Review of the Diversity, Differentiation and Physiological Function of "CD4+ T cells" in Animals

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ABSTRACT— The $CD4^+$ T cells are central orchestrators of adaptive immunity, playing critical roles in coordinating immune responses against pathogens, maintaining tolerance, and regulating inflammation. Naïve $CD4^+$ T cells, which haven't yet come into contact with their particular antigen, undergo differentiation into distinct effector subsets upon recognition of their cognate antigen presented by "MHC class II molecules". This differentiation process is strongly influenced by the surrounding cytokine milieu, co-stimulatory signals, and additional environmental cues. In vivo, the cytokine environment is highly complex and dynamic, often comprising overlapping, redundant, or even opposing signals. Nevertheless, well-defined T helper (Th) lineages can be generated in vitro through controlled cytokine-mediated polarization of naïve $CD4^+$ T cells, providing a powerful platform to investigate the molecular mechanisms that regulate T-cell fate decisions. Classic T helper subsets include "Th1, Th2, Th17, T follicular helper (Tfh), and regulatory T cells (Treg)" each defined by distinct transcription factor networks, cytokine profiles, and effector functions. "T helper-1 cells" are primarily involved in cell-mediated immunity against intracellular pathogens, Th-2 cells support humoral responses and defense against helminths, Th17 cells orchestrate mucosal immunity and inflammation, T-fh cells "promote B-cell maturation and antibody production" and Treg cells maintain immune tolerance and prevent autoimmunity. Recent advances in single-cell genomics, epigenomics, and proteomics have revealed that $CD4^+$ T-cell differentiation is highly plastic, with the potential for hybrid or intermediate phenotypes, reflecting the dynamic adaptability of these cells to changing environmental and immunological contexts. Animal models have been instrumental in elucidating the diversity, differentiation, and functional plasticity of "$CD4^+$ T cells" providing insights that are relevant to human immunity and the development of immunotherapies.

***Keywords* —** naïve T cells, CD4+ T cells differentiation , T helper subsets.

INTRODUCTION

Helper T cells" also known as CD4+Tcells, are crucial regulator of adaptive immune responses that are antigen specific and essential for defending animal against infections by pathogens. While humoral immune responses or antibodies (made by B cells) are largely in charge of controlling extracellular pathogens like the majority of bacteria and parasites, antigen-specific CD8+Tcell responses are largely responsible for controlling intracellular pathogens like viruses. The foundation for determining CD8+Tcell and antibody responses is CD4+Tcells (1). In order to produce an efficient antibody response and to support "cytotoxic T-cell" mediated defense against pathogen invasion, "$CD4^+$ helper T cells" are crucial for immune response coordination (2) "T-helper and regulatory T-cells" are two subtypes of CD4+Tcells, which are important modulators of adaptive immune system. T-helper cells change from a naive state to one of several effector subtypes with specific roles during an immune response. It is unclear what transcriptional processes underpin CD4+ T cells' distinct functional identity (3). To prevent phagosomal infections, support B cells, maintain tissue homeostasis, repair, or carry out immune regulation, specific subpopulations of CD4 T cells recognize and evaluate major histocompatibility complex class II–peptide complexes (4). The two main T cell subtypes that are crucial organizers of the adaptive immune system are CD4+ T helper (Th) cells and regulatory T (Treg) cells (5). The efficient Effective Th-2 regulates extracellular pathogens like the majority of parasites, while Th1 regulates intracellular pathogens like viruses (6). The majority of Th cells are naive and only develop into mature effector cells that secrete cytokines when antigen activates "T-cell receptor TCR" in presence of cytokines (7). Each T helper (Th) cell subtype performs specialized immune functions that correspond to its unique pattern of cytokine expression (8). Several Th effector subsets have been identified, with the best characterized being Th1, Th2, and

Th17 cells. T helper-1 cells are defined by the production of "IFN-γ" whereas Th2 cells primarily secrete "IL-4, IL-5, and IL-13. Th17 cells" are distinguished by their production of IL-17A, IL-17F, and IL-22 (9). In comparative studies of aging, stimulated $CD4^+$ T cells from older individuals exhibited markedly higher peak transcript levels of "IL-3, IL-4, IL-5, and IFN-γ. However, their peak expression levels of IL-2, TNF-α, and TNF-β" were comparable to those observed in young adult controls. (10). peak IL-2 levels were similar across the different age groups, consistent with cytokine secretion patterns observed later in culture (24–72 hours). In contrast, the elderly group demonstrated a markedly greater capacity to produce IL-3, IL-4, IL-5, and IFN-γ (11).

