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Review

Phenotypic and functional features of CD4+ T helper cells subsets

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ABSTRACT

CD4+ T helper cells represent an important cell population of immune system that render help to B cells. Their specialised cytokine-mediated effector functions help to shape the adaptive immune response. Depending on the environmental cytokine, which in turn is determined by the antigenic signal, CD4+ T helper cells is differentiated to different T helper cell subsets. Although, there is no discrete distinction between the CD4+ T helper subsets, reasonably distinct subsets were identified and characterised. In this review, we discuss the functional and phenotypic characteristics of the well-characterised CD4+ T helper cells subsets.

KEY WORDS: CD4+ T helper cells; Cytokine; B-lymphocytes.

INTRODUCTION

The human immune system is a complex system with the principal functions of recognising and subsequent elimination of foreign bodies (antigens). Immune system may also develop immunologic memory to some antigens as well as tolerance to self antigens. Defence against infectious agents is mediated by the innate (natural) immune system and the acquired (adaptive) immune system. The two immune responses work together to fight an infection with the innate immune system appearing early to fight the microbes while adaptive immune system is engaged later. The effector functions in the immune system are performed by various types of leucocytes. Cells of the myeloid lineage which consist of monocytes, macrophages, neurophils, eosinophils, basophils, mast cell and dendritic cells as well as natural killer (NK) cells are what mediate innate immune response, while the adaptive immune response is mediated by bone marrow-derived lymphocytes (B-lymphocytes or B-cells) and thymus-derived lymphocytes (T-lymphocytes or T-cells) both of which constitute the cells of lymphoid lineage. B-lymphocytes differentiate and mature to plasma cells which produce antibodies, a soluble substance, hence confer humoral immunity. T cells render help and regulatory functions in the adaptive immune response.[1] Based on the co-receptors for the major histocompatibility complex (MHC) molecules, T cells population is subdivided into CD8+ T cells (T cytotoxic cells) and CD4+ T cells (T helper cells) which are associated with MHC class-I and class-II respectively.[2,3] T helper (Th) cells are further divided into subsets based on the specific cytokines they produce, termed as signature cytokines and the expression of transcription factor.[4] Here we describe the phenotypes and the functions of these CD4 T helper cells subsets.

CD4+ T HELPER CELL SUBSETS

CD4 T helper cell becomes activated upon it encounter with an antigen presented by class II MHC molecule of antigen presenting cell (APC) and the receipt of co-stimulatory signal transduced by co-stimulatory molecules (CD28) on its surface. Depending on the cytokine signals it receives, it is differentiated to a distinct T-helper subset.[5] Therefore, the functional status of T helper cells al-

lows us to distinguish and group them into subsets. Each subset is induced by and produces distinct signature cytokines, express unique phenotype and programme by specific transcription factors.[6] Figure 1 summarises the properties of the well-studied and established T helper cells subsets.

Th1 AND Th2 cell SUBSETS

T helper cells subsets were first described by Mossman and colleagues in mono-specific assays to evaluate cytokines synthesis in two clones of mouse T helper cells.[7] The results revealed that one clone called Th1 produces interferon gamma (IFNγ) and interleukin-2 (IL-2), which are not produced by the other clone called Th2 but instead produced IL-3 and growth factors for mast cells and T cells that are distinct from IL-3 and IL-2, respectively. Th1 cells express IL-12Rβ2, an IL-12 receptor that is induced by T cell receptor (TCR) activation and maintained by IL-12 and IFNγ stimulation.[8,9] IL-4 blocks IL-12 signaling through inhibition of IL-12Rβ2 expression.[8] This provides an important switch point for Th2 pathway engagement. The differentiation and expression of chemokine receptors in T helper cells is not strictly coordinated, however CXCR3 and CCR5 are expressed preferentially on Th1 cells.[10] For Th2 cells, the main surface marker is IL-33Rα (T1/ST2). Also IL–4Rα is shown to be up regulated by IL-4 during differentiation. The chemokine receptors that are expressed on Th2 cells include CCR8, CCR4, CCR3 and CRTh2.[10-12] In human, T-bet and (STAT4 and STAT1 in mice) are the transcription factors that coordinate the differentiation of the Th1, whereas for the Th2 GATA3 and (STAT6 and IRF4 in mice) are the master regulator genes.[13] Since the identification of the Th1 and the Th2 cell subsets by Coffman and colleagues, the concept of T helper cell subsets was restricted to these two subsets, until 2005 when Th17 was discovered.[14]

It is believe that naïve CD4+ cells differentiate to Th1 in presence of IL-12 and produce IFNγ, IL-2, tumour necrosis factor alpha (TNFα) and lymphotoxin, whereas Th2 differentiation is induced by IL-4 and the th2 cells secrete IL-4, IL-5, IL-10, and IL-13.[1,2,4,5] Th1 and Th2 cells have antagonistic relationship, specifically IFNγ *in*hibits Th2 development while IL-4 on the other hand inhibits Th1 differentiation.[15] This is how the immune system controls the numbers of these cells base on the antigen and the environmental cytokine melieu in a specific immunological response.

