Mini Review

Induction of Th17 cells by dendritic cells

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The novel T helper subset, Th17, plays a critical role in the pathogenesis of a variety of chronic inflammatory disorders such as rheumatoid arthritis, Crohn's disease, and others. Although inflammatory dendritic cells (DCs), such as DCs producing TNF α and iNOS, have been suggested to act as an inducer of Th17-immunity both in humans and mice *in vivo*; it is unclear how DCs elicit Th17-immunity. Recently, some Th17-differentiation pathways, both TGF- β -dependent and -independent pathways, have been identified in mice. However, the DC biology to evoke Th17-immunity in chronic inflammation or autoimmunity has not yet been clarified, in particular, in human cells. This review summarizes and discusses the induction of Th17 cells by DCs.

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Introduction

Dendritic cells (DCs) are multifunctional professional antigen-presenting cells¹⁾. A subset of DC, inflammatory DC, is recruited into inflamed tissue and has been considered to evoke inflammation not only to clear invading microbes, but also to cause self-injury that potentially progresses to autoimmune diseases²⁾. Along with studies of TNF α and iNOS producing DCs (Tip-DCs) as putative inflammatory DCs²⁾, the role of inflammatory DC-produced IL-23 is correlated with autoimmunity rather than with IL-12 production³⁾. While DCs have the capacity to facilitate differentiation of naïve CD4⁺ T cells into a variety of T helper subsets, it is T helper 17 (Th17) cells that appear to be a unique player inducing granulocytosis and granulomatous inflammation, a histological sign specific for chronic inflamma-

tory diseases⁴⁾. In contrast, T helper type 1 (Th1) and type 2 (Th2) cells lead to the production of antigen-specific antibody via B cells without inflammation. This discovery has markedly advanced our understanding of not only anti-bacterial immunity, but also many inflammatory and autoimmune diseases. However, human studies on the initiation of Th17 by DCs have only advanced slowly because the procedures for the induction of Th17 from human cells are not as well defined as for murine CD4⁺ T cells²⁾. Studies of DCs that promote Th17-immunity substantially remain to be clear. In this review, we focus on studies involving Th17-inducing DCs. In human studies, we summarize reports describing Th17-differentiation from naïve CD4⁺ T cells and discuss cases of TGF- β -dependent or -independent induction of Th17 cells.

Cytokines Correlated to Promotion of Th17 Response

IL-23 is essential for IL-23-IL-17 axis inflammation, and the correlation of IL-23 with autoimmunity may relate to the role of IL-23 in regulating IL-17-production as IL-23 was initially thought to be important for the induction of IL-17^{7,8}). However, the finding that the combination of IL-6 and TGF- β induces the expression of a key transcription factor ROR γ t for IL-17-production⁵) changed the concept of the IL-23's major role from one of Th17-induction to one of Th17-maintenance. This discovery illustrates the paradigm of Th17-differentiation while TGF- β alone induces the expression of Foxp-3, a master gene for regulatory T cells (Treg)⁵). However, Chung et al. described an IL-1-dependent TGF- β -independent pathway in a murine model of experimental autoimmune encephalomyelitis (EAE)⁶). This has again raised a possible role for IL-23 produced by DCs during the initiation of Th17-responses.

