silencer function, and that different Runx family members are required to fulfill unique functions at each stage. Taniuchi and colleagues found that RUNX1 is required for active repression in CD4-negative/CD8-negative thymocytes, whereas RUNX3 is required for establishing epigenetic silencing in cytotoxic lineage thymocytes. Cytotoxic T cells deficient in Runx3, but not helper cells, had defective responses to antigen, suggesting that RUNX proteins have critical functions in lineage specification and homeostasis of CD8-lineage T lymphocytes [162]. Another nature paper found Runx1 are important in CD4 T cell's IL-2 producing processes [120], and also crucially required for normal

hematopoiesis including thymic T-cell development. In this study, several lines of evidence supported a model in which the interaction suppresses IL2 and IFN-gamma production, upregulates Treg cell-associated molecules, and exerts suppressive activity.

(d) **IRF4**

Another important transcript factor is one of IRF4 (IFN regulatory factor family members 4). IRF4 is a transcription factor essential for the development of Th2 cells, IL17—producing Th17 cells, and IL9—producing Th9 cells [153], and implies the potential relations among these three subsets. Negishi and colleagues demonstrated that IRF4 interacted with MYD88 (myeloid differentiation primary response gene 88) in the cytoplasm of human embryonic kidney cells. Mutation and coimmunoprecipitation analysis showed that IRF4 interacted with the TIR/IL1 region of MYD88, and IRF4 is also found inhibiting the interaction of MYD88 with IRF5 (IFN regulatory factor family members 5) Zheng and colleagues showed that in mouse T regulatory cells, high amounts of IRF4, a transcription factor essential for TH2 effector cell differentiation, is dependent on Foxp3 (forkhead box p3) expression. They proposed that IRF4 expression endows T regulatory cells with the ability to suppress Th2 responses. Indeed, ablation of a conditional *Irf4* allele in T regulatory cells resulted in selective dysregulation of Th2 responses, IL4-dependent immunoglobulin isotype production, and tissue lesions with pronounced plasma cell infiltration, in contrast to the mononuclear cell-dominated pathology typical of mice lacking T regulatory cells. Zheng concluded that T regulatory cells use components of the transcriptional machinery, promoting a particular type of effector CD4+ T-cell differentiation, to efficiently restrain the corresponding type of the immune response [184].

(e) **STAT family**

Activation of STAT1 by IFN- γ is important for the induction of T-bet during in vitro Th1 differentiation [1, 93]. STAT4 expression is higher in Th1 than in Th2 cells [165]. STAT4 expression is likely to be regulated positively by IFN- γ [50] and negatively by IL-4 and GATA3 [50, 165]. Activated STAT4 can directly induce IFN- γ production and expression of IL-12R β 2 and T-bet during Th1 differentiation [165, 166]. Low levels of STAT5 activation are sufficient for cell proliferation and survival; however, strong STAT5 signaling is required for Th2 differentiation [33, 185]. STAT5 directly binds to the DNase I hypersensitive sites (HS) II and HSIII in the second intron of the *Il4* locus in Th2 but not in Th1 cells [185]. STAT6 is the major signal transducer in IL-4-mediated Th2 differentiation and expansion [81, 150, 161]. In vitro, STAT6 activation is necessary and sufficient for inducing high expression levels of the Th2 master regulator gene, GATA3 [81, 188]. STAT6 may also be important for the amplification of Th2 responses at later stages and/or for the generation of Th2 memory cells in vivo [47].

(f) **Dec2**

Among the key co transcript factors, Dec2 (gene name: basic helix-loop-helix family, member e41; bhlhe41) is a transcriptional regulator that control a wide

variety of biological processes, including cell proliferation and differentiation [8, 80, 115]. The common structures shared among the members of this superfamily are the basic domain, which is required for DNA binding, and the helix-loop-helix domain, which is involved in dimerization. Dec2 is cloned by Fujimoto in 2001 [51]. In the same year, Garriga-Canut and colleagues showed that rat Sharp1 binds to the M1 muscarinic acetylcholine receptor and acts as a transcriptional repressor of both TATA-containing and TATA-less promoters. Repression occurs either via the bHLH domain or via a C-terminal domain that is sensitive to the histone deacetylase inhibitor trichostatin A, constructs the foundation of the transcript and binding function of Dec2 [52]. In a science paper, He and colleagues found that heterozygous P385R-mutant transgenic mice showed increased activity and less sleep time compared to wildtype mice. Under sleep deprivation, transgenic mice also showed less compensatory gain in non-REM sleep compared to wild type mice, suggesting a role for Dec2 in sleep homeostasis. There were no differences in circadian rhythm compared to wild type mice. The phenotype was not found in Dec2-null mice, suggesting a dominant effect of the heterozygous P385R mutation and a dominant increase in the quantity of wakefulness. Similar results were found in a *Drosophila* model [65]. It is surprisingly to find that Dec2 is highly expressed in murine T cells, while Dec1 does not exhibit such a preference, and overexpression of Dec2 leads to higher CD25 (IL-2 receptor α), thus more Th2 differentiation, both in vitro experiments and ex vivo results using Dec2 transgenic mice [94]. In the same year, it is also found that Dec2 is able to induce GATA3 expression, and Dec2 deficiency leads to impaired Th2 responses both in vitro and in vivo. GATA3 also regulates Dec2 expression, suggesting that Dec2 and GATA3 form a positive regulatory feedback loop during Th2 differentiation [179]. Considering that IL-2 is also required in Th2 development [33] just like IL-4 [156], and lack of IL-2/STAT5 signals leads to eliminated Th2 levels, even with normal IL-4 signals and STAT6 phosphorylation [185]. It is also found that IL-2 and STAT5 are necessary for maintain GATA3 expression [63] and regulates IL-4R α expression, in the very beginning of Th2 cell development [92]. It remains unsolved which mechanism is more significant of Dec2 in Th2 cells, as a co-factor of GATA3, or as a bridge of IL-4 and IL-2 signaling. With more and more finding in cytokines downstream signals crosstalk, it is for sure that more intermediary molecules like Dec2 will be found (Fig. 2.1).