The Th1 cells participate in pro-inflammatory cell-mediate immunity and phagocyte- dependent protective response.[4] IFNγ *is* found to be associated with stimulation of dendritic cells and macrophages to secret IL-12 and activate macrophages and natural killer (NK) cells which kill intracellular bacteria such as *Mycobacterium tuberculosis* and *Listeria monocytogen* and viruses.[16] TNFα activates neutrophils which induces inflammation.[2] Th1 cells were also found to induce delayed-type hypersensitivity, and help B cells in production of opsonising isotype

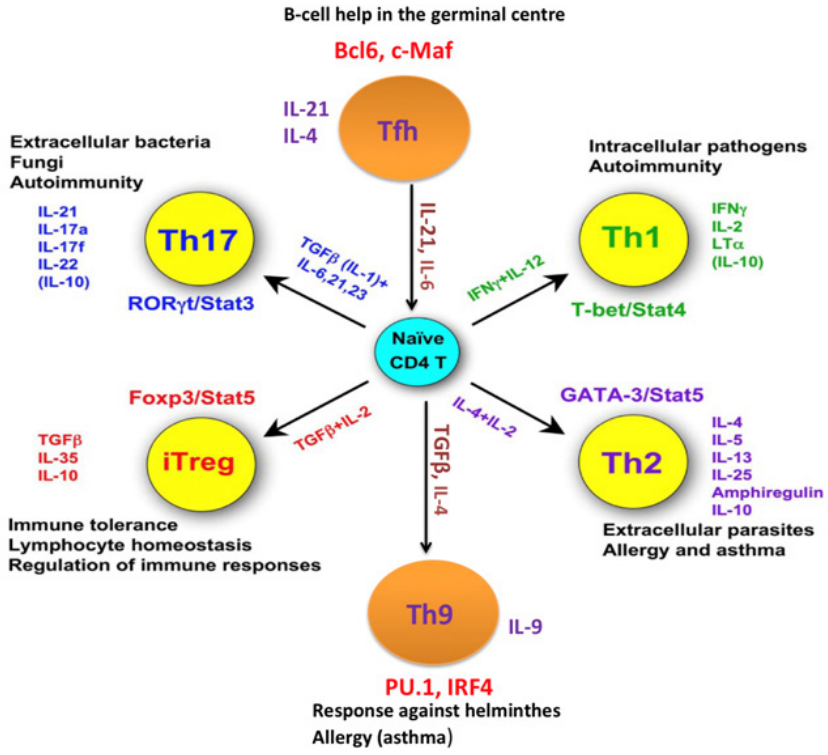

Figure 1: Diagrammatic summary of CD4+ T cell subsets. All the subsets originate from naive CD4+ T cells, the subsets are represented by coloured cycles with their designation inside the cycles. The transcription factors are on or below the cycles, cytokines they produced are by the sides and the function is written in black ink by the side of each subset. The arrows from the origin represent the pathway of the differentiation with the stimulating molecules by the sides.

immunoglobulin G, which mediate response to some protozoa such as *Trypanasoma.*[4] Th1 cells were found to be responsible for the autoimmune induction.[1]

Th2 cells on the other hand mediate non-inflammatory immediate immune response. They mainly provide help to B cell, which enables them to produce some antibodies like IgA, IgE and some IgG subclasses; and activate eosinophils which is associated with allergy. Th2 cells inhibit several functions of macrophages, hence confer non- phagocytic immune response.[16] Because antibodies are involved, therefore Th2 cells protect against extracellular parasites such as gastrointestinal nematodes.

