

CD4⁺ T-cell subsets in inflammatory diseases: beyond the T_h1/T_h2 paradigm

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Abstract

CD4⁺ T cells are crucial for directing appropriate immune responses during host defense and for the pathogenesis of inflammatory diseases. In addition to the classical biphasic model of differentiation of T-helper 1 (T_h1) and T_h2 cells, unexpected increases in the numbers of CD4⁺ T-cell subsets, including T_h17, T_h9, T follicular-helper (T_{fh}) and T-regulatory (T_{reg}) cells, have been recognized. In the present review, we focus on how these various T-helper cell subsets contribute to the pathogenesis of immune-mediated inflammatory diseases. In particular, we focus on multiple sclerosis, psoriasis and asthma as typical model diseases in which multiple T-helper cell subsets have recently been suggested to play a role. We will also discuss various unique sub-populations of T-helper cells that have been identified. First, we will introduce the heterogeneous T-helper cell subsets, which are classified by their simultaneous expression of multiple key transcription factors. We will also introduce different kinds of memory-type T_h2 cells, which are involved in the pathogenesis of chronic type-2 immune-related diseases. Finally, we will discuss the molecular mechanisms underlying the generation of the plasticity and heterogeneity of T-helper cell subsets. The latest progress in the study of T-helper cell subsets has forced us to reconsider the etiology of immune-mediated inflammatory diseases beyond the model based on the T_h1/T_h2 balance. To this end, we propose another model—the pathogenic T-helper population disease-induction model—as a possible mechanism for the induction and/or persistence of immune-mediated inflammatory diseases.

Keywords: epigenetics, memory T_h2 cells, pathogenic T_h2 (T_{path}2) cells, T-cell plasticity, T_h1/T_h2 paradigm

Introduction

CD4⁺ T cells are critical for immune responses during host defense against harmful microorganisms. In addition to their key roles as helper cells, they may play a pathogenic role as drivers of autoimmune diseases and allergies, including asthma. We herein discuss the diverse effects of T-helper cells on the pathology of immune-related disorders. Our discussion is based on two concepts: (i) the classical T-helper 1 (T_h1)/T_h2 paradigm and (ii) a new concept, about disease induction, based on recent evidence of the existence of heterogeneous memory T-helper cell subsets. We summarize the association between inflammatory diseases and the complexity of the CD4⁺ T-cell subsets, including T_h1, T_h2, T_h17, T_h9, T_h22, T follicular-helper (T_{fh}) cells and T-regulatory (T_{reg}) cells. We need to consider the impact of this complex fate decision on the multifaceted pathology of immune-related diseases.

Moreover, we review the epigenetic modifications including super-enhancers that influence cell identity, plasticity of the

T-helper cell subsets and genetic risk of diseases. The new insights are especially relevant to the development of new therapeutic strategies for immune-mediated inflammatory diseases, as we still face difficulties in treating most immune-mediated inflammatory diseases.

Beyond the T_h1/T_h2 paradigm

The T_h1/T_h2 paradigm and immune responses during host defense against microorganisms

For more than two decades, T-helper cells were thought to be limited to two major subsets, T_h1 and T_h2 cells. This paradigm is based on their production of specific cytokines (IFN- γ and IL-4, respectively) (1). The cytokines IL-12 and IL-4 are known to signal the induction of T_h1 and T_h2 cell differentiation, respectively. T_h1 cells are crucial for host defense against intracellular pathogens including *Leishmania major* and *Mycobacterium tuberculosis*, whereas T_h2 cells are

known to be important for the elimination of helminthic parasites such as *Nippostrongylus brasiliensis* (2, 3). The T_H1/T_H2 paradigm is useful for classifying the immune responses that occur in the elimination of microbial pathogens.

CD4⁺ T cell subsets and immune-mediated inflammatory diseases

The T_H1 cell subset and inflammatory diseases. IFN- γ -producing T_H1 cells have long been recognized to contribute to the pathogenicity of organ-specific autoimmune diseases such as autoimmune type 1 diabetes and multiple sclerosis (4–6). For instance, the pathogenic roles of T_H1 cells have been well described in several mouse disease models, including experimental autoimmune encephalomyelitis (EAE), which is a mouse model of multiple sclerosis (6). The adoptive transfer of T_H1 cells was found to exacerbate EAE (7). Moreover, the genetic abrogation of T-bet, which is a key transcription factor for T_H1 cell differentiation, resulted in resistance to EAE (8).