(g) **Other transcript factors**

There are other important transcript factors found in recent years, such as Gfi-1 (Growth factor independent 1) [42, 54, 189, 186], and Ikaros family members [53, 124, 135, 173], c-maf [15, 72, 76], Hlx (H.20 like—homobox) [113], Ets-1 (v-ets avian erythroblastosis virus e26 oncogene homolog 1) as a co-factor of T-bet [58, 108], JunB (v-jun avian sarcoma virus 17 oncogene homolog B) as a member of AP-1 (activator protein 1) family, collaborate with c-maf [90]. Blimp-1 (gene name: pr domain-containing protein 1; prdm1) is induced by

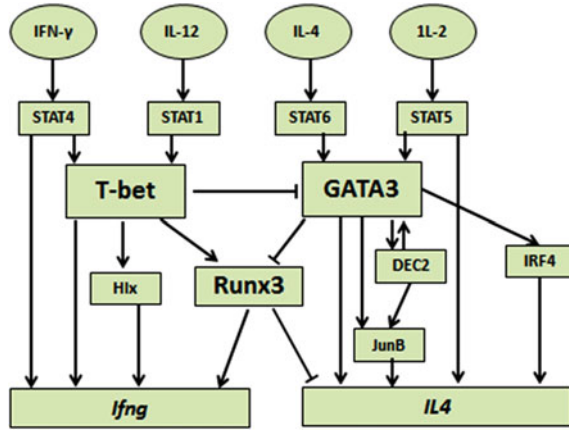


Fig. 2.1 The network of transcription factors in Th1/2 cells. The activation of signal transducer and activator of transcription (STAT) proteins by different cytokines plays a critical role in inducing the expression of the lineage-specific master regulators T-bet (Th1), GATA3 (Th2). The STAT proteins also collaborate with the master regulators and some secondary transcription factors, whose expression is controlled by the master regulators, for the induction of cytokine genes. Positive or negative regulation among these transcription factors occurs at the gene expression level and/or at the protein level through protein–protein interaction, forming a sophisticated transcriptional regulatory network during Th1/2 cell differentiation [191]

IL-2 signaling in CD4 T cells [34, 79, 103], and can directly suppress IFN γ and IL-2 production [27, 105, 104].

With the chip sequence and RNA sequence methods, the binding site and pattern, of transcript factors are widely and systematically identified, GATA3 is the first master regulator in approaching this thoroughly analysis. Zhu found that GATA3 binding exhibited both common and cell-specific patterns among divergent T-cell lineages, and it is surprisingly to find that many genes were either positively or negatively regulated by GATA3 in a cell type-specific manner, suggesting that GATA3-mediated gene regulation depends strongly on existing or not of cofactors in different T cells [172]. As the same researcher found that intergenic long non-coding RNAs are much more significantly changed in different T-cell subsets [69], in the future, this difference of lncRNA environment and other intracellular milieu of divergent T-cell subsets may successfully explain the subtle mechanisms in transcript factor function.

2.2.2 A Network of Cytokines Determines Th1/Th2

The induction of the distinctive patterns of gene expression may be achievable in several ways, but in vitro the major determinants of the differentiated state of the cell are the set of cytokines present during the T-cell receptor (TCR)-mediated

activation process. Several lines of evidence strongly support the notion that the cytokines play a major role in inducing the transcription factors that determine differentiation [60, 112]. Our understanding of this process has evolved over an extended period, and is described in detail below.

One major discovery of Th1/Th2 fate decision is the key role of IL-2. In most CD4 T-cell subsets, IL-2 is just help to proliferate, but in Th2 cells, IL-2 and the downstream STAT5 signals is essential in driving Naïve T cell toward Th2 differentiation [60, 112, 187]. With the finding that Th17 is negatively regulated by IL-2 [32, 170], while Treg need large amount of IL-2 in differentiation and maintenance, without produce any IL-2 [2, 3], and recent data showed Tfh cells also take IL-2 as its critical negative regulation pathway [11, 26].

Th1 cells predominantly produce interferon- γ (IFN γ) and are important for protective immune responses to intracellular viral and bacterial infection. Th1 cells are also responsible for the induction of some autoimmune diseases. In turn, IFN γ is important for the differentiation of IFN γ -producing Th1 cells [93] although IL-12 is also critical for this process [68]. Indeed, in vitro neutralization of IFN- γ will often markedly diminish Th1 development. Although IL-18 is not involved in the differentiation of Th1 cells, it can synergize with IL-12 in inducing IFN γ , implying that IL-18 plays an important role in Th1 responses [139, 180].

Th2 cells mediate host defense against extracellular parasites including helminthes. Similarly, when stimulated by a cognate antigen presented by accessory cells in the presence of IL-4, naive CD4+ T cells differentiate into IL-4-producing Th2 cells [67, 61, 149, 156]. CD25 (IL-2R α) expression is higher in Th2 cells than in Th1 cells, possibly due to the action of c-Maf [72]. Such higher expression of CD25 may confer hyperresponsiveness to IL-2.

2.3 Cross-regulation Among Transcription Factors and Cytokines During Th1/Th2 Differentiation

During Th cell differentiation toward one lineage, the other lineage fates are usually suppressed. An important cross-regulation during Th differentiation is through repression of transcription factors that are important for lineage determination. For example, GATA3 downregulates expression of STAT4, which is the important factor for mediating IL-12 signaling and Th1 differentiation [165]. GATA-3 deficiency results in spontaneous IFN- γ production, independent of IL-12 and IFN- γ [165]. A constitutively active form of STAT5 inhibits T-bet expression while it also promotes Th2 differentiation [185]. T-bet suppresses GATA-3 function by direct binding of the factors [71].

Th differentiation involves positive feedback by cytokines. The differentiation process also actively involves cross-inhibition of other lineage fates. Mutual suppression between IFN- γ and IL-4 signaling was the takeoff point for studies of

cross-regulation [110, 127]. IL-4 can suppress Th1, Th17, and Treg cell differentiation [189, 186]. TGF- β was also found to suppress both Th1 and Th2 differentiation [56], and both IL-4 and IFN- γ inhibit Th17 differentiation [64, 126].

