Change of paradigm: CD8+ T cells as important helper for CD4+ T cells during asthma and autoimmune encephalomyelitis

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Summary
The activation of naive CD4+ and CD8+ T cells in response to antigen and their subsequent proliferation and differentiation into effectors are important features of a cell-mediated immune response. CD4+ T cells (also known as T helper cells, Th) differentiate into several subpopulations including Th1, Th2, Th9, Th17, ThL and Treg cells, characterized by specific cytokine profiles and effector functions. However, recent evidence indicates that CD8+ T cells (termed cytotoxic T lymphocytes, CTLs or Tc cells) can differentiate into subpopulations with similar characteristics denoted as Tc2, Tc9, Tc17 and CD8+ Treg cells in addition to CTLs. Although these subpopulations accomplish important protective functions, their uncontrolled responses cause immunopathology including allergy and autoimmunity. Our recent findings indicate a change of paradigm: during these pathologic responses, CD8+ T cell subpopulations act as strong helpers for the activity of CD4+ T cells rather than being cytotoxic. In this review, we focus on the role of Th2, Th9, Th17 as well as Tc9 and Tc17 cells in asthma and autoimmune encephalomyelitis and on their interaction during these immunopathologic responses.

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Introduction
Cellular immunity is initiated after T cells recognize antigens presented by diverse cells. The antigens were displayed as peptides bound to major histocompatibility complex (MHC). At least, two functionally distinct types of T lymphocytes are part of the responding cellular compartment: CD4+ T cells get activated after antigen recognition presented by MHC class II by professional antigen-presenting cells (APC), whereas CD8+ T cells get activated after recognition of antigen displayed by MHC class I on the surface of all nucleated cells.

In response to antigen stimulation, CD4+ T cells (also termed T helper, Th cells) differentiate into several subsets such as Th1, Th2, Th9, Th17, ThL, iTreg cells. They secrete cytokines which influence the proliferation, function, and differentiation of cells including other T cells, B cells, and macrophages [1]. In contrast, the best characterized function of CD8+ T cells (also termed cytotoxic T lymphocytes, CTLs, or Tc1 cells) is to recognize and kill target cells expressing foreign peptide antigens by releasing cytotoxic molecules into the immunological synapse between CTL and target cell [2].
However, there is increasing evidence for the existence of subset diversification among CD8+ T cells, now described as CTL, Tc2, CD8+ Tregs, which can have cytotoxic function as well as Tc9 and Tc17 cells with low cytotoxic activity [3]. In this review, we discuss the role and interaction of Th2, Th9, and Tc9 cells in asthma and focus on Th17 and Tc17 cells during autoimmunity of the central nervous system (CNS).

Subpopulations of CD4+ T cells

CD4+ T cell subpopulations differentiate from naive precursor cells in response to signals induced by antigen recognition and cytokines of the microenvironment. They are characterized by the expression of distinct transcription factors termed master regulators, which regulate their specific cytokine profiles and effector functions. Th2 cells are induced by IL-4, secrete IL-4, IL-5 and IL-13, and express GATA3. IL-4 in combination with TGF-β induces the differentiation of Th9 cells, which produce high levels of IL-9 and IL-10. Although Th2 and Th9 cell subsets both contribute to immunity against helminths, Th9 cells are additionally involved in anti-tumor immunity [4]. When combined with TGF-β and IL-23, the cytokines IL-6 or IL-21 can induce Th17 cells to produce IL-17, IL-21 and IL-22, to express the lineage specific master regulators RORγt and RORα, and to protect from extracellular bacterial and fungal infections [5].

In contrast to these important protective functions, uncontrolled responses of the respective subsets cause immunopathology. Thus, Th17 cells have been implicated in autoimmune tissue inflammation, including autoimmune encephalomyelitis and inflammatory bowel disease, whereas Th2 as well as Th9 cells contribute to different forms of allergy including allergic asthma [1, 6].

Involvement of Th2 and Th9 cells in asthma

Asthma is a complex chronic airway disorder characterized by bronchial inflammation, airway obstruction, airway hyperresponsiveness (AHR), and by symptoms like wheezing and shortness of breath. This disorder affects 300 million people worldwide and is a major public health problem in western countries. Asthma is caused by multiple environmental factors in combination with numerous major and minor susceptibility genes and has many different forms and phenotypes.

