

## The role of T helper 9 (Th9) against infectious diseases

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### ABSTRACT

**Background and aims:** Infectious diseases are disorders caused by organisms such as bacteria, viruses, fungi or parasites. The Th9 subset develops in response to combined signals from TGF- $\beta$  and IL-4 among a cacophony of other cytokines in an extracellular milieu. T helper 9 (Th9) cells, as a novel CD4 T cell subset, seem to play a complex role in the outcome of specific immune responses. In this article, we aimed to review the role of these cells in infectious disease.

**Methods:** In this mini-review study, we study 25 novel articles since 2009 to 2014 about the role of T helper 9 in some Infectious Diseases.

**Results:** Pleural mesothelial cells promoted Th9 cell differentiation by presenting antigen. It significantly differentiated Th17, but not Th9 cells in the development of CVB3-induced VMC. The microenvironment of VMC seemed to contribute to the differentiation and proliferation of Th17 rather than to differentiation of Th9 cells. Having reviewed the limited number of articles considering this relevance, we came to this result that Lymphatic Filariasis and mycobacterium tuberculosis infections confirmed the existence of such relationship. In addition, Rapamycin resistant murine Th9 cells have a stable in vivo phenotype and inhibit graft-versus-host reactivity but concerning Viral Myocarditis, Th9 cells could not protect against it.

**Conclusion:** The accurate molecular mechanisms underlying the generation and differentiation of human Th9 cells are not elucidated completely. Th9 cells exhibit Ag specific expansion in a chronic helminth infection (lymphatic filariasis), but in relevance to viral myocarditis, Th9 cells did not play an efficient role against it. However; knowing that whether Th9 cells participate in the protection against infections needs further research.

**Keywords:** T helper 9, Infections, Helminthes, Mycobacterium tuberculosis.

### INTRODUCTION

Infectious diseases are disorders caused by organisms such as bacteria, viruses, fungi or parasites. Many organisms live in our

bodies and on our skin. They're normally harmless or even helpful, but under the certain conditions, some organisms may cause

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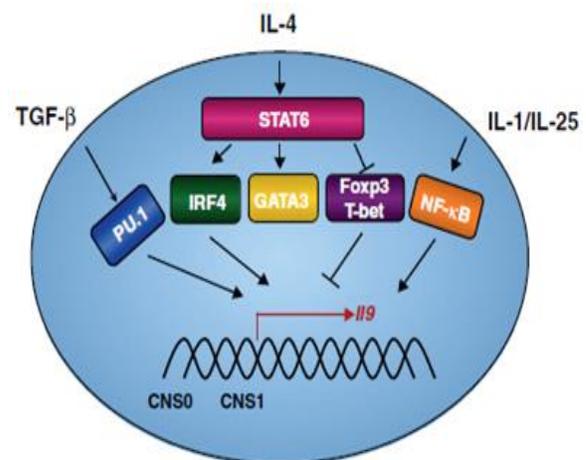
disease. Some infectious diseases can be passed from person to person. Some are transmitted by bites from insects or animals. Some others are acquired by ingesting contaminated food or water or being exposed to organisms in the environment. The specialized cytokine secretion profiles of T helper (TH) cells are the basis for a focused and efficient immune response.<sup>1</sup> CD4+T helper subsets can differentiate various lineages in response to environmental cues such as cytokines or ligand interactions. Of the various lineages of T helper subsets, Th9 cells are a recent addition. Th9 cells develop from CD4 precursors in response to TGF- $\beta$  and IL-4 and are primarily characterized by their increased secretion of IL-9.<sup>2</sup> IL-9 is critical for defence against invasion by helminthes and it is conceivable that certain unique features that characterize this cytokine and the cells that produce it are related to this primordial function. While IL-9 producing CD4+T cells were previously classified as Th2 cells, recent studies have shown that Th9 cells are a distinct lineage of T helper cells, Th9 cells have been implicated in various disease processes that have been associated with Th2 cells, including: helminthic infection, allergy and asthma.<sup>2-8</sup> In this article, we aimed to review the role of these cells in infectious disease. Having reviewed the limited number of articles considering this relevance, we came to this result that Lymphatic Filariasis and mycobacterium tuberculosis infections confirmed the existence of such relationship. In addition, Rapamycin resistant murine Th9 cells have a stable in-vivo phenotype and inhibit graft-versus-host reactivity but concerning Viral Myocarditis, Th9 cells could not protect against it. Further studies are, therefore warranted to determine the effect of the Th9 cells in infection.