Th17 CELLS

A study revealed that IL-17 production in activated CD4+ T cells is significantly increased in response to IL-23.[17] The IL-23 that is produced by activated dendritic cells, acts on memory T cells to up regulate the IL-17 secretion and expression of related cytokines, IL-17F. Like IL-12, IL-23 is a heterodimeric molecule each constituting a p40 subunit and p35 or p19 subunits, respectively.[18] These suggest the differentiation of a new T helper subset, with features distinct from the well characterised Th1 and Th2. Newer study showed that both IFNγ and IL-4 inhibit IL-17 producing T cells differentiation hence block IL-17 secretion.[19] The IL-17 producing T helper cells were further characterised by other studies and named Th17 as the third subset of T helper cells.[14,19] RORγt is identified as the transcription factor that triggers the transcription of the genes encoding IL-17 and related IL-17F in naïve CD4+ T cells, which are subsequently differentiated to Th17 in human.[20] In mice STAT3 or IRF4 in addition to the RORγt may serve as the master regulator. RORγ t-deficiency in mice T-cells lead to attenuated autoimmunity and lack of tissue infiltrating Th17 cells.[20] In terms of cytokine receptors, Th17 cells express IL-23R in high level and substantial amount of IL-1R1. Among the chemokine receptors, human Th17 express CCR4 and CCR6.[1] The identification of the Th17 subset breaks the Th1 and Th2 subset dichotomy of T helper cells and emphasise the existence of other T helper cell groups. However Mossman and Coffman raised the question of total diversity of T cell as far back as 1986 in the study that they identified Th1 and Th2.[7]

Th17 cells play very important role in responses against extracellular infections of bacteria and fungi.[21] They are first subset of T cells that are generated during an infection.[4] Epithelial cells, fibroblasts and keratinocytes express IL-17 receptors which on contact to IL-17 produce cytokines and chemokines that attract neutrohils and macrophages to the infection site.[4] Th17 found to be associated with the organ specific autoimmune diseases.[1]

Tfh CELLS

Follicular helper T (Tfh) cells were initially described as another distinct subset of T helper cell population in 2000 and 2001, when different studies reported that a number of CD4 T in the follicular area of lymphoid tissue possess unique phenotype, expressing high levels of chemokine receptor, CXCR5 and IL-21 secretion.[22,23] T cell that is deficient in Bcl6 was shown to be unable to develop into Tfh cells and could not sustain germinal center response, while it forced expression markedly induce the expression of tfh signature receptors, CXCR5 and CXCR4.[24] Thus, Bcl6 was suggested as the master transcription factor that regulates the Tfh cells differentiation. Also c-Maf was also found to be associated with regulation of the Tfh cells differentiation.[25] Although, the mechanisms by which the Bcl6 drives the Tfh cell differentiation is not yet completely elucidated.[26] Two mechanisms were proposed: Bcl6 can inhibit the differentiation of CD4+ T cells intoTh1, Th2 or Th17 by suppressing their transcription factors, thereby favouring Tfh cell differentiation indirectly.[26-28] or Bc16 inhibits terminal CD4+ T cell differentiation by suppressing Blimp1, thus indirectly favours the Tfh cells differentiation again.[26,27] Constitutive expression of Blimp1 by CD4+ T cells suppresses the expression of Bcl6.[27] This idea together with that of the first proposed mechanism by which Bcl6 regulate the Tfh cells differentiation, suggest an antagonistic relationship between the Bcl6 and Blimp- 1, hence provide the basis that control the CD4 T cells differentiation to the Tfh subset or other subsets (Th1, Th2 and Th17).

The primary function of Tfh cells is to provide IL-6 and IL-12 mediated help to B cells, in which way they are required for the eradication of pathogens and successful protection by vaccination.[22,25,29] During development, Tfh cells loss CCR7, the T cells zone-homing chemokine receptor; and express CCR5. Therefore, this allows mature Tfh cells to relocate from T cells zone to the B cells follicles, where they interact with and help the B cells.[25] Like any other effector cells, Tfh cells express a unique combination of effector molecules that are required for their functions and development. This includes high level of surface receptors e.g., inducible co-stimulator (ICOS which is a member of CD28 family), CD40L, BTLA, CD84, OX40 and PD-1; transcription factors and the IL-12 cytoplasmic adaptor.[13,25]

REGULATORY T (Treg) CELLS

Regulatory T (Treg) cells areconsidered as a subset of CD4+ T cells, which are important controlling pro-inflammatory and anti-inflammatory responses.[30,31] Treg cells are grouped into two functionally similar subsets base on the location of their differentiation and Foxp3 expression: Induced T regulatory (iTreg) cells which arise from the peripheral CD4+ CD25+ POXP3-T cell and natural T regulatory (nTreg) cell that are formed in the thymus with FOXP3 already expressed.[13,30,32] FOXP3 is the transcription factor that drives the specific differentiation of iTreg cells.[33,34] The expression of the FOXP3 is induced by TGFβ1 and IL-2 (Schmitt and Williams, 2013). In addition to FOXP3, other transcription factors such as Smad2 and Smad3, which are activated through TGFβ signalling pathway, contribute to the differentiation of iTreg cells.[35,36] Smad3 up regulates the expression FOXP3 and blocks RORγt, thus enhances iTreg development and inhibits Th17 differentiation, respectively. Also a study showed that Smad2 and Smad3 could induce FOXP3-independent iTreg differentiation.[37]