The most common form, allergic asthma, is associated with a typical Th2 cell cytokine mediated inflammation [7]. Cardinal features of the allergic Th2 cell response include IL-4-mediated allergen-specific synthesis of immunoglobulin E (IgE), interleukin-5 (IL-5)-mediated recruitment of eosinophils, and IL-13-mediated goblet cell hyperplasia, mucus overproduction, AHR, and fibrosis. Evidence for the central role of Th2 cells in allergic asthma has been provided by experimental models in mice, in which allergen-specific Th2 cells can be induced and, after recruitment into the lungs, drive key pathologic features of an allergic response, including eosinophilia, airway mucus production, and AHR [8].

In recent years, Th9 cells have been shown to contribute to allergic airway inflammation via secreted IL-9 [9, 10, 11]. In a mouse model, transfer of antigen-specific Th9 cells led to allergic airway inflammation which could be blocked by neutralization of IL-9 [9, 11]. More importantly, neutralization of IL-9 after induction of asthma also resulted in the reduction of asthma-associated pathology in the absence of cell transfer [10]. Moreover, Th9 cells promoted asthma with characteristics distinct from that induced by Th2 cells and the accumulation of Th9 cells preceded the accumulation of Th2 cells in the lungs of house dust mite-challenged mice [12].

Further evidence for the promotion of allergic asthma by Th9 cells was provided by studies in mice with deficiencies in the transcription factors BATF, IRF4 and PU.1, which interact during Th9 differentiation [9, 10, 11]. In mice with deficiencies in any of these transcription factors, CD4+ T cells failed to differentiate into IL-9 producers under Th9-inducing conditions and consequently, these mice were resistant to the induction of allergic airway disease. Importantly, reconstitution of disease resistant IRF4-deficient mice with wild-type Th9 cells restored asthma symptoms, further corroborating a crucial role for Th9 cells during allergic

### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AD           | Atopic dermatitis |
| AHR          | Airway hyperresponsiveness |
| EAE          | Experimental autoimmune encephalomyelitis |
| APC          | Antigen-presenting cells |
| CCR6         | Chemokine receptor 6 |
| CNS          | Central nervous system |
| CSF          | Cerebrospinal fluid |
| CTL          | Cytotoxic T lymphocyte, CD8+ T cell |
| IgE          | Immunoglobulin E |
| IL           | Interleukin |
| MHC          | Major histocompatibility complex |
| MOG          | Myelin oligodendrocyte protein |
| MS           | Multiple sclerosis |
| Tc           | Cytotoxic T lymphocyte |
| TGF-β        | Transforming growth factor-β |
| Th           | T helper lymphocyte, CD4+ T cell |
airway disease [9]. In support of these findings in mouse models, IL-9 expression is higher in lungs of asthmatic patients than in healthy controls [13, 14], arguing for the promotion of allergic asthma severity by Th cells not only in mice, but also in humans.

**Involvement of Th17 cells during autoimmunity of the CNS**

Multiple sclerosis (MS) is the most common autoimmune disease of the CNS affecting more than one million people worldwide, particularly women. The pathogenesis of MS is not fully understood. Immune cell infiltrates comprising T cells, B cells, and granulocytes as well as plaques of demyelization in the brain and spinal cord are key pathological features of MS. However, extensive heterogeneity in the clinical symptoms is seen among patients suggesting many pathways with distinct effector mechanisms involved in the onset of the disease [15, 16, 17]. MS has been widely studied by using the animal model experimental autoimmune encephalomyelitis (EAE). EAE is induced by immunization with myelin-derived antigens in an adjuvant or by adoptive transfer of activated myelin-specific T cells. The inflammatory infiltrates and demyelization seen in EAE have many similarities to the pathological hallmarks of MS. The ability to induce EAE by adoptive transfer of myelin-specific T cells supported the idea that MS is an autoimmune disease [16, 17].

Since its description in 2000, a novel cytokine termed IL-23 which is composed of the two chains p19 and p40, has dramatically changed the understanding of the contribution of CD4+ T cell subpopulations to EAE [18]. By generation of IL-23p19-deficient mice, it has been demonstrated that IL-23 is crucial for the induction of EAE [19] and that IL-23 expands IL-17-producing CD4+ Th17 cells which are capable of inducing EAE after adoptive transfer into wild-type mice. Accordingly, the expansion of endogenous Th17 cells was dramatically reduced in the CNS of IL-23p19 deficient mice [20]. Based on this and other studies, investigators proposed that Th17 cells are the “true” effector T cell subpopulation in EAE. The nuclear hormone receptors RORyt and RORα were identified as transcription factors important for Th17 development and accordingly, mice deficient in these transcription factors developed CD4+ T cell mediated EAE at a considerably reduced level [21, 22]. Additionally, the transcription factor RORyt regulates in CD4+ T cells the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) a cytokine with a strong encephalitogenic property [23].