Induction of Th17 Cells from Naïve CD4⁺ T cells in Mice

To date, the induction of murine Th17 cells by DCs has been well described through findings of both *in vitro* and *in vivo* studies. Murine naïve CD4⁺ T cells respond to TCR-signals and are easily differentiated to IL-17-producing cells under conditions in which the effects of IFN- γ and IL-4 are inhibited⁷). In the earlier studies, the induction of antigen-specific Th17 cells by antigen presenting cells (APCs) was proven by the use of OVA-specific TCR transgenic mice. When naïve CD4⁺ T cells were co-cultured with DCs in the presence of IL-23, TCR and ICOS signaling efficiently led to antigen-specific Th17-differentiation⁸). Thereafter, stimulation conditions under which DCs gain a Th17-inducing ability were explored along with the mechanism(s) of DC mediated differentiation. Dectin-1 is a receptor for β -glucan, a component of fungus, which preferentially stimulates DCs to induce Th17 cells as a result of the production of cytokines, including IL-1, IL6, IL-23, and TGF- β . The Syk-CARD-9 signaling pathway downstream of Dectin-1 was found to be the signal transduction route for DC-mediated Th17-induction⁹). Furthermore, DCs stimulate T cells through both the TCR and ICOS. These signals synergistically increase IL-21-production in an autocrine manner and increase IL-23 receptor expression. Both IL-21 and IL-23 accelerate the differentiation of Th17 cells through IL-21-IL-23 receptor positive loop stimulation¹⁰). Additionally, an IL-1-dependent, IL-6/TGF- β -independent pathway for Th17-differentiation has been found⁶). This pathway is associated with the activation of transcription factors, IRF-4 and

ROR γ t, and requires IL-23-stimulation⁶). The detailed molecular mechanisms remain to be clarified, as well as how the two individual pathways interact to regulate Th17-immunity. Recently, Lee et al. explored the presumed direct effect of TGF- β on Th17-differentiation by demonstrating that IL-6 alone can induce Th17-differentiation in Stat6^{-/-}T-bet^{-/-} mice, which have impaired Th1- and Th2-differentiation¹⁰). This suggests that TGF- β does not directly affect IL-17-production, but indirectly affects by its inhibitory effects on Th1 and Th2.

The characteristics of DCs that promote antigen specific Th17 cells also have been studied. Bailey et al. showed that a CD11b⁺ myeloid DC subset accumulates in the central nervous system in EAE models¹²). This DC subset has the strongest capacity to induce antigen-specific Th17 cells among plasmacytoid DCs and CD8⁺ DCs. These data are consistent with inflammatory DCs being derived from myeloid lineages including inflammatory monocytes²).

Induction of Th17 Cells from Naïve CD4⁺ T cells in Humans

Unlike naïve murine CD4⁺ T cells or human memory T cells, naïve human CD4⁺ T cells lack IL-23 receptors; additionally they do not respond to the conditions used in murine experiments that induce conversion to Th17 cells⁴). In order to analyze Th17-promoting conditions, we compiled a list of nine reports that study the induction of human Th17 cells in naïve CD4⁺ T cells¹³⁻²¹) (Table 1). Sources of naïve CD4⁺ T cells and the means of T cell stimulation are summarized. We chose not to include several reports in the list of studies that had negative control naïve CD4⁺ T cells which contained IL-17-producing cells (IL-17<1%) after PMA/ionomycin treatment. The majority of studies describe the negative control naïve CD4⁺ T cells as being completely negative for IL-17⁺ cells. Every reported method for Th17-induction is unique. Five reports used naïve CD4⁺ T cells from peripheral blood mononuclear cells (PBMC), and the others used those from umbilical cord blood (UCB). Naïve CD4⁺ T cells from UCB were more easily converted to Th17 cells than those from PBMC naïve CD4⁺ T cells²⁰). Presumably, the differently sourced naïve CD4⁺ T cells may cause the different responses. For example like human memory T cells and murine naïve CD4⁺ T cells, naïve human CD4⁺ T cells from UCB, but not from PBMC, express IL-23 receptors⁴). Interestingly, naïve CD4⁺ cells were re-stimulated in four of six reports using PBMC^{13,14,16,17}). We demonstrated that treatment with anti-CD3 induced IL-23 receptors on naïve CD4⁺ T cells (unpublished data). Furthermore, TGF- β -independent conversion into Th17 cells was described in a report using

Table 1 Sources and phenotypes of naïve CD4⁺ T cells, its treatments with antigen presenting cells (APCs), antibodies, and cytokines, and restimulation of T cells were summarized in the table from data in eight papers describing human Th17-induction from naïve CD4⁺ T cells.