CD4+T CELL DEVELOPMENT

The T cells are essential for preserving immunological function. preserving health, and preventing disease. They develop in the thymus through a gradual maturation process, resulting primarily in the generation of "$CD4^+$ T cells and $CD8^+$ T cells". T-cell development begins when bone marrow derived thymic seeding progenitors (TSPs) enter thymus, where they progress through distinct developmental stages: the double-negative DN; $CD4^-CD8^-$ stage, the double-positive DP; $CD4^+CD8^+$ stage and finally the "single-positive SP; $CD4^+CD8^-$ or $CD4^-CD8^+$ stage (12,13).To control phagosomal infections, support B-cell responses, maintain tissue homeostasis, facilitate tissue repair, and regulate immunity, specialized subpopulations of "$CD4^+$ T cells" recognize and interact with major histocompatibility complex class II (MHC II)–peptide complexes (14).The generation of mature, functional T cells is governed by the coordinated actions of transcription factors and cytokines, which regulate T-cell receptor (TCR) gene rearrangements, cellular proliferation, lineage restriction, and the selection processes that shape the T-cell repertoire (15). T cells develop into a diverse population of effector T cells that can mediate pathogen clearance after infection. A portion of these effector T-cells have the capacity to endure over an extended period of time and develop into memory T cells, which are capable of delivering sustained immunity. Designing vaccines that can induce T cell-based immunity requires a knowledge of the signals that control memory T cell development (16). CD4+ T cells play a crucial role in the development of protective immune memory. Following infection or vaccination, CD8+ T cells require signals and support from CD4+ T cells to properly differentiate into long-lived memory cells capable of mounting a rapid response upon re-exposure to the pathogen (17). Memory CD4+Tcells are found all over the body and play a role in chronic inflammation, graft rejection, autoimmunity, and allergy in addition to protecting the tissues from cancer and reinfection (18). The naïve CD4+Tcells respond to cytokines by developing an effector phenotype, which helps them coordinate the immune response. However, little is known about memory T cell cytokine responses (19).

T helper cell subsets

Recent advances in single-cell genomics, proteomics, and high-dimensional bioinformatic analysis have challenged the traditional classification of "T-helper cells" into the classic "Th1, Th2, and Th17" subsets. "$CD4^+$ T cells" as central regulators of the adaptive immune response, exhibit a remarkable degree functional specialization. Decades of research have identified five major $CD4^+$ T helper subsets: "Th1, Th2, Th17, Treg (regulatory T cells), and Tfh (follicular helper T cells)" (20). Th-1 cells mediate type 1 immunity against intracellular pathogens like viruses and mycobacteria by producing interferon-γ and expressing the master transcription factor T-bet. Conversely, Th2 cells are distinguished by the release of "IL-4, IL-5, and IL-13" and the expression of the lineage-determining transcription factor GATA-3 (21). Parallel to these adaptive subsets, innate lymphoid cells "ILCs" have been identified and classified into three major groups "ILC1, ILC2, and ILC3" each corresponding functionally to the "Th1, Th2, and Th17" effector lineages, respectively (22). The differentiation of naïve "CD4- T cells" toward the Th-1 lineage is driven by TCR activation in a defined cytokine environment. IL-12, created by antigen-presenting cells "APCs" activates STAT4, while interferon-γ created by NK cells and/or "T cells activates STAT1". The combined activation of "STAT1 and STAT4" promotes the expression of T-bet, the master regulator required for stabilizing and committing cells to the Th1 phenotype (23). In the tumor microenvironment, CD4+Tcells and the substances they secrete are also essential because they can coordinate immune responses that are both pro- and anti-tumor (24).

CD4+ T CELLS REGULATE ADAPTIVE IMMUNITY.

After leaving the thymus, naive CD4+Tcells examine secondary lymphoid tissues for pathogen-derived antigens that are displayed by "APCs". In order to create an infection in the local tissues, pathogens must penetrate the host's defenses (physical, chemical, etc.) (25). By attracting immune cells like neutrophils to the infection site, the host's immune system starts an inflammatory response that releases inflammatory cytokines and chemokines (26). These chemokines send out signals to bring more APCs to the infection site. APCs use their "pattern recognition receptors" "PRR" to identify "pathogen associated molecular patterns" "PAMP" on invasive pathogens in order to continuously search for them (27). Once the pathogen has been identified, "APCs" engulf it, break it down into tiny peptides, and then present the peptides to "CD4+Tcells" in the secondary lymphoid tissue. The initial activation signal is produced when the "TCRs" on the "naïve CD4+Tcells" recognize this peptide "MHC-II complex." (28). The second activation signal is produced concurrently by costimulatory molecules on "CD4-T cell surface" such as "CD28" which identify their matching ligands on Antigen Presenting Cell surface, such as "CD80 or CD86" (29). A vital component of the adaptive immune system are CD4+ T cells. They support important immune processes like "Cytotoxic CD8+T cells" developing into functional memory T cells and assisting in the production of B cell antibodies (30**).**

Response of "Cd8+ T Cells" to Intracellular Pathogens Is Coordinated By Th1 Cells.