2.4 Migration Signals as Guiding Light

The chemokine system controls CD4 T cell and other leukocyte migration in autoimmune disease, immune responses, virus infection and inflammation, and is implicated plays the key roles in the pathogenesis of many human diseases, and induced several successful strategies, which have been identified to develop drugs targeting chemokines and their receptors, but this has not yet resulted in many new therapeutics, because chemokine system is full of redundancy, pleiotropy, and differences among species, and single changes through chemical drugs will lead to a complex result. But on the other side, our understanding of chemokine biology is continuing to grow and so does drug discoveries. There are lots of findings in exploring the role of chemokines in health and diseases [169].

After differentiation, CD4 T cells need to migrate into focus of infection to exert its function. The research of T-cell migration control obtains lots of harvests, and leads to discovering some drugs and medical methods with significant therapeutic effects. The history of CD4 T-cell migration moves along with corresponding drug discoveries [169]. Critical determinants of the in vivo activities of chemokines in the immune system include their secretion by endothelial cells and together presenting with other extracellular matrix molecules, to recruit corresponding CD4 T helper cells and other immune cells. On the other side, there are cellular uptake via “silent” chemokine receptors (interceptors) leading either to their transcytosis or to degradation. Considering chemokines signals may contradict each other, and different chemokine receptors sometimes share the same chemokine ligand, the total image of T-cell migration regulation is extremely complicated and hard to uncover, represent the complexity of T-cell migration in tissue damage [6, 141]. But in recent years, the mosaic tiles of T-cells migration is on its way to finish.

2.4.1 CCR5, Discovery, Feature and Function

For Th1 cells, CCR5 (C–C chemokine receptor 5) will lead them into inflamed tissues [157]. CCR5 is first cloned in 1996 [144], from a human genomic DNA library based on its similarity to a murine C–C chemokine receptor clone. The human gene, which they designated ChemR13, encodes a 352-amino acid protein (designated CCKCR5 by them) with a calculated molecular mass of 40,600 Da and a potential N-linked glycosylation site. With a set of overlapping lambda clones, they showed that the gene is 17.5 kb from the CMKBR2 gene (CCR2; 601267). The 2 coding regions share 75 % DNA and amino acid sequence identity. One year

later, Mummidi et al. analyzed the genomic structure of CCR5, their conclusion shows CCR5 contains 4 exons, spanning approximately 6 kb, and only 2 introns. There is no intron between exons 2 and 3. Exon 4 contains the open reading frame, the complete 3-prime UTR, and 11 nucleotides of the 5-prime UTR. Transcripts are initiated from 2 distinct promoters, both of which are AT-rich and lack canonical TATA or CAAT motifs; one is upstream of exon 1 and the other downstream, including the 'intronic' region between exons 1 and 3. Complex alternative splicing patterns in the 5-prime UTR and in the 4 exons give rise to multiple CCR5 transcripts. The regulatory sequences and noncoding exons are polymorphic, whereas the protein sequence is not [114].

CCR5 is also found as a coreceptor for the human immunodeficiency virus-1 (HIV-1) in the same year as it was cloned [37, 41]. CMKBR5 (gene symbol of CCR5, also with the short form CKR5) was served as a secondary receptor on CD4 + T lymphocytes for certain strains of human immunodeficiency virus-1 (HIV-1) by Deng et al. and Dragic et al. CMKBR5 and fusin facilitate the fusion of HIV-1 with the plasma membrane of CD4+ cells. Deng et al. [37] found that CMKBR5, and not fusin, promotes entry of the macrophage-tropic viruses believed to be the key pathogenic strains in vivo. A 32-base pair deletion allele (CKR5Delta 32) was identified that is present at a frequency of sim 0.10 in the Caucasian population of the United States. An examination of 1955 patients included among six well-characterized acquired immunodeficiency syndrome (AIDS) cohort studies revealed that 17 deletion homozygotes occurred exclusively among 612 exposed HIV-1 antibody-negative individuals 2.8 % and not at all in the rest of 1343 HIV-1-infected individuals. The frequency of CKR5 deletion heterozygotes was much higher in groups of patients that had survived HIV-1 infection for more than 10 years, and, in some risk groups, twice as frequent as their occurrence in rapid progressors to AIDS. Survival analysis clearly shows that disease progression is slower in CKR5 deletion heterozygotes than in individuals homozygous for the normal CKR5 gene. All this results shows the CKR5Delta 32 deletion may act as a recessive restriction gene against HIV-1 infection and may exert a dominant phenotype of delaying progression to AIDS among infected individuals [36].

In short, CCR5 is found as a Th1 deviation chemokine receptor in 1998 [17]. Since related with HIV-1 infection, a lot of researches focus on this aspect [5, 25, 41, 46, 142, 181], which implies the relationship between virus infection and T-cell migration.

CCR5 is found important in directing T-cell migration from lymph node to disease location in 2001. By using bronchoalveolar lavage and flow cytometry, Campbell et al. proved that T cell migration is controlled by a combination of adhesion receptors and chemokines expressed on vascular endothelium and in the tissue, often in an organ-specific manner. T lymphocytes homing to the lung in both normal and asthmatic subjects express CCR5 and CXCR3 but not CCR9, which is found on T cells homing to intestinal mucosal sites, or selectin p ligand (SELPLG), the high-affinity counter-receptor for P-selectin, which is found on skin-homing T cells. No differences were observed between lung T cells from normal versus

asthmatic subjects. This study provides added support for the concept of a lung-homing pathway separate from other mucosal organs such as the gut and suggests that the chemokine pathways that control T-cell migration [20].

The ligand of CCR5 keeps on in 2003. By using mouse splenic dendritic cells and DCs from Ccr5 $-/-$ mice and Myd88 $-/-$ mice, Aliberti et al. found that *Toxoplasma gondii* stimulated IL-12 production not only through a classic Toll-like receptor/Myd88-dependent mechanism, but also through the release of an 18-kD protein, cyclophilin-18 (C18), that interacted directly with CCR5 on DCs. Cyclosporin A, a major ligand of cyclophilin, or anti-C-18 inhibited IL-12 production in DCs. Aliberti et al. concluded that C18 is a molecular mimic of a CCR5 chemokine ligand [4].