Naïve CD4 T cells		Stimulation				Ref.
Source	Phenotypes	APCs	Treatment with Ab	Additional Cytokines	Restimulation	
PBMC	CD4 ⁺ CD45RO ⁻	Cell-free, cDC Monocytes (LPS)*	Anti-CD2/CD3/CD28	IL-1+IL-23 No effects of IL-6+TGF- β	Anti-CD2/CD3/CD28 +IL-2	12
PBMC	CD4 ⁺ CD45RA ⁺ CD45RO ⁻	Cell free	Anti-CD3/CD28	IL-1+IL-6+IL-23 +TGF- β	Anti-CD3/CD28	13
PBMC	CD4 ⁺ CD45RA ⁺	Skin DC ex vivo	None	IL-15+IL-6+IL-23	None	14
PBMC	CD4 ⁺ CD45RA ⁺	CD1a+moDC (LPS+R848)	None	None	Anti-CD3	15
PBMC	CD4 ⁺ CD45RO ⁻	Monocytes (PGN)*	Anti-CD3	IL-1+IL-23+PGE ₂	IL-2	16
PBMC	CD31 ⁺ CD4 ⁺ CD45RA ⁺ CCR7 ⁺	Cell free	Anti-CD3/CD28	IL-1	None	17
UCB	CD161 ⁺ CD4 ⁺	Cell free	Anti-CD3/CD28	IL-1+IL-23	None	18
	CD4 ⁺ Thymocytes					
UCB	CCR7 ⁺ CD4 ⁺ CD45RO ⁻	Cell free	Anti-CD3/CD28	IL-1+IL-6+IL-21+IL-23 +TGF- β	None	19
UCB	CD4 ⁺ CD45RO ⁻	Cell free	Anti-CD2/CD3/CD28	IL-1+IL-6+IL-21+IL-23 +TGF- β	None	20

Data in the table were sourced from eight papers. PBMC; peripheral blood cells, UCB; umbilical cord blood, DC; dendritic cell, LPS; lipopolysaccharide; PGN; proteoglycan. () * indicates stimulatory substances for DCs or monocytes.

PBMC⁽¹⁴⁾. In contrast, re-stimulation was unnecessary for naïve UCB CD4⁺ T cells, and in 2 of 3 reports Th17-induction was dependent on TGF- β ^(19,20). Interestingly, Cosmi et al. described that CD161, a NK cell specific marker known as NK.1.1 in mice, was specific for human Th17 cells in both UCB and PBMC⁽¹⁸⁾. They described Th17 cells as CD161⁺CD4⁺ cell subsets from the thymus or UCB. However in adult peripheral blood naïve CD4⁺ T cells, CD161⁺ Th17 cells are not induced, while CD161⁺Th17 cells are detected in inflamed tissues of patients with psoriasis. Although precursors of Th17 appear to be different from Th1 or Th2⁽⁴⁾, subsets of naïve CD4⁺ T cells that inhibit Th17 induction may exist; depletion of a minor subset, CD25⁺ naïve CD4⁺ T cell, can effectively increase Th17-differentiation⁽²¹⁾. Additionally, Th17-induction is enhanced in the presence of prostaglandin E₂, which increases expression of IL-23 receptors on T cells. The effects of prostaglandin E₂ may be mediated by the inhibition of IFN- γ , which suppresses Th17-induction⁽¹⁷⁾. Along with the IL-23 receptor, expression of IL-1 receptor 1 (IL-1R1) on naïve CD4 T cells also is necessary for Th17-differentiation. A CD31⁺ naïve CD4 T cell subset expresses IL-1R1 and differentiates into Th17 cells in the presence of IL-1 β under CD3/CD28-stimulation, whereas a CD31⁻IL-1R1⁻ subset does not⁽¹⁸⁾. How-

ever, naïve CD4 T cells expressing IL-1R1 after stimulation with IL-7, IL-15, and anti-CD3/CD28 beads do not convert to Th17 cells. These data suggests that IL-1 β is necessary, but not sufficient, for naïve human CD4 T cells to differentiate into Th17 cells. The induction of Th17 cells in these listed reports is much lower than that seen in studies using murine cells and experimental models; therefore, additional investigation is needed to further elucidate the complex conditions under which naïve human CD4⁺ T cells undergo significant conversion into Th17 cells.

