Regulation of Memory T Cells by Interleukin-23

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Introduction

Cytokine immunity plays an essential role in the maintenance of health and in the development of clinical therapies [1, 2]. A great number of cytokines have been identified in humans, and their functions have been widely studied in animal models and human cells. Several cytokines have proved effective in the clinical treatment of specific diseases [3–7]. Understanding the molecular mechanism of cytokine function will help us better examine and treat different disease conditions.

Immune cells, such as T cells, B cells and dendritic cells, play critical roles in cytokine immunity [1]. Among them, memory T cells are a subset of specific T cells that have previously encountered their cognate antigen (e.g. during a previous infection) and elicited a response [8]. Memory T cells have critical functions in the immunological response to infection and cancer [9]. The study of the regulation of memory T cell function is thus a very promising area of research focused on the development of novel clinical treatments for inflammatory and infectious diseases as well as cancer.

Interleukin-23 (IL-23) was discovered by Oppmann et al. [10] in 2000. Its receptor, IL-23R, was identified 2 years later [11]. Since then, the expression and functions of IL-23 and its receptor have been extensively studied [12–14]. IL-23 has been used as a clinical indicator for a number of diseases, such as psoriasis and rheumatoid arthritis [15, 16], and has also been studied as a potential target for the
development of clinical treatments for some of these diseases [17, 18]. Although several reviews have covered the functions of IL-23 [19, 20], here, we review the current progress in the study of its role in the regulation of memory T cells.

**Discovery of IL-23**

In 2000, Oppmann et al. [10] searched DNA sequence databases with a computationally derived profile of members of the human IL-6 cytokine family, and identified a novel cytokine sequence which they called p19. p19 cDNA (encoding a 189/196 amino acid polypeptide of 18.7/19.8 kDa in humans and mice, respectively) shares 70% identity between human and mice and shows high protein similarity with the IL-12 p35 subunit [10]. Oppmann et al. [10] found that p19 required coexpression of the IL-12 p40 subunit to be secreted and biologically active, and named this heterodimeric cytokine IL-23. The main source of IL-23 is activated dendritic cells; IL-23 specifically functions on memory T cells [10]. In IL-23-deficient (p19−/−) mice, significantly low levels of antigen-specific IgG2a and IgA indicate a disruption of the humoral immunity [12]. Consistently, T cell-dependent, delayed-type hypersensitivity was significantly inhibited in these mice, indicating that the absence of IL-23p19 was associated with a reduction in the number of CD4+ memory T cells and a deficient immunological response [21]. These studies suggest that IL-23 plays a critical role in the regulation of memory T cell function.

**Discovery of the IL-23 Receptor**

IL-23R was discovered in a screen for subunits that could interact with IL-23 p19 using IL-12β1-expressing BaF3 cells [11]. A polypeptide similar to IL-12β2 was identified that was able to form a heterodimeric receptor with IL-12β1 [11]. This polypeptide, named IL-23R, specifically interacts with IL-23, and its expression on the cell surface of specific cells defines the functional targets of IL-23 [11]. Human (h)IL-23R is expressed in Th1 and Th0 cells as well as in a variety of natural killer cell lines [22]. Several types of monocytes and dendritic cells express extremely low levels of hIL-23R. Epstein B virus-treated B cells and anti-CD3/anti-CD28/LPS-stimulated peripheral blood mononuclear cells also express relatively low but still detectable amounts of hIL-23R mRNA. The hIL-23R expression pattern is similar to that of hIL-12Rβ2. On the other hand, mouse (m)IL-23R is expressed in polarized Th1 and Th2 cells and bone marrow dendritic cells. However, the macrophage cell line J774 and several mast cell lines show no detectable expression of mIL-23R mRNA. The different expression patterns observed for IL-23R in human and mouse cell lines suggest that IL-23 may have unidentified dissimilar functions in humans and mice [11]. IL-23 is the main factor involved in the induction of differentiation of naïve T cells into Th17 cells. IL-23R is highly expressed in Th17 cells in both human and mice [23–26].

In mice, memory (CD4+CD45RBlow) T cells express IL-23R mRNA, but very low amounts of IL-12Rβ2. In contrast, naïve (CD4+CD45RBlow) T cells express IL-12Rβ2, but show little or no IL-23R expression [11]. Similarly, human memory (CD4+CD45RA−CD45RO+) T cells express IL-23R, but human naïve (CD4+CD45RA+CD45RO−) T cells express very small amounts of IL-23R [27, 28]. These observations may explain why memory T cells respond to IL-23 treatment but show weak or no response to IL-12 treatment. On the other hand, naïve T cells show strong response to IL-12 treatment but no response to IL-23 treatment [22].