The CD8+Tcells are a crucial component of adaptive immunity that aids in the removal of intracellular infections and offers sustained defense. These functions are primarily

performed by the "cytotoxic T lymphocytes" The most characterized subpopulation of CD8+ T cells (31), because they can release cytokines like TNF-α and interferon-γ and kill infected cells. However, there is mounting evidence that, in the context of allergies, autoimmunity, and infections, different CD8+Tcell fates impact CD4+Tcell mediated responses (32). Therefore, similar to CD4+Tcell subpopulations, CD8+Tcells can also express IL-4, IL-5, IL-9, IL-13, IL-17 or suppressive activity under specific circumstances, which can affect immune responses. Co-stimulatory molecules, cytokines, and the course of CD8+ T-cell differentiation is determined by the antigen's strength (33). In response to intracellular pathogens during infection, antigen-presenting cells such as "macrophages and dendritic cells" produce cytokines like IFN-γ and IL-12, which further polarizes CD4+ T cells into a Th1 subtype (34). The transcription factor T-bet, which controls Th-1 differentiation in activated "naïve CD4+T cells" is expressed more when IFN-γ and IL-12 are present (35). In particular, these cytokines activate transcription factors STAT-1 or STAT-4 when they bind to their "receptors on naïve CD4+ T cells", which results in an upregulation of T-bet. Subsequently (36), T-bet increases the expression of interferon-γ by binding to the promoter region of "Th1-specific cytokine genes" and causing histone modification (37). Furthermore, by suppressing the transcription of "Th-2 specific genes", including GATA "Transcription Factor" that drives the expression of IL-4, T-bet also prevents Th2 differentiation. Therefore, Th-1 differentiation is induced by interferon-γ and IL-12, which results in the production of "IFN-γ" and the suppression of Th2 differentiation (38). Th1-produced IFN-γ can cause activated B cells to switch to the IgG subtype during infection brought on by intracellular pathogens. The species may vary in this subtype switching, though. For instance, it causes mice to produce IgG2a and cattle to produce IgG2, but humans only produce IgG1 and IgG3. These IFN-γ-induced IgG subtypes can aid in a number of processes, including Antibody-Dependent Cellular Cytotoxicity , that eliminate intracellular infections (39).

Extracellular Humoral Response to Pathogens is Coordinated By Differentiated Th2 Cells.

The "CD4+ T cells" are a crucial part of adaptive immune responses. "Naive CD4+ T cells" differentiate into at least "four types of T helper cells" "TH1, TH2, TH17, and inducible regulatory T cells" in response to "various infectious agents, commensal microorganisms, or self-antigens." TH-2 cells are crucial for the host's immunity against extracellular parasites like helminths (40). Several TH2 cell-associated cytokines are produced by the TH2 cells, including "IL-4, IL-5, IL-9, IL-13, and IL-25 also called IL-17E," by adhering to specific tissue compartments. "TH2 cells" regulate the conversion of Bcell classes to IgE by generating IL-4 (41). When extracellular pathogens cause infections, innate immune cells such as "basophils, eosinophils, and innate lymphoid cells" produce and release "IL-4" (42). The IL-4 signaling on "naïve CD4+Tcells" upregulates "GATA-3" GATA binding protein-3 a crucial "transcription factor" for Th-2 differentiation, in conjunction with the first and second signals (43). Mice lacking GATA-3, displayed compromised Th2 responses. Upon binding to its corresponding receptor on the surface of naive CD4+Tcells IL-4 triggers "STAT-6" this in turn triggers the expression of GATA-3 (44). After differentiating, Th2 cells can stimulate B cells to generate antibodies that protect the host from extracellular infections. Th2 cells provide co-stimulation via CD40L and identify "peptide MHC-II complexes" expressed on B cells during B cell activation, both of which are essential for B cell activation (45). Crucially, IL-4 signaling causes B cells to switch isotypes and subtypes in order to produce Ig-E and IgG1, two essential antibodies for regulating extracellular infections in animals (46), Antibodies are crucial in preventing infections brought on by extracellular pathogens, even though they can support CD8+ T cell responses during intracellular infections (47).

CONCLUSION

The $CD4^+$ T cells are a central component of adaptive immune system in animals, having a vital role in orchestrating immune responses and coordinating other immune cells. Their diversity and differentiation into multiple subsets, like "Th1, Th2, Th17, Tfh, and Treg" enable immune system to effectively react to numerous pathogens while maintaining tissue integrity. Recent studies, particularly those using single-cell analyses, proteomics, and high-dimensional bioinformatics, suggest that traditional Th classifications may oversimplify reality, with cells displaying a dynamic spectrum of functions rather than rigidly defined lineages. Understanding this complexity is vital for developing targeted therapies for immune-related diseases, including autoimmunity, infections, and cancer. Therefore, exploring "$CD4^+$ T cell" diversity and differentiation remains essential for advancing our knowledge of immune regulation and improving therapeutic strategies.

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