

# Modulation of autoimmune diseases by interleukin (IL)-17 producing regulatory T helper (Th17) cells

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Following the discovery of interleukin (IL)-17 producing T helper (Th17) cells as a distinct lineage of CD4<sup>+</sup> T helper cells it became clear that these cells play an important role in the host defense against extracellular fungal and bacterial pathogens and participate in the pathogenesis of multiple inflammatory and autoimmune disorders. Depending on the microenvironment, Th17 cells can alter their differentiation programme ultimately giving rise to either protective or pro-inflammatory pathogenic cells. We found that besides the conventional *in vitro* protocol for Th17 differentiation by transforming growth factor-beta (TGF- $\beta$ ) plus IL-6 cytokines, a combination of IL-23 plus IL-6 can also induce Th17 cells. The Th17 cells induced by IL-23 plus IL-6 (termed as effector Th17, Teff17 cells) are pathogenic upon adoptive transfer into non-obese diabetic (NOD) mice contributing to the development of type 1 diabetes (T1D) while cells induced by TGF- $\beta$  plus IL-6 (termed as regulatory T cells, Treg17 cells) are non pathogenic and regulatory, and suppressed the pathogenic T cells in T1D. These cells differentially expressed a number of cytokines where Teff17 cells exhibited an increase in granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-22 whereas Treg17 cells demonstrated increased expression of IL-21 and immunosuppressive cytokine IL-10. Differentiation of Th17 cells is controlled by a transcription factor, ROR $\gamma$ T although these cells also express variable levels of T-bet and FoxP3 transcription factors. This points to a dual functional role of Th17 subsets in autoimmune diseases particularly T1D. We suggest that similar to conventional regulatory T cells (Treg), induction of regulatory Treg17 cells could play an important role in modulating and preventing certain autoimmune diseases.

**Key words** Autoimmunity - cytokines - immunoregulation - helper T cell plasticity - regulatory T cells - Th17 cells - Type 1 diabetes

## Introduction

Type 1 diabetes (T1D) is a T cell mediated autoimmune disease in which insulin producing  $\beta$  cells are selectively destroyed in the pancreatic islets of Langerhans. Both CD4 and CD8 T cells have been implicated in T1D and the disease pathogenesis is primarily driven by CD4<sup>+</sup> Th1 (T helper type 1)

cells<sup>1</sup>. Several studies including our own have shown that CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (Treg) induced by various immunotherapy approaches are strongly protective in the NOD (non-obese diabetic) mouse model of T1D and in human subjects<sup>2-4</sup>. The pathogenic role of Th17 cells has been implicated in many autoimmune diseases but direct evidence

supporting their involvement in T1D pathogenesis is lacking<sup>5-7</sup>. Th17 cells infiltrating the pancreas of NOD mice showed plasticity and could alter their phenotype *in vivo*<sup>8</sup>. Moreover, interleukin (IL)-17 the signature cytokine produced by Th17 cells, is dispensable in the pathogenesis of autoimmune diabetes and NOD mice lacking IL-17 still develop T1D<sup>9</sup>.