**IL-23 Promotes Proliferation of Memory T Cells**

Oppmann et al. [10] reported that IL-23 could promote mouse memory T cell proliferation. They used a p40-p35 fusion protein with similar activity to IL-12 to treat mouse naïve and memory T cells in vitro. They observed proliferation of naïve (CD4+CD45RBlow) T cells, but the memory T cells were not significantly stimulated. In contrast, when they used a Hy-p40-p19 fusion protein, with similar activity to IL-23, proliferation of memory (CD4+CD45RBlow) T cells, but not naïve T cells, was strongly induced. Moreover, an anti-IL-12p40 neutralizing antibody decreased the effect of Hy-p40-p19 on memory T cells 100-fold. Consistently, addition of the supernatant from 293T cells cotransfected with p19 and p40 induced a similar proliferative response in mouse memory T cells to that promoted by p19-p40 fusion proteins [10]. Furthermore, in human memory T cells, Hy-p40-p19 promoted stronger proliferation of CD45RO+ T cells than CD45RA+ T cells, while IL-12 showed a similar effect on both CD45RO+ and CD45RA+ T cells [10]. Importantly, IL-23-mediated proliferation of memory T cells was dose-dependent [28]. Contrary to these observations, a study of IL-23R gene polymorphisms found that IL-23 could not promote proliferation of CD4+CD45RO+
T cells in normal human peripheral blood in vitro [29]. It was also reported that cord blood naïve T cells could be polarized toward a Th17 phenotype upon IL-23 stimulation.

The contradictory results of these studies may be attributable to the use of different doses of IL-23, cell activation methods and/or incubation times.

Memory T cells hyperproliferate during disease; therefore, the role of IL-23 in the promotion of memory T cells may be important for the immunological response to certain diseases. For example, gastric cancer patients classified as stage I using the TNM cancer staging notation system showed higher levels of IL-23 in affected tissues than patients at TNM stages II, III and IV. Meanwhile, in gastric carcinoma, tumor-infiltrating lymphocytes, the number of memory CD4+ T cells and the proportion of effector memory T cells decreased at higher TNM stages. IL-23 levels were positively correlated with the number of effector memory T cells, suggesting that IL-23 promoted memory T cell proliferation in gastric cancer patients [30].

IL-23 Induces Memory T Cell Secretion of IL-17

Memory (CD4+CD45RO+) T cells secrete IL-17 in normal peripheral blood after activation in vitro [31]. Addition of IL-23 alone to cultured memory (CD4+CD45RO+) T cells induces a slight increase in IL-17 secretion [27]. However, when other factors, such as activating anti-CD3/anti-CD28, are added together with IL-23, the level of IL-17 secreted from memory T cells significantly increases [23, 28, 29]. Consistently, intracellular IL-17 mRNA levels significantly increase in these conditions [31].

Further analysis of memory T cells has shown that different memory T cell subsets secrete diverse levels of IL-17. After activation, memory (CD4+CD45RO+IL-23R+) T cells produce more IL-17A than IL-23R− cells. CD4+CD45RO+IL-23R+ cells also express higher levels of IL-17A, IL-17F, IL-22, IL-26 and CCL20 mRNA than IL-23R− cells, as shown by real-time PCR [28]. These results support the notion that the interaction between IL-23 and IL-23R is key for IL-23 function in memory T cells.

Memory T cells can be further subdivided into central type memory (CD4+CD45RO+CCL20+) T cells (TCM) and effector memory (CD4+CD45RO+CCL20+) T cells (TEM). TEM cells are the main source of IL-17, with levels of IL-17 secretion 4-fold higher than those of TCM cells. The addition of neutralizing antibodies against Th1 cytokine IFN-γ and Th2 cytokine IL-4 does not affect the amount of IL-17 secreted by memory T cells or the number of IL-17-producing cells [31]. TCM and TEM cells can be further divided into 2 subgroups according to the presence or absence of the CCR6 marker. In both CCR6+ and CCR6− TEM cells, IL-23 promotes IL-17 secretion. However, IL-23 does not promote IL-17 secretion in either CCR6+ or CCR6− TCM cells [31]. Three types of TEM cells have been described: those that secrete only IL-17, those that secrete IL-17 and IFN-γ and those secreting IL-17 and IL-22. IL-23 promotes the proliferation of all 3 CCR6+ TEM types 2-fold. In contrast, IL-23 is able to promote the proliferation of CCR6− TEM 6-fold.