Our group has demonstrated the critical role of the transcription factor IRF4 for the development of Th17 cells. Thus, CD4+ T cells from IRF4-deficient mice failed to differentiate into IL-17 producers under Th17-inducing conditions in vitro. Accordingly, IRF4-deficient mice were totally resistant to the induction of EAE. This resistance correlated with lack of Th17 cell differentiation in vivo. Reconstitution of IRF4-deficient mice with wild-type CD4+ T cells restored their susceptibility to the disease and the transferred cells developed a Th17 phenotype, again pointing to the crucial role of Th17 cells for the development of EAE and to the critical role of IRF4 in the CD4+ T cell intrinsic regulation of Th17 development [24]. Further support for a pathogenic role of Th17 cells derives from studies of patients with MS. Increased numbers of IL-17 transcripts are detectable in chronic MS lesions as compared with acute lesions or control tissue from individuals without pathology [15, 17]. Accordingly, a phase II trial including 73 patients with relapsing-remitting MS revealed that a fully humanized antibody that neutralizes IL-17A (secukinumab) significantly reduced the number of new active lesions as compared to placebo-treated patients. In addition, a trend towards a reduction of annualized relapse rate was noted [5, 25]. An extension of this study assessing adverse events and another phase II study will end 2015, respectively. This trial suggests an involvement of IL-17A in lesion formation and that IL-17A could be an important target in MS treatment. The analysis of therapeutic effectiveness in MS is also planned for another IL-17A neutralizing antibody (CJM112) [25]. Besides trials in MS, a clear effectiveness of anti-IL-17A and anti-IL17RA antibodies is demonstrated in psoriasis. The IL-17A targeting analyses include the treatment of further autoimmune and chronic inflammatory conditions for example rheumatoid arthritis, ankylosing spondylitis, asthma, type I diabetes, and Crohn’s disease [5, 25].

**Subpopulations of CD8+ T cells**

There is growing evidence that CD8+ T cells, like CD4+ T cells, differentiate under specific conditions into separate subsets such as CTLs or IL-4- and IL-13-producing Tc2, IL-9-producing Tc9, IL-17-producing Tc17, IL-22-producing Tc22 cells, and CD8+ Treg cells [3]. In this review, we focus on the role of these “alternative CD8+ T cell subsets”, in particular Tc9 cells in asthma and Tc17 cells in autoimmune encephalomyelitis. Tc9 cell development parallels that of Th9 cells and is also induced by TGF-β and IL-4. These cells are detectable in the lamina propria of mice and in the periphery of mice and humans with atopy [26, 27]. Like their Th9 counterpart, they also display strong anti-tumor activity in a mouse system [28].
As for Th17 cells, TGF-β in combination with IL-6 or IL-21 acts to promote differentiation of IL-17-producing and RORγt-expressing Tc17 cells, which are detectable during viral infections, in tumor environments and during autoimmunity [3]. Importantly, in contrast to “canonical” CTLs, Tc9 and Tc17 cells display low cytotoxicity which might have important implications for their presence in tumor tissue [3]. Because TGF-β is a common product within the tumor-microenvironment, a shift from CTL to Tc17 may represent a tumor escape mechanism.

**Involvement of Tc9 cells in asthma**

Not only CD4+, but also CD8+ T cells are involved in asthma pathology: An increase of CD8+ T cells is detectable in sputum of severe asthmatics [29] and increased numbers of bronchial CD8+ T cells correlate with decline in airway function [30]. However, the role of CD8+ T cells in the disease is unclear. Conflicting evidence from mouse models denote pathogenic [31, 32] or protective functions [33, 34], arguing that different CD8+ T cell subsets are responsible for these contradictory effects. Whereas CTLs are beneficial in allergic airway inflammation, Tc2 cells aggravate disease. This enhancement of inflammation is at least partially dependent on the capacity of Tc2 cells to produce IL-13 [35].

In an own study, we analyzed the biology and role of Tc9 cells in allergic airway disease. We demonstrated that, similar to the induction of Th9 cells, the presence of IL-4 plus TGF-β caused IL-9 production by CD8+ T cells in vitro. These cells expressed less of the CTL-associated transcription factors T-bet and eomes, as well as less of the cytotoxic molecule granzyme B. In agreement with low amounts of granzyme B, Tc9 cells displayed diminished cytotoxic activity in vitro, suggesting that functionally they rely on IL-9 production rather than on cytotoxicity [27]. Using adoptive transfer of antigen specific Tc9 cells into T and B cell deficient RAG2-knockout mice, we found that Tc9 cells failed to induce key features of asthma, revealing that they are not pathogenic by themselves. However, in combination with sub-pathogenic numbers of Th2 cells, which failed to evoke disease symptoms, Tc9 cells elicited key features of allergic airway disease, including increased eosinophil numbers in the bronchoalveolar fluid, increased numbers of mucus-producing cells in the lung, and elevated lung inflammatory score. The capability to promote Th2-mediated airway inflammation was associated with Tc9 properties, because CTLs failed to fulfill this function. Thus, Tc9 cells but not CTLs cooperated with Th2 cells for airway infiltration and for induction of key features of asthma.