The T_H17 cell subset as a new player in the pathology of inflammatory diseases. The finding of the pathogenic role of IL-23 in EAE suggested that another T-helper cell subset, distinct from T_H1 cells, is involved in this inflammatory neural disease (9). Both IL-12 and IL-23 are heterodimeric cytokines and consist of p40–p35 and p40–p19, respectively. Mice that lack the IL-12p35 subunit have been shown to develop much more severe disease. In sharp contrast, the genetic abrogation of the p19 subunit of IL-23 resulted in resistance to EAE (9).

Around the same period, it was appreciated that the selective production of IL-17 by a distinct T-helper cell subset was induced by IL-23 stimulation (10), although the pathophysiological roles of the cytokine IL-17 in human arthritis have been in focus since the late 1990s (11). These findings led to the identification of an IL-17-producing population of CD4⁺ T cells, termed T_H17 cells (9, 12–14), and the recognition of T_H17 cells has helped to understand the contrasting findings in EAE. IL-27 is an important negative regulator of T_H17 differentiation, which also induces T_H1 differentiation (15–18). The hyper-susceptibility of IL-27R-deficient mice with elevated numbers of T_H17 cells to EAE also suggests the importance of T_H17 cells in the pathology of EAE (19).

Psoriasis, a chronic inflammatory immune-mediated disease of the skin and joints, is another example of a disease in which both T_H1 and T_H17 cells are involved (20). Patients with psoriasis develop erythematous scaly papules and plaques. Around one-third of patients develop a so-called psoriatic joint, which is sometimes accompanied by severe joint destruction (21). For more than 20 years, type 1 responses were thought to play a central role in the pathology of psoriasis, because of the presence of IL-12-expressing dendritic cells and T_H1 cells, which secrete IFN- γ and TNF (22, 23). Recently, however, accumulating evidence suggests that IL-17-producing T_H17 cells may play a crucial role in the pathology of psoriasis (20), and monoclonal antibodies that interfere with IL-17 action such as ixekizumab and secukinumab (both of which bind IL-17A) appear to be effective for reducing the symptoms of psoriasis (24, 25).

Thus, T_H17 cells have been demonstrated to have critical pathogenic roles in the pathogenesis of human autoimmune diseases and a variety of mouse models of autoimmune

diseases. T_H17 cells also contribute to the host defense against extracellular bacteria such as *Staphylococcus aureus* and *Klebsiella pneumonia* and against fungi (26, 27).

The T_H2 , T_H9 and T_H17 cell subsets and allergic diseases. The pathophysiological importance of cytokines produced by T_H2 cells, namely IL-4, IL-5 and IL-13, has been demonstrated in allergic inflammation, such as human asthma, as well as in animal models of allergic airway inflammation (28–31); the involvement of T_H2 cytokines in the pathophysiology of airway inflammation, eosinophilia, the hyper-production of mucus, fibrosis and other responses is well recognized (32–34). In addition to T_H2 cells, IL-9-producing T-helper cells, namely T_H9 cells, make up another key T-helper cell subset, which is involved in the pathology of airway inflammation (35, 36).

In addition to the pathogenic role of T_H2 and T_H9 cytokines in airway inflammation, emerging evidence suggests that T_H17 cells are also key players in chronic lung inflammation, including asthma (37–41). The massive infiltration of neutrophils in the lung is a well-known clinical feature of steroid-resistant asthma and is an important pathogenic action of IL-17; the recruitment of neutrophils to the lung during airway inflammation likely plays a key role in steroid-resistant asthma (37–40, 42–45). IL-17 also directly affects the airway smooth muscle by inducing allergen-induced airway hyper-responsiveness (40).

Recent research has highlighted that the expression patterns of the T_H2 and T_H17 signature genes in the bronchial epithelial cells of asthmatics are mutually exclusive, which suggests that the etiology of asthma is heterogeneous and indicates the reciprocal regulation of the T_H2 and T_H17 inflammatory pathways in asthma (46). Interestingly, IL-21, a cytokine that is known to be a potent inducer of T_H17 cell differentiation (47), can also promote T_H2 cell responses together with IL-25 in a mouse model of house-dust asthma (48).