Naïve (CD4+CD45RA+) T cells do not normally secrete IL-17 and can only secrete very small amounts of IL-17 in the presence of IL-23 from dendritic cells [31, 32]. However, to mimic and induce the immune response, the addition of thymic stromal lymphopoietin and Toll-like receptor 3 ligand poly (I:C)-treated dendritic cells to naïve T cells for 7 days induces naïve (CD45RA+) T cells to differentiate into IL-17-secreting (CD45RA−CD45RO+CD62L+CCR7+) TCM cells. Stimulation of these cells with anti-CD3 and anti-CD28 in the presence of IL-2 for 3 days induces them to display a (CD45RA−CD45RO+CD62LlowCCR7low) TEM phenotype. These results indicate that, in the presence of IL-23, naïve T cells can differentiate into Th17 memory T cells [33]. The effect of IL-23 on Th17 cell generation has also been observed in vivo [34, 35]. Although naïve T cells express low levels of IL-23R, the presence of IL-23 could increase the IL-23R expression level by simultaneous activation with anti-CD3 and anti-CD28. This effect could be inhibited by Th17 culture conditions (TGF-β+ IL-6, anti-IFN-γ and anti-IL-4); however, Th0 (neutral), Th1 (IL-12 and anti-IL-4) and Th2 (IL-4, anti-IL-12 and anti-IFN-γ) culture conditions did not affect the expression of IL-23R [27]. These studies strongly suggest a function for IL-23 in the regulation of IL-17 secretion.

The induction of memory T cell IL-17 secretion by IL-23 has been observed in a number of diseases. In systemic sclerosis, the number of IL-17-secreting CD45RO+ and CD45RA+ cells is significantly increased, with the former expressing significantly higher levels of IL-17 than the latter, as shown by median fluorescence intensity detection, suggesting that the induction of IL-17 secretion by IL-23 is stronger in memory T cells [36]. In Behçet’s disease associated with uveitis, a higher serum level and higher mRNA expression level of IL-23 were observed. Additionally, recombinant IL-23 could increase the amount of IL-17 secreted from peripheral blood mononuclear cells in patients compared to healthy controls, suggesting that IL-23 may...
promote the production of IL-17 by memory T cells in this disease [37]. In addition, IL-23 was able to promote IL-17 secretion by Th17 cells, which promotes the gathering of neutrophils to the airways during asthma [38].

**IL-23 Induces Memory T Cell Secretion of IFN-γ**

In healthy people, activated memory (CD4+CD45RO+) T cells secrete moderate amounts of IFN-γ (fig. 1) [10]. While CD4+CD45RO+CCR7− TEM cells are the main IFN-γ-secreting cell population, TCM cells secrete low amounts of IFN-γ [31]. In the absence of anti-CD3 and anti-CD28 stimulation, neither Hy-p40-p19 nor IL-12 can induce secretion of IFN-γ in CD45RO+ T cells [10]. After the addition of activating factors, IL-23 can induce memory (CD4+CD45RO+) T cells to secrete IFN-γ in a dose-dependent manner [22, 28, 29, 39]. After treatment with IL-23 for 3 days, CD45RO+ T cells secrete large amounts of IFN-γ [10]. Even though IL-23 can induce IFN-γ-secretion via both CD4+ and CD8+ memory T cells, its effect is more significant on CD4+ memory T cells than on CD8+ memory T cells. IL-23 induction of memory T cells to produce IFN-γ could be inhibited by the Th2 cytokines IL-4, IL-10 and IL-12Rβ1 [40]. Interestingly, phosphorylation of ERK1/2 and p38 MAPK is increased in dendritic cells concomitantly with the promotion of IFN-γ secretion by memory (CD4+CD45RO+) T cells by IL-23, suggesting that these kinases are important signaling components of this pathway [41].

