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Review Article

IL-35- and IL-10-Dependent Immunoregulatory Lymphocytes in Rheumatoid Arthritis

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ABSTRACT

Several studies investigated the different therapeutic roles of T and B lymphocytes subsets in Rheumatoid Arthritis (RA). The disease pathogenesis implicates various cells subpopulations regarding T_H1 and T_H17 cells that produce pro-inflammatory cytokines as well as autoantibodies production by B cells. On the other hand, certain T and B lymphocytes negatively regulate the immune response by producing regulatory cytokines or by interaction with pathogenic subsets. Recent studies investigate that the therapeutic function of these cells is exerted by IL-10, IL-35 and TGF-β regulatory cytokines production. IL-35 showed to restrict T_H1/T_H17 differentiation and function, and expand the suppressive function of T_{Reg} and B_{Reg}. Furthermore, IL-35 promotes the conversion of T_{Reg} and B_{Reg} to IL-35-producers (iTr35 and IL-35$^+$ B_{Reg}, respectively). B cell-derived IL-10 not only Suppresses IL-6 and IL-12 production by dendritic cells, but also it indirectly effects on T cell response by suppress the innate immunity. This review focuses on the current knowledge of the immune regulatory modulations of IL-10 and IL-35 to understand the potential therapeutic consequences of RA for further prospective studies.

Keywords: Rheumatoid arthritis; IL-10; IL-35; iTr35; IL-35$^+$ B_{Reg}; Cytokines; Immunoregulation

INTRODUCTION

Rheumatoid Arthritis (RA) is a progressive inflammatory autoimmune disease with articular and systemic effects with unknown cause but recent findings suggest a genetic basis for disease development [1]. The rheumatoid joint contains numerous cell types that are involved in these inflammatory and destructive processes [2]. Synovitis is caused by the influx or local activation, or both, of mononuclear cells (including T cells, B cells, etc.) and by angiogenesis. The synovial lining then becomes hyperplastic, and the synovial membrane expands and forms villi. The osteoclast-rich portion of the synovial membrane, or pannus, destroys bone, whereas enzymes secreted by neutrophils, synoviocytes and chondrocytes degrade cartilage [3].

T cells, B cells and the orchestrated interaction of pro-inflammatory cytokines play key roles in the pathophysiology of RA [3,4]. The cytokines most directly implicated in this process are TNF-α and IL-6; IL-1 and IL-17 may also play important roles in the disease prognosis [3]. To prevent inflammatory diseases, some therapeutic methods including chemical drugs (Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)), molecular target drugs (Disease-Modifying Anti-Rheumatic Drugs (DMARDs)), and surgery have been developed. However, these methods revealed many problems; i.e., high cost and insufficient effect [5,6].

There is thus a need to investigate more effective treatments for RA. Several studies investigate the negative regulatory function of Interleukin-35 (IL-35) and IL-10 in autoimmune diseases. B cells generally considered to have dual regulatory functions. Their capability to produce antibodies, antigen presentation as well as other functions in immune responses modulation, make them as positive regulators. However, different B cell subsets i.e. $B_{Reg,}$ can also negatively regulate immunity either by cell contact-dependent mechanism or by regulatory cytokines secretion [7]. B cells functions have been observed in mice lacking B cells which suffered from severe Encephalomyelitis (EAE), a mouse model indicated the regulatory properties of B cells [8]. Furthermore, in Ra patients, psoriasis was developed after B cell depletion with rituximab [9]. This regulatory function was primarily associated with IL-10 as it protects from autoimmune diseases [10, 11] until IL-35-producing B cell have been identified as a negative regulator [11]. This was confirmed by IL-10-independent immune inhibition in mouse models [12,13].

IL-35 regulatory roles had been investigated severally in RA models. It showed to induce different Regulatory T cell (T_{Reg}) cell subsets which have suppressive activity against pro-inflammatory mediators. On the other hand, IL-35 dealt with Helper T cell (T_H17) proliferation inhibition as well as attenuation of RA progression [14-17].

Natural regulatory T cells (nT_{Reg}) were specifically identified as a CD4$^+$ CD25$^+$ Foxp3 T cells [18] and were found to suppress effector immune responses including CD4$^+$ and CD8$^+$ T cells, dendritic cells as well as macrophages. However, nT_{Reg} considers a mediator of self-tolerance, it is not the primary one encountered in the periphery where another cells class were identified as inducible T_{Reg} (iT_{Reg}) to mediate tolerance against pathogens and other antigens. Either, it has been recorded that T_{Reg} and B_{Reg} cells expanded and converted into a subpopulation iTr35 [19]; and IL-35$^+$ B_{Reg} cells by the action of IL-35, a potent cytokine generated from both T_{Reg} and B_{Reg} cells [20].