The phenotype analysis of transferred cells from the lung revealed loss of IL-9 production by Tc9 cells, in favor of IFN-γ and to small extent IL-13, revealing plasticity of these IL-9-producing CD8+ T cells [27]. Similar support for Th2 cells has been described for innate lymphocytes [36] and Th9 cells [37]. Thus, our data described a supportive „helper cell“ function of CD8+ Tc9 cells for the initiation of CD4+ Th2-mediated airway inflammation at a stage, when the frequencies of Th2 cells are not sufficient to induce disease pathology by themselves. Therefore, we speculated that Tc9 cells might accumulate in diseases, which precede airway inflammation. Accordingly, we found Tc9 cells in the lymph nodes (but not in the skin) of mice and in peripheral blood of patients with atopic dermatitis (AD) [27], a skin disease which is Th2-associated and often precedes asthma in humans [38]. In contrast, another alternative CD8+ T cell subset, Tc22 cells, is increased in the skin of patients with AD and its frequency is positively correlated with disease severity [39]. Tc22 cells drive probably directly via secreted IL-22 specific clinical features of AD pathology including keratinocyte proliferation and modulation of terminal differentiation [39]. Since Tc9 cells presumably fail to directly influence AD pathology, we suppose that the peripheral Tc9 cells arising during AD might contribute to the subsequent onset of asthma by promoting the pathogenic function of Th2 cells. Probably, in the periphery, Tc9 cells use IL-9 and/or other so far unknown Tc9-associated cytokines or surface molecules to activate Th2 cells directly and/or via innate immune cells to enhance allergic inflammation. In agreement with this hypothesis, peripheral blood mononuclear cells of atopic infants produce more IL-9 than those of healthy controls [40], suggesting that increased IL-9 production is an early event promoting the development of allergic disease.

**Involvement of Tc17 cells in autoimmunity of the CNS**

Apart from the importance of CD4+ T cells during EAE and its human counterpart MS, several observations suggest that CD8+ T cells may also be involved in disease pathogenesis. First, CD8+ T cells are present in inflammatory lesions. Second, a higher frequency of CD8+ T cells recognizing myelin proteins was demonstrated in patients with MS than in healthy controls. Third, in brain tissue from patients with MS, clonal expansion of CD8+ T cells was shown [15, 17]. Finally, CD8+ T cell involvement in EAE was described [41]. In this model, a particular peptide of myelin oligodendrocyte protein (MOG 37-50) representing a MHC I epitope was characterized as pathogenic. By using MHC I tetramers loaded with this peptide, pep-
tide-specific CD8+ T cells were identified in the CNS of mice with EAE [41]. Others and our group have found that similar to Th17 cells, the cytokines IL-6 or IL-21 along with TGF-β determine the differentiation of IL-17-producing CD8+ T (Tc17) cells. In addition, IL-23 stabilizes their phenotype. Similar to Th17 cells, Tc17 cells produce the cytokines IL-17 and IL-21 and express the receptor for IL-23 and the lineage-specific transcription factors RORγt and RORα [42, 43, 44]. In comparison to CTLs, Tc17 cells expressed the transcription factors T-bet and Eomes at diminished levels. Probably therefore, they produced fewer proteins characteristic for CTLs such as IFN-γ, granzyme B, and perforin, again resulting in impaired cytotoxicity [42, 43, 44].

These findings indicated that the function of Tc17 cells is associated with IL-17 production rather than with cytotoxicity. As we detected IL-17-producing CD8+ T cells in mice diseased from EAE [42] and other groups found these cells in lesions of patients with MS [45], we sought to understand their function during autoimmune CNS inflammation. In these studies, we again used IRF4-deficient mice, which not only failed to generate Th17, but also Tc17 cells in vitro, and were totally resistant to EAE [46]. By performing transfers of wild-type CD8+ T cells into IRF4-deficient mice and induction of EAE, we observed that the transferred CD8+ T cells developed a Tc17 phenotype in peripheral lymphatic organs, but failed to migrate into the CNS and consequently to induce inflammation. Thus, in an IRF4-deficient environment Tc17 cells were not pathogenic. Most interestingly, the help of small numbers of wild-type CD4+ T cells was sufficient to enable the entry of Tc17 cells into the CNS of IRF4-deficient but also in RAG-deficient mice [46]. In this setting, CD4+ T cells migrated in the first wave dependent on the expression of the chemokine receptor CCR6, confirming the hypothesis proposed by Sallusto et al. [47], while Tc17 cells entered the CNS independent of CCR6 in a second wave.