Naive CD4+T helper (Th) cells, after encountering specific antigen, become activated and differentiate into T helper subsets effectors, each characterized by a

distinct pattern of cytokine secretion and function, including Th1, Th2 and Th17.<sup>9</sup> Recently, a new subset of CD4+T helper cells that predominantly secrete cytokine interleukin-9 (IL-9) is identified, termed Th9 cell.<sup>10</sup> It has been reported to participate in tissue inflammation and immune-mediated diseases ranging from autoimmunity to asthma.<sup>11</sup>

In this review, we propose to investigate the relationship between Th9 and different infections including viral, bacterial and fungi infections. At first, we review some mechanisms of differentiation and function of Th9, as well as their regulation, and then we go over some studies investigating the role of Th9 in some infectious disease.

The Th9 subset develops in response to combined signals from TGF- $\beta$  and IL-4 among a cacophony of other cytokines in the extracellular milieu.<sup>12</sup> The transcriptional network that regulates Th9 development includes TGF $\beta$ -induced Sfp1, and IL-4-induced STAT6 that induces IRF4 as it represses Foxp3 and T-bet (Figure 1).



**Figure 1:** Transcriptional network in Th9 cells

Transcription factors including PU.1, downstream of TGF- $\beta$  signals, and IL-4-activated STAT6 that promote expression of GATA3 and IRF4 contribute to the expression of the II9 gene in Th9 cells.<sup>13</sup>

Additional transcription factors, possibly downstream of them and additional cytokines, undoubtedly harmonize in efficient transcription of the IL9 gene.<sup>8</sup> IL-9 promotes inflammation by stimulating growth of hematopoietic cells, particularly mast cells, and the secretion of factors including chemokines that recruit additional cells to inflamed sites.<sup>14</sup> Th9 cells are capable of promoting autoimmune inflammation, although whether Th9 cells are required as a source of IL-9 for autoimmune inflammation is not still clearly established. Among the obstacles to defining these functions is the lack of a more detailed understanding of sensitization conditions that prime IL-9-producing T cells.<sup>15</sup> More evidence supports the important role for Th9 cells in allergic inflammation, but how Th9 cells contribute to allergic disease, and how they cooperate with Th2 cells in promoting inflammation is the focus of ongoing investigations.<sup>16</sup>

A study carried out by Zhi-Jian Ye and et al investigated the differentiation and recruitment of Th9 cells stimulated by pleural mesothelial cells in human mycobacterium tuberculosis infection. Their data showed that increased Th9 cells with the phenotype of effector memory cells were found to be intuberculous pleural effusion as compared with blood.<sup>17</sup> Majority of these pleural Th9 cells displayed the phenotype of effector memory cells. TGF- $\beta$  was essential for Th9 cell differentiation from naive CD4+ T cells stimulated with PMA and ionomycin in vitro for 5 h, and more addition of IL 1 $\beta$ , IL-4 or IL-6 leads to augmented of Th9 cell differentiation.<sup>18</sup> The findings of this study also showed that the numbers of Th9 cells in TPE positively correlated with that of Th17 cells, but not of Th1, Th2, or Tregs. It is supposed that IL-9 together with TGF- $\beta$  promoted Th17 differentiation from naive CD4+T cells might account for this correlation.<sup>19</sup> Tuberculous pleural effusion and

supernatants of cultured pleural mesothelial cells were chemotactic for Th9 cells.<sup>20,21</sup> IL-9 significantly improved PMC wound healing and long term restoring and inhibited IFN- $\gamma$ -induced PMC apoptosis.<sup>22</sup>

Moreover, in their study, pleural mesothelial cells promoted Th9 cell differentiation by presenting antigen. Collectively, these data provide new information concerning Th9 cells, in particular the collaborative immune regulation between Th9 cells and pleural mesothelial cells in human *M. tuberculosis* infection.

In particular, pleural mesothelial cells were able to function as antigen-presenting cells to stimulate Th9 cell differentiation.<sup>17</sup>

In another study with the subject of distinct different expression of Th17 and Th9 cells in coxsackie virus B3-induced mice viral myocarditis (VMC), Kong Qing et al. have reported that it significantly differentiated Th17, but not Th9 cells elevated in the development of CVB3-induced VMC. The microenvironment of VMC seemed to contribute to the differentiation and proliferation of Th17 rather to differentiation of Th9 cells. Their preliminary data implied that Th9 cells could neither protect against VMC nor promote the disease because no change of Th9 cells had been observed in VMC.<sup>23</sup>