### Pathogenic and protective Th17 cells in type 1 diabetes

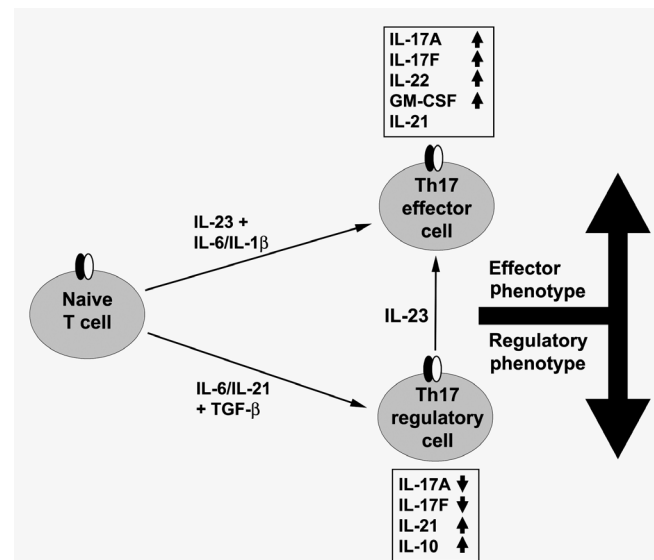
*In vitro* treatment of naive CD4<sup>+</sup> T cells with transforming growth factor (TGF)- $\beta$  alone induces Treg cells, while TGF- $\beta$  plus IL-6 generates Th17 cells<sup>10</sup>. It is not clear what drives the maturation of Th17 *in vivo* cells but is likely that commensal microbiota-derived metabolites may be involved in the induction of Th17 cells and also in mediating defense against pathogens<sup>11</sup>. Several studies point to the fact that *in vitro* generated Th17 cells can have a pathogenic and non-pathogenic phenotype. Th17 cells that have been matured with TGF- $\beta$  and IL-6 have high expression of the genes encoding the transcription factor ROR $\gamma$ T and cytokines IL-17A, IL-17F and IL-21 and IL-10. These can express both ROR $\gamma$ T and FoxP3 transcription factors and have been shown to be capable of immunoregulatory functions<sup>12,13</sup>. FoxP3 is a marker of highly immunosuppressive Treg cells<sup>14,15</sup> and Th17 cells that express both transcription factors have the capacity for immunoregulatory functions. The discovery of human IL-17-producing ROR $\gamma$ T<sup>+</sup>Foxp3<sup>+</sup>T cells that retain their ability to suppress effector lymphocytes supports the dichotomous nature of Th17 regulatory cells<sup>12,16-19</sup>. These studies suggest that cells co-expressing ROR $\gamma$ T and Foxp3 exist *in vivo* and are not simply an intermediate stage in the differentiation of inducible Treg or Th17 cells<sup>20</sup>. We have previously reported that NOD mice immunized with mycobacterial adjuvants, complete Freund's adjuvant (CFA) or BCG vaccine leads to the induction of similar regulatory Th17 cells. These cells produce IL-17, IL-22, IL-10, and interferon (IFN)- $\gamma$  and support the idea of regulatory Th17 cell existence *in vivo* in T1D prone NOD mice<sup>21</sup>.

Depending on the microenvironment, Th17 cells can alter their differentiation programme, ultimately giving rise to either protective or proinflammatory pathogenic cells. Besides the conventional protocol for Th17 differentiation by TGF- $\beta$  plus IL-6 cytokines, we found that a combination of IL-23 plus IL-6 can induce Th17 cells. The population of Th17 cells induced by IL-23 plus IL-6 and [termed as effector T (Teff17) cells]

were pathogenic upon adoptive transfer into NOD mice causing type 1 diabetes while cells induced by TGF- $\beta$  plus IL-6 (termed as Treg17 cells) were protective and suppressed the pathogenic T cells. The Teff17 cells differentially secreted granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-22 while Treg17 cells produced increased levels of IL-10 and IL-21 (Fig.). The identification of two distinct Th17 populations one with pathogenic properties (Teff17) and another with regulatory and protective properties (Treg17) may explain the controversial nature of Th17 cells in autoimmunity particularly T1D.

### Plasticity of Th17 cells

To determine the adoptive transfer capacity of Th17 cells in T1D two different approaches were used. The TGF- $\beta$  plus IL-6 polarized Th17 cells when transferred into wild type NOD mice promoted pancreatic inflammation but no overt diabetes<sup>6-8</sup>. In lymphopenic NODSCID (severe combined immunodeficiency) recipients Th17 cells mediated transfer of T1D but this was due to the plasticity of Th17 cells. It was found that Th17 cells converted into IFN- $\gamma$  producing Th1 cells in the lymphopenic environment of NOD.SCID mice<sup>21</sup>. Th17 cells generated *in vivo* have been found to co-produce IL-17 and IFN- $\gamma$  and these cells express Th1 transcription factor T-bet in addition to ROR $\gamma$ T.