IL-16, which signaled through CD4 on eosinophils in a dose-dependent manner and induced the release of leukotriene C4 (LTC4), as well as eotaxin (CCL11) and RANTES (CCL5), in an autocrine manner, RANTES and eotaxin signaled through their membrane receptor, CCR3, and enhanced eosinophil secretion of LTC4 and IL-4 [12]. IL-16 binds to CD4 and induces a comparable migratory response in CD4-positive T cells. Lynch et al. observed a preferential migratory response in mouse Th1 cells, which express CCR5, but not happened in Th2 cells, which express little CCR5. T cells from CCR5-deficient mice were unable to migrate in response to IL-16. In transfected human osteosarcoma cells, the presence of CCR5 significantly increased IL16 binding activity as compared with CD4 alone; however, IL-16 could not bind CCR5 alone. Lynch et al. [99] concluded that augmentation of IL-16 stimulation by CCR5 plays a role in regulation of Th1 cell recruitment and activation at sites of inflammation.

There are also some clinical researches regarding CCR5. Gulati et al. investigated the expression of chemokine receptor CCR5 in the conjunctival epithelium in 45 patients' cells with dry eye syndromes, a disease of millions of people, primarily women [146], compare with 15 control individuals with normal syndromes. By using 2-color flow cytometry using fluorescein-conjugated anti-CCR5 and phycoerythrin-conjugated anti-CD45 and realtime PCR, they found a significant up-regulation in cell surface expression of CCR5 in patients with either aqueous tear-deficient (e.g., Sjogren syndrome;) or evaporative forms of dry eye syndrome in both protein and mRNA level. The majority of the cells expressing CCR5 were non-bone marrow-derived resident epithelial cells of the conjunctiva, suggesting a role of ocular surface epithelial cells in modulating immunoinflammatory responses in dry eye syndromes. Gulati et al. suggested that chemokine receptor CCR5 or its ligands might serve as useful targets for modulation of these responses [62]. Considering increasing levels of chemokine ligand RANTES with concomitant increase in expression of CCR5 receptor has been described in the lacrimal glands in an animal model of Sjogren syndrome [164]. The expression of CCR5 is very important in the pathogenesis and treatment of dry eyes syndromes.

In 2007, when Th17 cells are started to be considered as a new subsets of CD4+ T cells [22, 35, 85], Sato et al. shows CCR5 is a key marker in distinguishing Th1 cells and Th17 cells in human. By analyzing healthy donors PBMC (Peripheral blood mononuclear cell) with flow cytometry and ELISA, Sato et al. found that

CCR2+CCR5⁻, but not CCR5⁺, CD4⁺ T cells produced large amount of IL17 and little IFN γ . Within the CCR2⁺ population, CCR5⁺ cells produced IFN γ and CCR5-negative cells produced IL-17. Sato et al. concluded that human Th17 cells are CCR2-positive/CCR5-negative. This research provides a simple cell surface marker of human Th1 and Th17 cells.

Recently a research proves CCR5 is an important target in immune responses toward bacterial pathogen *Staphylococcus aureus* that causes significant morbidity and mortality worldwide. *S. aureus* secretes a number of protein products that allow the organism to effectively subvert the host immune system. Such factors include super-antigens, antibody binding proteins, cytolytic peptides, and pore-forming cytotoxins [48]. Alonzo et al. identified the HIV coreceptor CCR5 as a cellular determinant required for cytotoxic targeting of subsets of myeloid cells and T lymphocytes by the *Staphylococcus aureus* leukotoxin ED (LukED). They found that LukED was cytotoxic to a human T-cell line ectopically expressing CCR5 (HUT-R5); whereas another T-cell line (Jurkat), which lacks detectable CCR5, was insensitive [7]. In other respect, when CCR5 levels were reduced in HUT-R5 cells using lentiviral CCR5 shRNA, the cells were protected from LukED-mediated killing. And CCR5 antagonist, maraviroc, Vicriviroc and TAK-779, are found potently blocked LukED killing of CCR5⁺ cells. Alonzo et al. further demonstrated that cell killing is blocked by CCR5 receptor antagonists, including the HIV drug maraviroc, in a LukED-dependent manner, and direct interaction between Luke with CCR5 is also proved. Remarkably, CCR5-deficient mice are largely resistant to lethal *S. aureus* infection, highlighting the importance of CCR5 targeting in *S. aureus* pathogenesis, suggests that CCR5 could provide much-needed therapeutic alternatives in the treatment of *S. aureus* infections.

2.4.2 CXCR3, Discovery, Feature and Function

Another chemokine receptor preferentially expressed by Th1 cells and is critically involved in Th1 recruitment to inflamed tissue is CXCR3 (cxc motif, receptor 3). Under IFN γ stimulation, tissues will produce CXCL9 (cxc motif, ligand 3), CXCL10 (cxc motif, ligand 10), or CXCL11 (cxc motif, ligand 11), all three are ligand of CXCR3, which could recruit Th1 cells moving toward and infiltrated into inflamed tissue [49, 86, 97], such as lung [20].

As important chemokines mediating migration of immune cells into infected or inflamed tissues to initiate effective immune responses, the chemokine receptor CXCR3 is preferentially expressed by Th1 cells and CD8⁺ cytotoxic T cells [97, 98, 177] and is critically involved in their recruitment to inflamed tissue. Tissue infiltration of T cells expressing high levels of CXCR3 depends on IFN γ induced release of the CXCR3 ligands CXCL9 (also known as monokine induced by gamma-interferon, MIG), CXCL10 (interferon-induced protein of 10 kDa, IP-10), or CXCL11 (interferon-inducible T-cell alpha chemoattractant, I-TAC) [29, 117]. CXCR3 is also expressed on nonlymphoid tissue-homing CD4⁺CD25⁺ regulatory

T (Treg) cells [83]. Naïve T cells does not constitutively express CXCR3, but is rapidly upregulated following DC-induced T-cell activation [143].