Some research studies have initiated an evaluation of the in vitro phenotype and in vivo stability of rapamycin resistant Th9 cells during graft-versus-host-reactivity.<sup>24</sup> However, it is not known that whether Th9 differentiation occurs in the presence of rapamycin or adoptively transferred donor Th9 cells would augment or restrict allo reactivity after experimental bone marrow transplantation.<sup>25</sup>

They found that CD4+ T cells that were co-stimulated and polarized with TGF- $\beta$  and IL-4 in the presence or absence of rapamycin each yielded effector cells of Th9 phenotype that secreted increased IL-9 and expressed a

transcription factor profile characteristic of both Th9 and Th2 cells (high GATA-3/low T-bet).<sup>24</sup> Augmentation of T cell replete allografts with manufactured rapamycin resistant Th9 cells markedly reduced both CD4+ and CD8+ T cell engraftment and strongly inhibited allo-specific T cell secretion of IFN- $\gamma$ .<sup>26</sup> The potency of Th9 cell inhibition of alloreactivity was similar to that of rapamycin resistant Th2 cells. Importantly, rapamycin resistant Th9 cells persisted and maintained their cytokine phenotype, thereby indicating limited differentiation plasticity of the Th9 subset.<sup>27</sup>

As such, Th9 differentiation proceeds in the presence of rapamycin to generate a cell therapy product that maintains high IL-9 expression *in vivo* while inhibiting IFN- $\gamma$  driven alloreactivity.<sup>24</sup>

A study was carried out about the association between IL-4-, TGF- $\beta$ -, and IL-1-dependent expansion of parasite antigen-specific Th9 cells and clinical pathology in human lymphatic filariasis.

This study demonstrates that these Th9 cells exhibit Ag-specific expansion in a chronic helminth infection (lymphatic filariasis). Comparison of Th9 responses reveals that individuals with pathology associated with filarial infection exhibit significantly expanded frequencies of filarial Ag-induced Th9 cells, but not of IL9+ Th2 cells in comparison with filarial-infected individuals without associated disease.<sup>28</sup> Moreover, the per cell production of IL-9 is significantly higher in Th9 cells compared with IL9+ Th2 cells, indicating that the Th9 cells are the predominant CD4+ T cell subset producing IL-9 in the context of human infection.<sup>29</sup> This expansion was reflected in elevated Ag-stimulated IL-9 cytokine levels in whole blood culture supernatants.<sup>30</sup> Finally, the frequencies of Th9 cells correlated positively with the severity of lymphedema (and presumed inflammation) in filarial-diseased

individuals.<sup>31</sup> This expansion of Th9 cells was dependent on IL-4, TGF- $\beta$ , and IL-1 *in vitro*. They have therefore identified an important human CD4+ T cell subpopulation coexpressing IL-9 and IL-10, but not IL-4, the expansion of which is associated with disease in chronic lymphatic filariasis and could potentially have an important role in the pathogenesis of other inflammatory disorder.<sup>28</sup>

## CONCLUSION

Th9 cells are a new subset of T helper cells, and the signature cytokine for Th9 cells is IL-9. Both Th9 cells and Th9 products are implicated in multiple disease settings. There are different molecular pathways identified thus far in the induction of Th9 cells, and activation of such diverse pathways requires integration of signals from TGF- $\beta$  and IL-4 cytokine receptors as well as costimulatory molecules. These signals converge on the induction of multiple transcription factors that collectively drive the development of Th9 cells. However, the accurate molecular mechanisms underlying the generation and differentiation of human Th9 cells are not elucidated completely. Th9 cells have been shown to be important in allergy, autoimmunity, and antitumor responses. However, their role in human infectious diseases has not been explored in detail. Although some studies have demonstrated that Th9 might elicit inflammation<sup>13</sup> and contribute to the development of allergic diseases.<sup>14,15</sup> For example, the numbers of Th9 cells with the phenotype of effector memory cells in TPE (tuberculous pleural Effusion) were significantly increased when compared to their compartments in blood. Some studies indicated that the Th9 cells are the predominant CD4+ T cell subset producing IL-9 in the context of human infection. They significantly improved PMC wound healing and long term restoring and inhibited IFN- $\gamma$ -induced PMC apoptosis.<sup>32</sup> Moreover, Th9 cells exhibit Ag-specific expansion in a

chronic helminth infection (lymphatic filariasis), but in relevance to viral myocarditis, Th9 cells did not show efficient role against it. Anyway, whether Th9 cells participate in the protection against infections warrants further research. For obtaining more concise results, it is suggested to study and compare more articles in this field and compare the signals pathway of Th9 in various infectious diseases.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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