T_H17 cells can also produce IL-22, which has been shown to have protective roles in the epithelial barrier functions of the lung and the gut (49). There are positive correlations between the expression level of IL-22 and the severity of allergic diseases such as asthma and atopic dermatitis (50, 51). In humans, the identification of a population of CD4⁺ T cells that produce IL-22 without the expression of IL-17, IL-4 or IFN- γ has led to the notion of ' T_H22 ' cells (52). However, recent research using a fate-mapping mouse model has suggested that, in mice, IL-22 induction might not be strongly associated with a particular T-helper cell subset (53).

Taken together, these findings indicate that the clinical features of asthma are heterogeneous (46, 54, 55), and that the involvement of several T-helper cell subsets in asthma might be one of the reasons for the heterogeneity of this disease.

The T_H cell subset and immune-mediated inflammatory diseases. T_H cells contribute to both host protection and immune-mediated inflammatory diseases by providing B-cell help and antibody production. These cells are identified on the basis of their location in the germinal centers *in vivo* and by the surface expression of the CXCR5 chemokine receptor 5 (CXCR5) and programmed cell death 1 molecules (56). T_H cells are critical, for example, for the production of neutralizing antibodies against HIV (57, 58).

T_{H} cells are also involved in various immune-related diseases. As lineage-specific cytokines including IFN- γ , IL-4 and IL-17 can be produced by T_{H} cells (56), it may be possible that T_{H} cells contribute to immune-mediated inflammatory diseases both redundantly and distinctly. For example, IgE is well known as a key player in the pathophysiology of allergies and asthma (59) and T_{H} cells are important for providing B-cell help to generate IgE-producing plasma cells. Moreover, patients with systemic lupus erythematosus (SLE) have increased numbers of IL-17-producing T cells, which stimulate B-cell antibody production and infiltrate the kidney (60, 61). The expression of OX40 ligand on antigen-presenting cells has been recently revealed to control the activity of T_{H} cells and the pathogenicity of SLE (62).

The T_{reg} cell subset as a regulator of immune-mediated diseases. In contrast to effector T-helper cell subsets, T_{reg} cells are essential for the maintenance of immunological self-tolerance and the prevention of various autoimmune diseases (63–67). The importance of immunological tolerance is particularly evident in mice and humans that are missing transcription factor forkhead box P3 (Foxp3), which drives the specification of this subset (64–67). T_{reg} cells can be divided into thymus-derived T_{reg} (tT_{reg}) cells and peripherally induced T_{reg} (pT_{reg}) cells (68); these two sub-populations of T_{reg} cells are distinguished by several markers, including Neuropilin 1 and Helios (69–71). pT_{reg} cells are known to control allergic inflammation in the lung and gut (65). Various mechanisms, such as the consumption of IL-2 by T_{reg} cells and CTLA-4 expression on T_{reg} cells, are suggested to be crucial for their suppressive activity (64, 72).

Taken together, it has become clear that CD4⁺ T cells execute host defense immune responses and that they are involved in immune-mediated inflammatory diseases through their multiple distinct fates. These new insights will provide a more sophisticated understanding of immune-mediated inflammatory diseases and new curative approaches.

Heterogeneity of CD4⁺ T-cell subsets and immune-mediated inflammatory diseases

Heterogeneity of CD4⁺ T-cell subsets and key transcriptional factors. As mentioned above, the diversity of CD4⁺ T cells— $T_{\text{H}}1$, $T_{\text{H}}2$, $T_{\text{H}}17$, $T_{\text{H}}9$, $T_{\text{H}}22$, T_{H} cells and T_{reg} cells—is now widely acknowledged (36, 56, 64, 73, 74). Extrinsic factors, in particular cytokines, are crucial for the appropriate differentiation of these CD4⁺ T-cell subsets because they activate transcription factors, including signal transducers and activators of transcription (STATs) (75). The combination of TCR and STAT signals is important for the induction of subset-specific transcription factors (75). $T_{\text{H}}1$, $T_{\text{H}}2$, $T_{\text{H}}17$, $T_{\text{H}}9$, T_{H} and T_{reg} cells express T-bet (encoded by *Tbx21*), GATA-binding protein 3 (GATA3), retinoic acid receptor-related orphan receptor γ t (Roryt), SFFV proviral integration 1 (PU.1), B-cell lymphoma 6 (Bcl6), and FoxP3, respectively (14, 76–80). Beyond these transcription factors, there are several key transcription factors for the differentiation of CD4⁺ T cells. For instance, Runt-related transcription factor (Runx3) is crucial for $T_{\text{H}}1$ differentiation and Blimp-1 (encoded by *Prdm1*) is a crucial negative regulator for T_{H} differentiation (79, 81).