Like other wide spectrum of receptors, intercellular migration signals is mediated by peptide ligands interacting with specific cell surface receptors in CXCR3. As many peptide-binding receptors, CXCR3 also belong to the G protein-coupled receptor family and exhibit common structural features, including the presence of 7 transmembrane domains and a number of conserved amino acid residues. CXCR3 is the first chemokine receptor identified that is highly induced by T-cell activation. The ORF was first identified in incomplete form in 1995 on a genomic clone isolated by polymerase chain reaction-based homology hybridization. The gene was named GPR9 and was originally mapped incorrectly to human chromosome 8p11.2-12. Marchese et al. used PCR and genomic DNA library screening to clone 2 novel human genes, GPR9 and GPR10, and a rat gene, GPR14. Each of these encodes a G protein-coupled receptor. The receptor encoded by GPR9 was found to share the highest identity with human IL8 receptor type B (IL8RB) (38 % overall and 53 % in the transmembrane regions), followed by IL8RA (36 % overall and 51 % in the transmembrane domains) [102] and map to chromatin 13 by Loetscher et al. [97].

As found in 1996, the human CXCR3 receptor cDNA has an open reading frame of 1104-bp encoding a protein of 368 amino acids with a molecular mass of 40,659 dalton [10]. The sequence includes seven putative transmembrane segments characteristic of G-protein coupled receptors [21]. It shares 40.9 and 40.3 % identical amino acids with the two IL-8 receptors, and 34.2–36.9 % identity with the five known CC chemokine receptors. The CXCL9/CXCL10 receptor is highly expressed in IL-2-activated T lymphocytes, but is not detectable in resting T lymphocytes, B lymphocytes, monocytes and granulocytes [40]. The superfamily of T-cell chemokines is made of an array of chemoattractant proteins that has been divided into 4 branches (C, CC, CXC, and CXXC) on the basis of the relative position of the cysteine residues in the mature protein [118]. Structural variants of chemokines are associated with differences in their ability to regulate the trafficking of immune cells during hematopoiesis and inflammatory responses. Chemokines exert their attractant properties after binding to distinct membrane receptors. Because a single chemokine receptor binds several chemokines, it is often difficult to evaluate the activity of these structures in lymphocyte homing at that time. For instance, CXCL10 and CXCL9, CXC chemokines that are induced by IFN-gamma, bind the CXCR3 receptor and are shown to be specifically chemotactic for activated lymphocytes [97].

The research of CXCR3 activities moves on, trying to figure out the importance of persistence expression of CXCR3. Although FACS analysis demonstrated that 40 % of resting T lymphocytes and low numbers of B cells and natural killer cells expressed CXCR3, Loetscher et al. found that these cells did not have detectable CXCR3 transcripts and did not respond to CXCL9 or CXCL10. However, exposure to IL2 with or without addition of phytohemagglutinin for 10 or more days results in cultures of fully responsive CXCR3. Treatment with anti-CD3 antibodies in the presence or absence of soluble anti-CD28 antibodies was inhibitory [98].

Using Northern blot analysis, Bonecchi and colleagues showed that polarized Th1 cells preferentially express CXCR3 and CCR5 among four CXC (CXCR1-4)

and five CC (CCR1-5) chemokine receptors analyze. In contrast, Th2 cells preferentially express CCR4 and, at least in a subpopulation of Th2 cells, CCR3. Th1s and Th2s selectively migrate in response to the corresponding chemokines. It is an early work systematically compared Th1 and Th2 chemokines receptor regarding their migration [17].

In recent years, new ligand of CXCR3 is still found. The human CXC chemokine platelet factor-4 (CXCL4) is encoded by 2 genes, located on chromosome 4 and probably arose through duplication [44, 57]. The 2 genes are indeed highly related and give rise to mature proteins that differ in only 3 amino acid residues in the carboxylic acid (COOH)-terminal part. Analysis of conditioned media from thrombin-treated platelets revealed that both CXCL4 genes are translated into proteins [154]. Different cell have different splice form of CXCL4. Afterward, tumor cells and smooth muscle cells were identified as alternative cellular sources for CXCL4L1 but not for CXCL4, indicating that not every cell type that produces CXCL4 also releases CXCL4L1 and vice versa [86, 167]. By binding analyses and using CXCR3 blocking antibody with function assay, Struyf and colleagues found that human CXCL4L1, the potent inhibitor of angiogenesis, had lower affinity for heparin and chondroitin sulfate-E than did CXCL4 and that CXCL10 and CXCL4L1 could displace each other on human microvascular endothelial cells. CXCL4L1 bound to both CXCR3A and CXCR3B. Neutralization antibodies to CXCR3 blocked CXCL4L1 antiangiogenic activity, and human CXCL4L1 activity was reduced in mice treated with anti-human CXCR3 or in mice lacking *Cxcr3*, as assessed by tumor growth and vascularization of Lewis lung carcinoma. Like CXCL4, CXCL4L1 attracted activated T, natural killer, and dendritic cells, but preincubation with CXCL10 and CXCL11, pertussis toxin, or anti-CXCR3 reduced or neutralized this activity. Struyf and colleagues concluded that CXCR3A and CXCR3B are involved in the chemotactic and vascular effects of CXCL4L1, and provide evidence that both PF-4 forms can attract immature DCs in a CXCR3-dependent manner. Moreover, the angiostatic and antitumoral effects of CXCL4L1 in vivo are also mediated through CXCR3 [155].