However, the most surprising finding was, that the co-transfer of high numbers of CD8+ T cells together with sub-pathogenic very low numbers of CD4+ T cells caused very rapid onset and severe course of EAE accompanied by high accumulation of CD4+ (not CD8+) T cells in the CNS. These CD4+ cells were characterized by a strongly pathogenic phenotype, because they co-produced IL-17 and IFN-γ [46]. These findings suggested that CD8+ T cells accelerated the pathogenicity of CD4+ T cells during autoimmune inflammation of the CNS. For this “reverse help” provided by CD8+ T cells toward CD4+ T cell pathogenicity, the production of the Tc17 hallmark cytokine IL-17 was important. Tc17 cells induced in CD4+ T cells a Th17

![Fig. 1: Reciprocal interaction of Tc17 and Th17 cells for EAE onset. 1st step occurring in the lymphnodes (upper panel marked in green): Tc17 cells use IL-17A to endow Th17 cells with stronger pathogenicity (such as production of more IL-17A and probably other cytokines). 2nd step: pathogenic Th17 cells to enable entry of Tc17 cells into the CNS via CCR6.](image-url)
transcriptional program and IL-17 production via direct cell-cell contact. Probably, surface IL-17 expressed by Tc17 cells contributed to the IL-17-induction in CD4+ T cells. When evaluating whether these findings obtained in the EAE are compatible with the human disease, we found that patients with early-stage MS show enhanced Tc17 cells selectively in the cerebrospinal fluid (CSF) and harbor significantly higher percentages of Tc17 cells in CSF as compared to the control group [46]. This indicated, that also in humans Tc17 cells are involved in the initiation of disease – perhaps when Tc17 and Th17 cells in the periphery have already interacted and Tc17 cells start to enter the CNS. Our findings also suggested selective expansion and CNS-recruitment of Tc17 cells in early-stage MS and that targeting of Tc17 cells may be relevant for the therapy in early MS.

Conclusions

There is strong evidence that besides CD4+ T cell-subpopulations, CD8+ T cells also contribute to the pathology of asthma and autoimmune encephalomyelitis. The recent work of our group indicates that during some types of immunopathology including allergy and autoimmunity, “alternative” CD8+ T cell subsets like Tc9 and Tc17 cells arise, with functions relying on cytokine production rather than on cytotoxicity, in contrast to “canonical” CTLs. These alternative CD8+ T cells seem to get in touch with CD4+ T cells and to accelerate the Th2-mediated pathology in asthma as well as Th17-mediated autoimmune encephalomyelitis. Particularly for autoimmune encephalomyelitis, we propose two sequential steps during induction of this disease by interacting CD4+ and CD8+ T cells: the first event is crucial to endow CD4+ T cells with stronger pathogenicity and requires direct cell-contact between Tc17 cells, CD4+ T cells, and probably also APCs. During this initial process, Tc17 cells might promote directly and indirectly – via IL-17A-dependent APC-activation – the differentiation of CD4+ T cells towards the pathogenic Th17 phenotype and thus regulate the “first wave” of CD4+ cell migration into the CNS via CCR6. These Th17 cells in turn facilitate the CCR6-independent migration of Tc17 cells in the second wave (Fig. 1). However, the exact details of the in vivo interaction between IL-17A-competent CD8+ T cells and CCR6-competent CD4+ T cells during autoimmune CNS inflammation deserve further exploration.

Furthermore, we have detected higher amounts of Tc17 cells in the CSF of patients with early-stage MS as compared to health individuals. For the contribution of Tc9 cells to Th2-mediated asthma, further studies are needed to exactly understand the mechanisms of this interaction. Thus, our data suggest that Tc9 and Tc17 cells arise and accelerate immunopathology at earlier stages of the disease when the respective CD4+ T cell subsets Th2 and Th17 cells are present at low sub-pathogenic frequencies. Therefore, therapeutic targeting of Tc9 and Tc17 cells in the early-stage of disease may prevent the development of full-blown asthma and MS, respectively.

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Conflict of interest
The authors states that there are no conflicts of interest.

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