Studies of human DCs in Induction of Th17

Some reports have described studies of human Th17-induction by DCs *in vitro* or *in vivo*. However, methods using a cell-free system to study the conversion of naïve CD4⁺ T cells into Th17 cells are not always useful for DC-mediated Th17-induction⁽²²⁾. Mathers et al. reported that *in vitro* skin migratory Langerhans cells (LCs) and DCs induced naïve peripheral blood CD4⁺ T cells (CD4⁺CD45RA⁺>90%) to become IL-17-producing cells, which was dependent upon IL-15 and IL-6, but independent of TGF- β or IL-23⁽¹⁵⁾. Conversely, LC-like cells derived from monocytes cultured in the presence of TGF- β and GM-CSF were

dependent upon TGF- β for Th17 induction²¹). According to studies by Trinchieri et al.³), stimulation through Dectin-1, TLR-2, and NOD-2, receptors that recognize pathogen associated molecular patterns, converted immature DCs into mature DCs, which promoted Th17-induction. Combined stimulation of monocyte-derived DCs by TLR4 and TLR7/8 also convert naïve CD4⁺ T cells into Th17 cells¹⁶).

In addition, the condition of monocytes prior to DC-differentiation is important. Previously, we reported that LPS-stimulated DCs derived from monocytes cultured in the presence of GM-CSF and TNF (TNF-DC) efficiently induce human Th17 from total CD4⁺ T cells²⁴). We compared TNF-DCs to DCs derived from monocytes cultured in the presence of GM-CSF and IL-4; TNF-DCs predominantly produced TNF and IL-23 rather than IL-12, and had the capacity to induce Th17 cells (unpublished data).

Zaka et al. provided data on the *in vivo* characteristics of skin migratory DCs in patients with psoriasis²⁵). They found that CD11c⁺CD1c⁻DCs rather than CD11c⁺CD1c⁺DCs (resident DCs) accumulated in the inflamed tissues. The accumulated cells included a Tip-DC population and possessed low levels of macrophage markers, CD14 and CD163. This could be the definitive phenotype specific for human inflammatory DCs, which may be distinct from other subtypes of DCs. However, further *in vitro* studies using such inflammatory DCs, or Tip-DC-like cells, are necessary.

Concluding Remarks

The discovery of Th17-immunity represents a major step in our understanding of infectious immunity and autoimmune pathologies. To date, murine experimental disease models have strongly facilitated a better understanding of Th17-immunity; however, studies of human inflammatory DCs have not satisfactorily explained the mechanism(s) by which these DCs initiate and facilitate Th17-immunity. Therefore, to advance our understanding of Th17-immunity in human, a well-defined experimental model using human inflammatory DCs is needed. Human Th17-immunity seems to be normally limited under emergency conditions⁴), and Th17-induction by DCs may be unusual in comparison with former experiments of the induction of Th1 or Th2. According to the reports listed in Table 1, at least two individual pathways, IL-1/IL-23 or IL-6/TGF- β dependent Th17-induction, appear to exist in humans as well as in mice; human naïve CD4⁺ T cells from PBMC appear to require recurrent stimulation to differentiate into Th17 cells. The role of human inflammatory DC biology is complex and investigation of the multiple

steps involved in Th17-differentiation may lead to greater understanding of the etiology of autoimmune diseases. While not discussed in this review, understanding the conversion of regulatory T cells into Th17 cells or Th17 cells into Th1 cells is another aspect of Th17-immunity that remains to be elucidated. The regulatory system that controls the balance of these effector T cells may be influenced by DCs²⁶). Furthermore, the discovery of natural Th17 cells that recognize self-antigens will alter the Th17/Treg balance²⁷). There are likely differences between adult and neonatal Th17-immune responses and this has yet to be fully investigated. In the future, the advance of inflammatory DC biology will contribute to a better understanding of chronic inflammatory diseases and autoimmune diseases, and provide insight for designing targeted therapies or for diagnosis of these diseases.

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