And some studies found CXCR3 is essential and as a marker in deciding precise cell fate. The regulation of memory CD4+ helper T-cell function, such as polarized cytokine production, remains unclear. Endo and colleagues in 2011 examined expression of cell surface markers to identify functionally distinct subpopulations of mouse memory Th2 cells. They use FACS analysis to demonstrate 4 Th2 subpopulations based on high or low expression levels of CD62L (SELL) and *Cxcr3*. All 4 subpopulations produced moderate levels of IL4 and IL13, but Th2 cells with low levels of both CD62L and *Cxcr3* (Cd62l-lo/*Cxcr3*-lo cells) selectively produced IL-5. IL-5 production in Cd62l-lo/*Cxcr3*-lo cells was together with histone H3-K4 methylation, a marker for the permissive conformation of chromatin [175], at the IL5 promoter. DNA microarray analysis and quantitative RT-PCR showed that CD44+ memory Th2 cells expressing IL-5 had lower levels of *Eomes* and *Tbx21* and higher levels of *Rora* and *Pparg* than memory Th2 cells lacking IL5 expression. RNA silencing demonstrated that *Eomes* downregulation was required for IL5 expression and that *Eomes* had no effect on H3-K4 methylation at the IL5

promoter. Instead Eomes suppressed Gata3 transcriptional activity by inhibiting Gata3 binding to the IL5 promoter. Depletion of Cd62L-lo/Cxcr3-lo cells ameliorated memory Th2 cell-dependent airway inflammation in mice. Endo et al. concluded that IL5 production preferentially occurs in the CD62L-lo/CXCR3-lo subpopulation regulated by EOMES expression and Eomesodermin was shown to interact with the transcription factor GATA3, preventing GATA3 binding to the IL5 promoter. Memory Th2 cell-dependent airway inflammation was attenuated in the absence of the CD62L(lo)CXCR3(lo) population but was enhanced by Eomes-deficient memory Th2 cells. Thus, IL-5 production in memory Th2 cells is regulated by Eomesodermin via the inhibition of GATA3 activity [45].

2.4.3 CCR4, Discovery, Feature and Function

Th2 cells also have specific chemokine receptors, CCR4 (C-C chemokine receptor 5) [107, 129, 145], cloned in 1999 [116], as well as CCR8 [23, 78]. As other C-C receptor family members, CCR4 are a kind of small, mostly basic, structurally related molecules that regulate cell trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane, G protein-coupled receptors [138, 143]. Chemokines also play fundamental roles in the development, homeostasis, and function of the immune system, and they have effects on cells of the central nervous system as well as on endothelial cells involved in angiogenesis or angiostasis. Chemokines are divided into 2 major subfamilies, CXC and CC, based on the arrangement of the first 2 of the 4 conserved cysteine residues; the 2 cysteines are separated by a single amino acid in CXC chemokines and are adjacent in CC chemokines. The International Radiation Hybrid Mapping Consortium mapped the CCR4 gene to chromosome 3 (sts-X85740). Several CCR genes, including CCR1, map to 3p22-p21.

CCR4 is cloned in 1995 By RT-PCR analysis using degenerate oligonucleotide primers corresponding to conserved sequences of CCR1 and the IL8 receptors alpha (IL8RA) on an immature basophilic cell line, followed by screening of a spleen cDNA library, Power and colleagues isolated a cDNA encoding CCR4 [133]. The deduced 360-amino acid CCR4 protein contains 3 potential N-glycosylation sites and multiple potential phosphorylation sites. Northern blot analysis detected a 4.0-kb CCR4 transcript at high levels in thymus and peripheral blood leukocytes and at lower levels in spleen; no expression was detected in prostate, testis, ovary, small intestine, and colon. RT-PCR analysis detected CCR4 expression in stimulated and unstimulated T and B cells, basophils, and monocytes but not in T-cell lines or pulmonary macrophages. Hence in the future, this work will be necessary to characterize postreceptor signaling pathways in order to define the precise function of these receptors in different leukocyte populations and their relevance in inflammatory diseases.

An early report in 1998 showed that polarized Th1 cells preferentially express CXCR3 and CCR5. In contrast, Th2 cells preferentially express CCR4 and, at least

in a subpopulation of Th2 cells, CCR3. It is found selective expression of CCR3 by cells of the Th2 subset. However, the extremely low level of expression, as compared for example with CCR4, may reflect a minority of positive clones in the Th2 population. Consistent with receptor expression, MIP-1 α (CCR1 agonist) and MCP-1 (a selective CCR2 agonist) showed comparable chemotactic activity for Th1s and Th2s. In contrast, MDC (19; a selective CCR4 agonist) was at least 10 times more active on Th2s versus Th1s, whereas MIP-1 β (CCR5) and IP-10 (CXCR3) were more active on Th1s. Eotaxin (a selective CCR3 agonist) was inactive or weakly active only on Th2s [17].

The ligand of CCR4 in first found in 1997. Thymus and activation-regulated chemokine (TARC) is a previously identified CC chemokine that is expressed constitutively in thymus and transiently in stimulated peripheral blood mononuclear cells. By receptor binding analyses with secreted alkaline phosphatase (SEAP) and used it for specific binding, Imai et al. showed that CCR4 is a specific receptor for TARC-SEAP. Besides that, macrophage-derived chemokine (MDC) is a recently identified member of the CC chemokine family, which contains 37 % identical amino acids similar with TRAC, and Imai et al. [74] showed that CCR4 is a specific receptor for MDC. They confirmed that MDC is also a functional ligand for CCR4. Since MDC and TARC are both expressed in the thymus, one role for these chemokines may be to attract CCR4-bearing thymocytes in the process of T-cell education and differentiation. By northern blot analysis, they detected CCR4 expression in human T-cell lines and peripheral blood T cells, but not in B cells, natural killer cells, monocytes, or granulocytes [75, 73].

2.4.4 S1P Receptor Signals as Treat Target

Among all the chemokine signaling, there is an overwhelming one sphingosine-1-phosphate (S1P), S1P is formed by phosphorylation of sphingosine, a major component of all sphingolipids, the production of S1p depends on a reaction catalysed by two isoforms of sphingosine kinase, SPHK1 and SPHK2, which have distinct and overlapping functions [96, 119]. The expression of SPHK1 is depend on the activation from numerous stimuli, including pro-inflammatory cytokines, and promotes the formation of S1P, while SPHK2 is ubiquitously expressed [100]. The level of S1P is dynamically and tightly regulated by kinase and lyase of S1P, the collaboration of these two enzyme in most tissues keep S1P concentration stable in low [148], while in erythrocytes and platelets, the lack of S1P lyase made these two kind of cells the main source of S1P in the body [125]. This difference in producing and maintaining S1P cause the concentration gradients of S1P in blood and tissues, especially in the part with wound, inflammation or infection, and this gradients is very important in directing immune cells to migrant to where they are needed [24, 128, 130, 137, 147].