An intact and healthy immune system is the function of its ability to discriminate between self and non-self antigens. Failure in this critical function of immune system results to autoimmune disease, a condition where an immune system recognises self antigens as non-self, and hence destroys them. Treg cells are found to mediate this function, thus they play key role in immune tolerance and homeostasis.[32] Takahashi, *et al.* findings which revealed that elimination of CD25+ CD4+ T cells present in mice is associated with the spontaneous development of various autoimmune diseases,[38] lead to various studies including the one which reported that CD25+ CD4+ POXP3+ (nTreg) cells inhibit the development of autoimmune disease.[39] However, till date the molecular mechanism by which Treg cells control the effector function of lymphocyte is not completely clear.

Th9 CELLS

T helper 9 (Th9) cells where initially characterised as a subset of Th2 cells, as the data from early studies associated IL-9 production, which is the signature cytokine produced by the Th9 to Th2 cells.[13,40] Transforming growth factor-β, which is a cytokine induces the differentiation of Th17 and iTreg, singly or in combination with IL-4 redirect th2 cell to lose their distinct characteristic profile and switch to IL-9 production.[41] Mast cell and eosinophils were also reported to produce this cytokine.[42] Schmitt et al reported that CD4+ T cells are strongly stimulated to produce IL-9 by TGFβ and enhance further by IL-4 which, alone has minimal influence. They further showed that IL-2 is essential for IL-9production; and CD4 T cells isolated from IL-4 knockout mice produced elevated level of IL-9 in presence of TGFβ, suggesting that (TGFβ + IL-2) mediated IL-9 production is independent of IL–4. They also reported that IFNγ suppresses the IL–9 secretion probably by neutralising the effect of IL-4. These findings were supported by many current studies. It was found that addition of IL-21 significantly elevate intracellular IL-9 concentrating and also similar effect of IL-1 was observed in mice and human CD4 T cell culture.[40] The fact that the TGFβ diverts CD4 T cells that are programmed to ward Th2 pathway to an IL-9 producing one,[41] probably form the basis for the earlier believe that Th9 cells population is a subset of Th2 cells.

The existence of Th9 cells as a distinct subset of CD4 T helper cells was further characterised and established by the identification of interferon regulatory factor 4 (IRF4) and PU.1 which serve as the master regulators in the Th9 lineage differentiation.[40] Staudt et al showed that, CD4 cells which are IRF4 – deficient did not differentiate to Th9 cells.[42] Similarly, Chang et al demonstrated that mice with PU.1 – deficient T cells presented response similar to the normal Th2 response in vivo and showed attenuated allergic pulmonary inflammation that mimic the response produced due to low expression of IL-9.[44]

The IL-9 produced by Th9 cells were found to participate in the immune response against helminthes and are involve in the pathological process of allergy, particularly asthma. However, IL-9 deficiency does not affect the allergic reaction development in respiratory tract.[40]

UNCHARACTERISED CD4+ T HELPER CELLS POPULATION

Different research groups reported the existence of a number of CD4 T helper cells with so-called distinct properties and proposed them as distinct CD4 T helper cell subsets. Kurowska-Stolarska et al reported that a newly identified IL–33 derives the differentiation of CD4 cells to a population which mainly produce IL–5 and IL–13 but not IL-4.[45] This population contrast the well-characterised Th2 cells in that it produces different signature cytokine IL-5 rather than the IL-4 produced by Th2 cells. However no any molecule yet identified to be the transcription factor that regulates the differentiation of this IL-5 producing cell population. Also Eyerich et al reported CD4 cell population which they designated Th22 cells as they are characterised by IL–22 secretion. In addition to IL–22 this population of cells were demonstrated to produce IFNα,[46] but not IFN*γ, IL*–4 or IL–17 which are the signature cytokines for Th1, Th2 and Th17 cells respectively. For this reasons Eyerich and his colleague proposed Th22 as a subset of CD4 T helper cells. However, also no transcription factor was identified to be associated with the Th22 cells differentiation.

CONCLUSION

Generally, CD4+ T-helper cell is a complex cell population. Although, some cells within the population possess similar attributes, which distinguish them from other cells of the population, hence classified as a subset of the T helper cells. However different subsets may shear some properties such as production of similar cytokines; and products of one subset may inhibit or even stimulate the differentiation of other subsets. Therefore, there is no absolute distinction between the T helper cell subsets.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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