IL-23-mediated induction of IFN-γ secretion by memory T cells is also important in a number of diseases. IL-23 promotes the proliferation of CD4+ and CD4+CD45RO+ cells in vitro and induces IFN-γ secretion more pronouncedly in cells from tuberculosis patients than in healthy cells. Mice infected with *Schistosoma mansoni* were found to exhibit Th1 immune response against SEA (*Schistosoma* egg antigens) in CFA (complete Freund's adjuvant) and elevated the level of IFN-γ, which caused worse lesions. After infection with *S. mansoni*, IL-12p40−/− mice, which cannot produce IL-23 and IL-12, were incapable of eliciting a pathological response. SEA-stimulated lymphocytes could not produce significant amounts of IFN-γ or IL-17. In contrast, SEA/CFA-immunized IL-12p35−/− mice showed severely increased levels of IL-17 and IFN-γ, associated with the hyperproliferation of activated memory CD4+ T cells that secreted high levels of IL-17 and IFN-γ, playing a catalytic role during inflammation and pathological changes.

It should be noted that IL-23 is not the only factor capable of promoting IFN-γ secretion by memory T cells. In the presence of IL-2, IL-23+CD4+CD45RO+ and IL-23R−CD4+CD45RO+ T cells activated with anti-CD3, anti-CD28 and anti-CD2 produce large amounts of IFN-γ [28]. Even though Oppmann et al. [10] did not detect IFN-γ expression by CD45RA+ T cells stimulated with anti-CD3 and anti-CD28 antibodies, Liu and Rohowsky-
Kochan [31] detected IFN-γ in cell supernatants from CD4+CD45RA+ T cells (which do not produce IFN-γ if not activated) after T cell receptor/anti-CD28 activation. Similarly, IL-12 significantly increased IFN-γ levels by activation of CD45RO+ T cells [10]. Furthermore, IL-23 promoted IFN-γ secretion not only in memory T cells but also in naïve T cells. CD45RA+ T cells secreted moderate amounts of IFN-γ 6 days after addition of IL-23 (in the absence of other activators), indicating that naïve T cells can respond to IL-23 after prolonged stimulation [10].

In addition to promoting IL-17 and IFN-γ secretion, IL-23 also functions in the production of other cell factors, such as GM-CSF in mice [43] and humans [44].

**IL-23 Clinical Applications**

The increase in the expression of IL-23R on the cell surface of memory T cells during the immunological response to some diseases suggests a specific role for IL-23 in disease response. For example, in patients with systemic sclerosis, CD3+, CD45RO+ and CD45RA+ cells in peripheral blood show increased levels of IL-23R [36]. In a number of diseases, memory T cells in the local lesion show higher levels of IL-23R than those in the circulating blood. In patients with arthritis, >95% of CD4+ T cells in the synovial fluid of local joints are memory CD45RO+ T cells. IL-23R-expressing CD4+CD45RO+ T cells increase by 34.1%. In contrast, only 40% of CD4+ T cells are memory CD45RO+ T cells in the peripheral blood. The percentage of IL-23R-expressing CD4+ memory T cells is lower (33.3%) than that in synovial fluid [45], suggesting a role of IL-23 on memory T cells for local inflammation.

Complexity arises when we consider IL-23R gene polymorphisms. These have been associated with changes in memory T cells numbers, proliferation and cytokine secretion [29]. R381Q (c.1142G>A; p.R381Q) is one of the most studied IL23R gene polymorphisms and has been associated with a protective role in many inflammatory diseases. The effect of the R381Q IL23R gene polymorphism on memory CD4+ T cells can be summarized as follows: individuals carrying the R381Q IL23R gene polymorphism contain fewer memory T cells. Peripheral memory T cells (either R381Q or wild-type IL23R) did not proliferate in vitro, regardless of IL-23 presence. Peripheral R381Q IL23R memory T cells secreted significantly lower amounts of both IL-17 and IL-22 in vitro after IL-23 administration and during allergen stimulation. However, IFN-γ secretion by R381Q IL23R memory T cells was not affected. Additionally, the presence of R381Q IL23R gene polymorphism has been associated with unresponsiveness to IL-23 by human memory Th17 cells, impairing the Th17 response in psoriasis patients [46].

One of the future challenges in the development of clinical applications for IL-23 will therefore be focused on overcoming the complexity of the interaction between IL-23 and its receptor.

**Perspective**

Memory T cells play critical roles in a variety of diseases. IL-23 can promote the proliferation of memory T cells and the secretion of related cytokines (fig. 2). IL-23 is thus likely to play a regulatory role in a number of diseases. Further validation and in-depth study of the mechanisms of IL-23 function in physiology and during disease will likely lead to the development of therapeutic approaches for the treatment of memory T cell-associated inflammatory diseases and cancers.

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**Disclosure Statement**

The authors declare there are no conflicts of interest.