At the same time, the flexibility and heterogeneity of CD4⁺ T-cell subsets have recently been described (82–84). In fact, CD4⁺ T cells can express multiple ‘subset-specific’ transcription factors. For example, it is now known that T-bet is expressed in GATA3⁺ $T_{\text{H}}2$ cells, Roryt⁺ $T_{\text{H}}17$ cells, Foxp3⁺ T_{reg} cells and Bcl6⁺ T_{H} cells (85–88).

The expression of multiple transcription factors can help to provide specialized functions to sub-populations of CD4⁺ T-cell subsets. In the case of T_{reg} cells, diverse and unique sub-populations, in which Foxp3 is co-expressed with T-bet, GATA3, Bcl6 or STAT3, work to regulate the $T_{\text{H}}1$, $T_{\text{H}}2$, T_{H} or $T_{\text{H}}17$ responses *in vivo* (87, 89–92). For example, IL-10R-expressing T_{reg} cells can respond to IL-10 signaling to activate STAT3 and are crucial for the regulation of $T_{\text{H}}17$ immune responses *in vivo* (91).

A member of the IL-1 family, IL-33 is expressed constitutively in epithelial cells and works as an alarmin in response to tissue damage (93). Colonic T_{reg} cells expressing the IL-33 receptor (ST2) can also express GATA3 and control intestinal inflammation via IL-33 signaling (92). After stimulation by IL-33, ST2⁺ T_{reg} cells show a protective role against viral infection in the lung through the induction of amphiregulin, which is a member of the epidermal growth factor family and is known to be a key protein for tissue repair (94, 95). Another type of T_{reg} cell with a unique *in vivo* activity are Roryt⁺Foxp3⁺ pT_{reg} cells, which are induced by the microbiota and vitamin A in the gut and which regulate type-2 immune responses (96, 97).

Heterogeneity of the memory-type T-helper cell subsets and organ-specific chronic inflammatory diseases. Recent technological progress has allowed us to investigate genetic expression at the single-cell level among T-cell subsets (98, 99). These investigations have shown that the populations of T-cell subsets, including memory CD8⁺ T cells, are more heterogeneous than we anticipated (99). In the case of CD4⁺ T cells, various types of effector and memory $T_{\text{H}}2$ cells are involved in the type-2 immune responses (98, 100–103).

Among these sub-populations, IL-5-producing $T_{\text{H}}2$ cells are known to contribute to the pathogenicity of immune-mediated inflammatory diseases in both humans and mice (100–107). For example, a certain population of memory $T_{\text{H}}2$ cells, which shows low expression of both CXCR3 and CD62L, plays a crucial role in the pathogenicity of chronic allergic airway inflammation, since this memory $T_{\text{H}}2$ subset, namely pathogenic $T_{\text{H}}2$ ($T_{\text{path}}2$) cells, has the ability to produce a large amount of IL-5 (100, 101). IL-33 confers the induction of memory-type $T_{\text{path}}2$ cells from effector $T_{\text{H}}2$ cells through the enhancement of ST2 expression on T cells both *in vitro* and *in vivo* (101), whereas CCR8⁺ memory-type $T_{\text{H}}2$ cells are key pathogenic players, which induce the production of high levels of IL-5 during chronic skin inflammation (102, 108). G-protein-coupled receptor 15 (GPR15) is known as a lymphocyte trafficking receptor (109). It has recently been demonstrated that CD4⁺CD45RO⁺GPR15⁺ memory-type $T_{\text{H}}2$ cells are found in increased numbers in the colon of patients with ulcerative colitis (106).

These results indicate that the heterogeneity of memory-type $T_{\text{H}}2$ cells among different organs could be crucial for the pathology of chronic immune-mediated diseases (Fig. 1).