The regulation of immune cells movement is precisely controlled by S1P receptor expression [151]. S1P receptor is specifically expressed in divergent

immune cells, in CD4⁺ T cells, S1Pr1 (sphingosine-1-phosphate receptor 1), and S1Pr4 (sphingosine-1-phosphate receptor 1) are primarily expressed [77, 168]. S1P receptors are all GPCR (G protein-coupled receptors). S1Pr1 and S1Pr4 play the key role in driving naive CD4 T-cell egress out of thymus and migrate into secondary lymph node after their maturation [151]. During the activating, S1Pr1 and S1Pr4 quickly down regulated under TCR (T-cell receptor), which keeps naive T cells stay in the lymph node, waiting the APC (antigen presenting cell) with the pathogen to activate them [59]. Then S1Pr1 and S1Pr4 re-expressed with their transcript factor KLF2 (kruppel-like factor 2) [174], driving effector T cells to lesion tissues. The mechanism of S1Pr1 and S1Pr4 stay uncovered until recently, Li and colleagues found that ECM1 (extracellular matrix protein 1), which is specifically expressed in Th2 cells, is responsible for KLF2 and S1Pr1 revival in Th2 cells, through interacting with CD122, IL-2 receptor β , and blocking late IL-2 signals [91]. These results showed that Th2 cells are under significant control of IL-2 signals, not only in the early differentiation, but also in the late activation.

S1Pr1 is first named as EDG1 in 1998, in a Science paper [87], Lee and colleagues showed EDG-1 bound S1P with high affinity and high specificity. Over-expression of EDG-1 induced exaggerated cell-cell aggregation, enhanced expression of cadherins, and formation of well-developed adherent junctions in a manner dependent on SPP and the small guanine nucleotide binding protein Rho. 3 years later, another Science paper proved that cell migration toward platelet-derived growth factor (PDGF), which stimulates sphingosine kinase and increases intracellular S1P, was dependent on expression of EDG-1 [66]. With more and more findings, the importance of sphingosine-1-phosphate receptors in both science research and clinical trial will be found, and so do other chemokine receptors.

2.5 New Fields in Th1/Th2 Differentiation

Recent years are the revolutionary period for biological science discoveries, and immunology, both innate immunology and adaptive ones, also takes advantages of these progresses. Several new fields is open in CD4⁺ T helper cells research, and contributes a lot to the uncovering of total immune system network, and provide important implies in disease pathobiology and drug design. Patients with auto immune disease or allergy disease are already benefit from these discoveries. One example is high resolution profiling of histone methylations in systematically research T-cell epigenetics [13].

2.5.1 Progress in Epigenetic Research of Th1 and Th2 Cells

Histone modification in Naïve T-cell differentiation is found the key point and marker in recent years. The modification locis includes H3K27, H3K4, H3K9,

H3K14, H3K18 [106, 113]. Epigenetic modification functionally relevant changes to the structures of genome that do not involve a change in the DNA nucleotide sequence. The most common phenomena of epigenetic modification are DNA methylation and histone acetylation, these modifications results divergent gene expression and regulation [175]. Gene expression can be controlled through the action of repressor proteins that attach to silencer regions of the DNA. These epigenetic changes may last through cell divisions for the duration of the cell's life, and may also last for multiple generations even though they do not involve changes in the underlying DNA sequence of the organism; instead, nongenetic factors cause the organism's genes to behave differently, which makes epigenetic modification very important in immune systems [16], since immune cell needs quick and complicated regulation of gene expression and repression based on the same DNA nucleotide sequences [175]. Th cell differentiation involves epigenetic modification and chromatin remodeling at specific loci [182]. Epigenetic regulation includes modification of both DNA and histones, including DNA CpG methylation, histone methylation and acetylation, as well as DNase I HS induction. Epigenetic modification and chromatin remodeling play critical roles in determining specific gene expression induced by common transcription factors such as NFAT [101]. Indeed, NFAT binding to the Il4 promoter increases, whereas its binding to the Ifng promoter decreases during Th2 differentiation, possibly owing to opposite epigenetic modifications at these two loci during Th2 differentiation [182]. The large image of epigenetic research of CD4+ T helper cell is that chromatin DNA modification happened in the traditional key loci of master regulator or other important transcript factors, but also leads to much wide range, some of which do not even have function reports or any transcript products. Considering that most of chromatin DNA sequence is untranscribed, the future of epigenetic research in CD4+ T cell is bright.

2.5.2 *MicroRNA in Th1 and Th2 Cells*

Most genome of organisms are not exon, and the importance of nontranslate RNA is proved plays important roles in Th1 and Th2 cells. One recent topic of CD4+ T-cell research is MircoRNA (miRNA). MiRNAs are small, approximately 21-nucleotides RNAs [84, 95], usually target 3'UTR region of objective molecule genes, and shows a lot of function in many physiological processes. In the biogenesis of miRNA, Drosha DGCR8 and Dicer take part in cutting the pri-miRNA to pre-miRNA and mature miRNA, MiRNA is lately found quite important in immune cells, such as dendritic cells, macrophages [31], and CD4+ helper and CD8+ cytotoxic T cells. Different miRNAs target universal and unique molecules in T cells, help to regulate different Th cell develop stage. In 2005, a brief report shows a requirement for Dicer in the generation and survival of normal numbers of $\alpha\beta$ T cells, but not stable shutdown of a developmental stage-specific gene (developmentally regulated gene silencing) in the T-cell lineage [70], and suggest that