**Fig.** Functionally distinct Th17 cell subsets are induced by different cytokines. Transforming growth factor-beta (TGF)- $\beta$  plus interleukin (IL)-6 promote the maturation of regulatory (Treg17) cells and IL-23 drives the pathogenic (Teff17) cells. These cells differentially produce increased ( $\uparrow$ ) or reduced ( $\downarrow$ ) levels of pro-inflammatory and/or regulatory cytokines.

The expression of IFN- $\gamma$  by Th17 cells is dependent on IL-23<sup>22</sup>.

### IL23 promotes effector pathogenic Th17 cells

IL-23 appears to be the key player in the terminal differentiation of Th17 cells and generation of pathogenic Th17 cells (Fig.)<sup>12,23</sup>. Treg17 cells can be converted to Tef17 cells by culturing with IL-23 *in vitro*. Several investigators have shown that TGF- $\beta$  plus IL-6 induced Th17 cells produce IL-17 but do not transfer autoimmunity. IL-23 is required for these cells to acquire a pathogenic phenotype<sup>10,12,24</sup>. This supports the results that IL-23 and IL-23R deficient mice are highly resistant to autoimmune diseases<sup>24</sup>. IL-23 has emerged as the key factor in determining if Th17 cells can become pathogenic during the differentiation process. This also influences the expression of pathogenic cytokines particularly GM-CSF. This effector phenotype is a result of the network of transcription factors that include ROR $\gamma$ T, T-bet and FoxP3. Treg17 cells produce variable amounts of IL-10 and IL-23 which diminish IL-10 production by Th17 cells while TGF- $\beta$  promotes the production of IL-10<sup>25,26</sup>. GM-CSF is important for the pathogenicity of Th17 cells and TGF- $\beta$  suppresses its production<sup>27,28</sup>. The proinflammatory cytokines IL-1 $\beta$  and IL-23 drive the production of pathogenic cytokine GM-CSF. A recent study has confirmed that IL-23-induced GM-CSF is essential for the pathogenicity of Th17 cells in the autoimmune model of multiple sclerosis (MS) namely experimental autoimmune encephalomyelitis (EAE)<sup>24</sup>. Moreover, Th17 cells that lack the ability to produce GM-CSF do not transfer autoimmune disease<sup>28</sup>. Thus the effector function of Tef17 cells is driven by GM-CSF via IL-23, and IL-10 drives the effector function of Treg17 cells via TGF- $\beta$ . In summary, high TGF- $\beta$  levels contribute to the maturation of Treg17 cells and IL-23 supports induction of Tef17 cells in autoimmunity.

### Conclusion

The initial focus of Th17 studies was to determine their pathogenic role in inflammatory and autoimmune diseases. In the case of T1D, the published work has provided conflicting results either supporting their role in the destruction of islet  $\beta$  cells or having no effect or protecting animals from adoptive transfer of disease. Recent studies support the regulatory function of Th17 cells induced by mycobacterial adjuvants *i.e.* BCG and CFA. The *in vitro* conditions used for the generation of the Th17 cells for these studies contribute

to the functional differences observed by various investigators. The Th17 subsets induced by TGF- $\beta$  and IL-6 and/or IL-23 produce immunosuppressive to proinflammatory cytokines. In addition, the plasticity of Th17 cells has been discovered where these can give rise to Th1 cells *in vivo*. Immunization with adjuvants suggests that it is possible to change the functional phenotype of Th17 cells from pathogenic to protective by altering the cytokine microenvironment. This may also be feasible by immunization with autoantigens to modulate autoimmune diseases. In summary, the role of Th17 cells in autoimmune diseases is not simply to be proinflammatory and cause tissue damage but these can also act in an immunoregulatory fashion leading to prevention of autoimmunity by inhibiting pathogenic T cells.

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