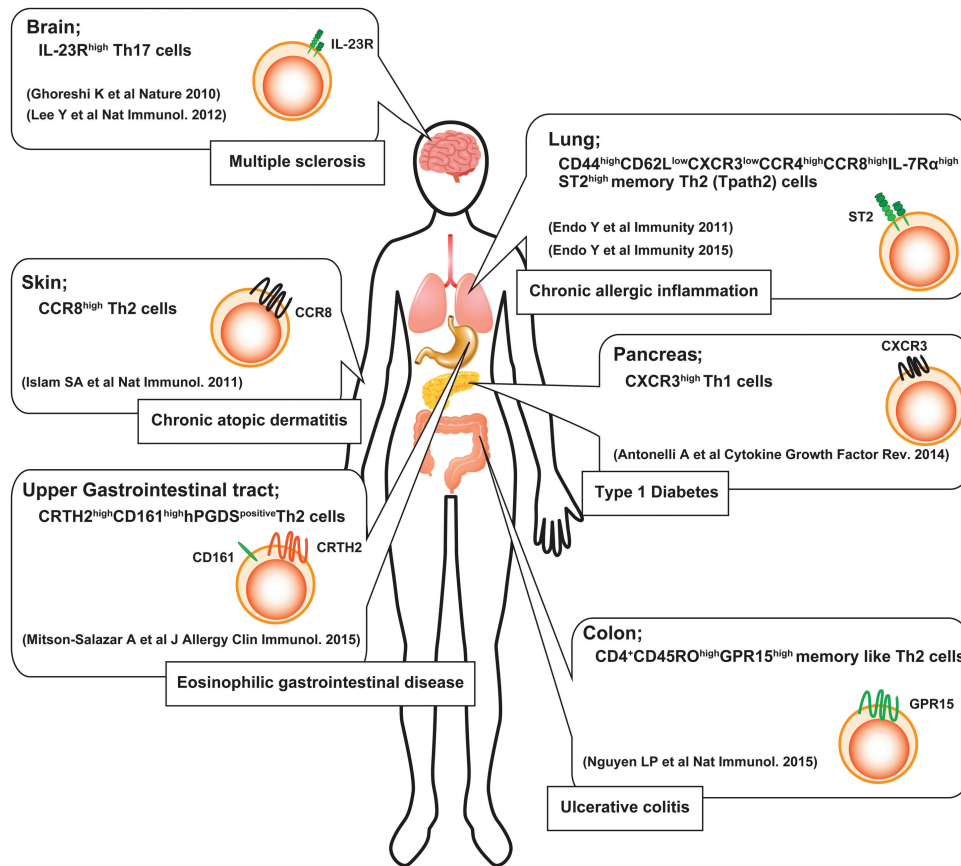


Fig. 1. The heterogeneity of pathogenic T-helper (T_{path}) cells. Recent advances in research point out several lines of evidence of the heterogeneity of T_{path} cells, which play a critical role in the pathogenesis of various immune-mediated diseases in an organ-specific manner.

Interestingly, a recent single-cell analysis revealed that a certain population of effector T_H2 cells had a suppressive function through the *de novo* synthesis of steroids (98).

Taken together, the evidence from recent studies implies that T-helper cells show far more plasticity and heterogeneity than we previously imagined. Precise assessments regarding the nature of T-helper cells will be indispensable in the development of new therapeutic approaches for these immune-related diseases.

Molecular mechanisms underlying CD4⁺ T-cell stability—the intersection of epigenetics and immune-mediated inflammatory diseases

The term 'epigenetic' was originally used to refer to inheritable changes in genetic expression that are not due to changes in the DNA sequence (110). At present, however, the term 'epigenetic change' refers to different chromatin alterations, such as histone tail modifications, DNA methylation and chromatin conformation and interaction. Regarding histone tail modifications, histone H3 lysine 36 trimethylation (H3K36me3) is a marker of an actively transcribed coding region and histone H3 lysine 4 trimethylation (H3K4me3) is associated with active promoters. Histone H3 lysine 4 monomethylation (H3K4me1) and histone H3 lysine 4 dimethylation (H3K4me2) are enhancer-related marks, whereas histone H3 lysine 27 trimethylation (H3K27me3) and histone H3 lysine 9 trimethylation (H3K9me3) encompass silenced genes.

Consistent with the plasticity of T-helper cell differentiation, genes encoding key transcription factors, including *Tbx21*, *Gata3*, *Bcl6*, *Runx3* and *Prdm1* exhibit both accessible and repressive marks (111). These bivalent modifications might be one of the mechanisms underlying the plasticity of the T-helper cell subsets. These histone marks are mediated by a variety of enzyme complexes including the Polycomb and Trithorax complexes (112–116).