Dicer may not be required continually for heterochromatin maintenance in thymocytes. It is remarkable that Dicer appears to be dispensable for CD4/8 lineage commitment and the implementation of lineage-specific gene expression programs [28]. Other article in the same year made the mice with conditional knock out Dicer in T-cell lineage, in that case, pre-miRNA can not develop into duplex and mature miRNA. In this study, Muljo and colleagues found fourfold reduction on average in spleen of CD8+T cells and twofold reduction on average in spleen of CD4+ T cells, and there is an overall decrease of CD3+ peripheral T cells, but no changes in B220 + B cells [140]. These results suggest miRNA-deficient CD4+ T cells are lack in their ability to repress IFN- γ production and are predisposed to become Th1 cells. Even after two consecutive rounds of activation under Th2 conditions, 18 % of Dicer-deficient T cells produced IFN- γ but not IL-4, and 15 % of Dicer-deficient cells expressed both IFN- γ and IL-4. The Dicer-deficient Th2 culture behaved very differently: after 5 d in Th1 growth conditions, 87 % of the remaining cells were IFN- γ +IL-4- Th1-like cells, and nearly all of the IL-4cells also expressed IFN- γ . These data reinforce the notion that Dicer is required to repress the Th1 genetic program and raise the possibility that Dicer deficiency may also impair stable commitment to the Th2 lineage. Alternatively, lack of Dicer may render terminal Th2 differentiation so inefficient that a residual pool of uncommitted cells remains after 5 d of culture in Th2 conditions, and these cells expanded preferentially as Th1 cells when switched to Th1 growth conditions [111].

An early report in 2008 prove miR-17-92 is existed in CD4+ T cells, and miR17-92 transgenic mice exert completely disrupted follicular structure in spleen and lymph node, with B cell and T cell mixed, compared with separated B and T-cell zone in WT mice. Moreover, the number of T cells also increased in miR-17-92 TG mice when ages grow up. And cytokine express assay shows IL-4 and IL-10 are higher in TG mice, consisting with the antibody IgG1 and IgG2a styles. Finally, PTEN and Bim are taken as the most possible targets of miR-17-92, but not all of the phenomenal could be explained by that, implies the existence of other targets [176]. This is a typical miRNA research, shows the significant function of this small RNA regulation mechanism, regarding to almost every important aspects of Th cells, including develop, follicular formation, facilitate antibody production, proliferation, and cytokine production [14]. Comparing with protein encoded by gene exon, miRNA exhibits more vague and wide function. One reason for this is the exact mechanism of miRNA is still ambiguous, the sequence analysis of certain miRNA and its potential 3'UTR target only provide an imprecise relations between, which did not give the explanation why other gene expression with coincide mRNA region not affected by this miRNA, and why other miRNA with the same predicted binding site did not have the same function. These problems are not only in immunology research, but also in every aspect miRNA involved in. The miRNA research is just on its beginning; in the future, after precise work mechanism of miRNA is uncovered, the answers, as to how miRNA work in immune systems, can be found.

2.5.3 *LnRNA in Th1 and Th2 Cells*

The last but not least important field is the found of long noncoding RNA (lnRNA). For a long time, lnRNA is not considered as a key role in physiological processes, but in recent years, it is proved not true [38]. LnRNA lacks of conservation between species, which implies its evolution-trend, and may execute important functions in higher species [131, 132]. But the real estate of lnRNA in immunology is still in mist and far from understood. Even the unique definition and database of lnRNA is waiting to be reached. Even so, some research work has already pulled the lnRNA out of the starting line. In 2011, RNA sequence technologies and relevant computer methods have helped to discovery and define tens of thousands of human lnRNAs [18], which provide the basic database and methods for lnRNA research. Fortunately, with these high throughput ways, the foundation of lnRNome is preliminarily built within a few years, compared with the tens of years for human genome program. Another paper reveals that similar to the way of protein-coding RNAs production, lnRNAs are generated through classic pathways, with similar histone-modification profiles, splicing signals, and exon/intron lengths. On the other side, different from protein-coding RNAs, lncRNAs display a striking preference toward two-exon transcripts, and are predominantly localized in the chromatin and nucleus sites, with a fraction appear to tend to processed into small RNAs. This character implies lnRNAs are much easier to degrade, thus under stronger selective pressure and display levels of selection comparable to protein-coding genes. Comprehensive analysis of their expression in multiple human organs and brain regions shows that lncRNAs are generally lower expressed than protein-coding genes, and display more tissue-specific expression patterns [38, 39]. This tissue specific expression is also confirmed in divergent CD4+ T helper cell subsets [69]. Within the same study, 1,524 genomic regions expressing lincRNAs in 42 samples from T cells at various developmental and differentiation stages and identified, and Hu and colleagues found that the lincRNAs were much higher stage specific or lineage specific, than mRNAs. This work is the foundation of lnRNA research in CD4+ T cells, and serves as a resource for the study of transcriptional regulatory networks during T-cell development and differentiation by comparison of the existing dynamic expression of genes encoding proteins, including transcription factors, cell surface markers and signaling molecules, with the help of these combination. Some findings related lnRNA and epigenetic modification of key cytokines gene loci of CD4+ T helper cells [55]. A series of significant findings of lnRNA and key molecule can be expected in the development, differentiation, and immune responses of T cells (Fig. 2.2).

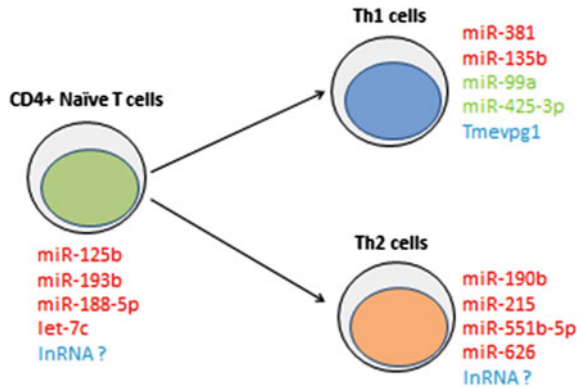


Fig. 2.2 Th1/2 cells differentiation and their micro-/lncRNA signatures. A schematic Th1/2 cells differentiation map is shown and signatures. MicroRNAs in red (overexpressed) and green (downregulated) are the ones with the strongest subset-specific expression pattern [123]. Tmevpg1 in blue is a long intergenic noncoding RNA has the influence on the Th1 cell [30]. More functional lncRNAs are still undiscovered

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