It is now well appreciated that epigenetic regulation through the Trithorax and Polycomb complexes is important for the functions of T-helper cells (113, 114). For example, CD4⁺ T cells that lack the Polycomb proteins Mel-18, Bmi-1 and Ring1B exhibit impaired T_H2 cell differentiation (117–119). The deletion of the Polycomb protein Ezh2 results in spontaneous cytokine production of both IFN- γ and T_H2 cytokines (120). Moreover, the loss of Ezh2 has been associated with enhanced pathology and the accumulation of memory T_H2 cells in an allergic airway inflammation model (120). The disruption of Trithorax impairs the maintenance of the T_H2 cell signature in memory T_H2 cells (121).

Another important factor in determining whether or not genes can be 'read' is DNA methylation. For example, the DNA-methylation adaptor protein, Uhrf1, controls the homeostasis of intestinal T_{reg} cells through the silencing of *Cdkn1a* (122).

Cis-regulatory elements are well appreciated as the DNA elements that control genetic expression and which constitute

an integral part of gene structure. Among the many regulatory elements in non-coding DNA, enhancers play key roles in appropriate gene expression. Enhancers are non-coding DNA sequences that enhance the transcription of cognate target genes. Originally evidence of enhancer function relied on certain conserved non-coding sequences (CNSs) and DNase hypersensitivity sites (123). In T-helper cells, the T_H2 cytokine loci have been well investigated as part of the complex architecture of reasonably regulated lineage-specific genes (124, 125). In particular, the *IL4/IL4* loci are composed of a number of enhancers and a silencer element (125).

In addition to CNS and DNase hypersensitivity sites, it is now appreciated that H3K4me1^{high} and H3K4me3^{low} are chromatin signatures of 'active' and 'poised' enhancers in human cells (126). H3K4me2 modification also reveals both 'active' and 'poised' enhancers and this histone tail marker allows for the more precise localization of enhancers in comparison to that which is provided by an analysis of H3K4me1 (127).

Additional indicators such as the binding of acetyltransferase p300 or H3K27Ac have been related to 'active' enhancers. Emerging data show that these epigenetic modifications of the *cis*-regulatory region are closely related to human diseases including asthma, ulcerative colitis and allergy (128–131). For example, a genome-wide study of histone modifications identified disease-associated enhancers in CD4⁺ T cells in samples from asthmatic patients (129–131). Enhancers that gain the H3K4me2 mark during T_H2 cell differentiation in asthmatic patients reveal the enhanced enrichment of asthma-associated single nucleotide polymorphisms (130). Moreover, super-enhancers, which consist of clusters of enhancers, were recently identified as key DNA regulatory

elements for the control of cell-type-specific gene expression and genetic risk of diseases (131–133).

In summary, all of these epigenetic modulators work together to influence the accessibility of genes to the actions of transcription factors and promote appropriate gene expression. The breakdown of the orchestrated systems causes immune-mediated inflammatory diseases. It is therefore crucial for us to elucidate the detailed molecular mechanisms underlying the T-helper cell fate decisions.

The pathogenic T-helper population disease-induction model—toward the development of specific immune therapy

In accordance with the increased complexity and heterogeneity of T-helper cell subsets, we realized that we have to reconsider the pathogenicity of inflammatory diseases beyond the 'classical T_H1/T_H2 balance disease-induction model'. Specifically, the heterogeneity of T-helper cells implies that a specific population of T-helper cells dictates the pathology of immune-mediated inflammatory diseases. We proposed a 'pathogenic T-helper population disease-induction model' in 2014 (134). In this model, regardless of the balance of T_H1/T_H2 , the pathogenesis of so-called T_H1 -mediated or T_H2 -mediated diseases is largely dependent on the 'pathogenic sub-populations' of each T-helper cell subset that are generated *in vivo* and possess a distinct feature of effector function. In particular, in T_H2 -type pathology such as asthma or chronic dermatitis, IL-5-producing or IL-17-producing T_H2 cell sub-populations are considered to be pathogenic populations.

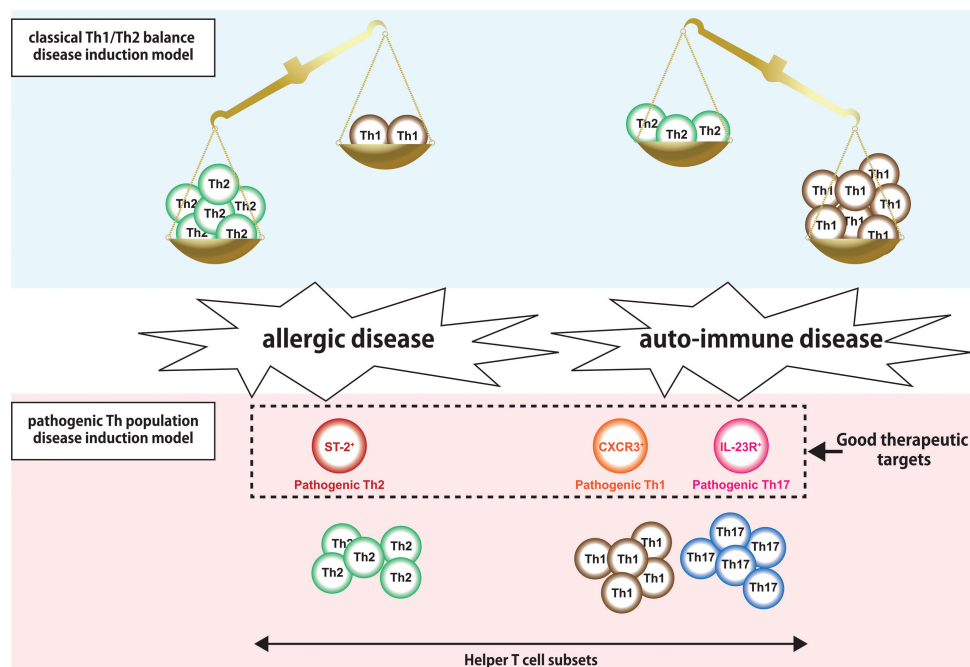


Fig. 2. A schematic representation of the 'classical T_H1/T_H2 balance disease-induction model' and the 'pathogenic T-helper population disease-induction model'. Since the 1980s, the balance among the T-helper cell subsets has been considered a key component for the pathogenesis of immune-mediated inflammatory diseases such as allergic diseases and autoimmune diseases. In the 'pathogenic T-helper population disease-induction model', regardless of the balance among T-helper cell subsets, a pathogenic sub-population of T-helper cells plays a critical role in the pathogenesis of these immune-mediated inflammatory diseases.

Consistent with this new disease-induction model, several groups including us have reported that various distinct sub-populations of T-helper cells are identified in mice and humans, and play a big role in the pathogenesis of certain types of immune-mediated inflammatory diseases (Fig. 1). For example, $T_{\text{path}}2$ cells are involved in chronic allergic inflammation of the airway, eosinophilic chronic rhinosinusitis, chronic atopic dermatitis, ulcerative colitis and eosinophilic gastrointestinal diseases in both mice and humans (100–103, 106). In the case of $T_{\text{h}}1$ cells, CXCR3^{high} $T_{\text{h}}1$ cells are known to be crucial for the pathogenicity of type 1 diabetes (135). Moreover, pathogenic $T_{\text{h}}17$ cells are induced under conditions without TGF- β 1 or with TGF- β 3 (86, 136). A recent report shows that AIM (CD5L) restrains $T_{\text{h}}17$ cell pathogenicity through regulation of lipid biosynthesis (137).

Taken together, we consider a key to understand the pathogenicity of immune-mediated inflammatory diseases is a 'pathogenic T-helper population disease-induction model'. In the model, a pathogenic sub-population of T-helper cells, which is induced under certain conditions, is rather critical for the pathogenesis of immune-mediated diseases than the balance among the T-helper cell subsets (134) (Fig. 2). Therefore, pathogenic T cells (T_{path} cells; $T_{\text{path}}1$, $T_{\text{path}}2$ and $T_{\text{path}}17$) could be good specific-therapeutic targets for immune-mediated intractable inflammatory diseases.

Conclusions

We have briefly reviewed the recent progress of research on the association between various CD4⁺ T-cell subsets and immune-mediated inflammatory diseases. There has been increasing evidence regarding the heterogeneity of T-helper cell subsets, particularly T_{path} cells. The heterogeneity of T_{path} cells might be involved in treatment resistance (to various types of treatment) that is observed in various immune-mediated inflammatory diseases. In order to develop new therapeutic strategies for the intractable immune-mediated inflammatory diseases, it will therefore become increasingly important for immunologists and clinicians to understand the precise features of T_{path} cells.

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