IL-35-DEPENDENT REGULATION

IL-35 is the latest member of IL-12 family of heterodimeric cytokines. Opposed to other family members, IL-35 was identified as a potent immunosuppressive cytokine [19,21]. It composes of Ebi3 (Epstein-Barr virus-induced gene 3), a β chain subunit encoded by IL27b, and p35 α subunit encoded IL12α [22,23]. IL-35 is signaling through a unique IL-12R β 2:gp130 heterodimer receptor, gp130:gp130 or IL-12R β 2:IL-12R β 2 homodimers. This signaling pathway depends on STAT1 and STAT4 transcription factors that bind to specific sites on IL27b and IL12 α promoters and promote their transcription. Thus, the anti-inflammatory capability may be referred to the STAT1:STAT4 heterodimerization [19].

Another study revealed that IL-35 signals activates STAT1, STAT3 and STAT4 in T cells while STAT1 and STAT3 are preferentially activated in B cells [20]. It was reported that IL-35 be secreted by T_{Reg} [24,22] and activated B_{Reg} [11] (Table 1). As well, IL-35 promotes the suppressive function of T_{Reg} and optimize homeostasis *in vitro* and *in vivo* [22], as it induces naïve T cells conversion into iTr35 cells that maintain immune tolerance [24]. IL-35 production by B cells (IL-35$^+$$B_{Reg}$) was triggered by the high active effector CD4$^+$T cells and macrophages beside their role as Antigen Presenting Cells (APCs) [11], which results in further B cells differentiation into B_{Reg} that secret IL-10 [11,20].

IL-35/ T_{Reg} / iTr35

iT_{Reg} be activated from naïve one in an immunosuppressive environment in the periphery then, they gain their suppressive and immune regulatory functions. They maintain their functional mechanisms through various surface molecules such as CTLA-4 or PD-1 while other populations mediate the suppressive role by

Table 1: IL-35 producing cell subsets.

Cell type	Phenotype	Features	Reference
nT_{Reg}	$CD4^+CD25^+Foxp3$	- Suppress $CD4^+$ and $CD8^+$ T cells, DCs and macrophages. - Mediate self-tolerance.	[18]
T_{Reg}	$CD8^+CTLA\text{-}4^+$	- Suppress effector T cells	[24,33]
iT_{Reg}	iTr35	- IL-35-dependent stimulation - Suppress $CD4^+$ T cells	[29]
Plasma cell	$CD138^{hi}$ $B220^+$	- Suppress effector T cells - Suppress NK cells	[11]
B_{Reg}	$IL\text{-}35^+$	- IL-35- and IL-10-dependent stimulation - Suppress effector T cells	[20]

utilizing TGF-β and minimal secreting of IL-4 and IL-10 [26]. Dario Vignali and colleagues identified an inducible T_{Reg} class, iTr35 that mediate suppression through the expression of regulatory cytokine IL-35 [22]. Several studies report that IL-35 has been expressed in thymus-derived and peripheral nT_{Regs} in either human and mice [21,27] while, Bardel et al. investigated that human $Foxp3^+$ or $CD25^+$ cells didn't express Ebi3 but in activated effector T cells. This study concerning stimulation of Ebi3 by CD3/CD28 in different $CD4^+$ T cell subsets and there was no detected Ebi3 in stimulated T_{Reg} [28].

In a co-culture of conventional T cells (T_{Conv}) with nT_{Reg}, Collison, et al. [29]. in 2009 reported that higher levels of IL-35 has been expressed and T_{Conv} have been converted into an IL-35-expressing inducible T_{Reg}, iTr35. This conversion was IL-35- and IL-10-dependent till T_{Conv} gained their regulatory function, then IL-10 was no longer required for their activity. Also, it has been confirmed that T_{Conv} was converted into iTr35 in a contact-independent manner through the IL-35 activity. In the same study, T_{Reg} showed maximal suppressive activity depending on both, IL-35 expression and contact with T_{Conv} [21]. IL-35-dependent iTr35 given that the nature of these induced regulatory cells which though to be transient, they depend on their microenvironment through gaining their immunosuppressive phenotype [25]. From the fact that iTr35 has been naturally generated following the onset of various infectious and autoimmune diseases, IL-35 levels showed to be modest in the spleen whereas there was a significant increase in the infection site [24].

In addition to suppressive activity of T_{Reg} by cytokines secretion, it also maintains their action by cell-cell contact with target cells or APCs [30,29]. *In vitro* T_{Reg}-mediated suppression studies suggested that it is a contact-dependent process and there are no cytokines required [30,31] however *in vivo* studies indicated that secreted cytokines are important mean in T_{Reg}-mediated suppression [29,32]. Collison, et al. [29] confirmed that T_{Reg} optimal suppressive activity is potentiated by signaling pathway with conventional T cells including inhibitory cytokines, IL-35 and $IL\text{-}10^{low}$, secretion. Another cellular subset, $CD8^+CTLA\text{-}4^+$ T_{Reg}, maintain their suppressive activity in a contact-independent but IL-35-dependent manner [33].

IL-35/ B_{Reg} /$IL\text{-}35^+$ B_{Reg}

B cells' suppressive activity was maintained through Toll-Like Receptors (TLR). TLR4 and CD40 were established as the mediators of B cells regulatory functions in EAE [34] while, CD40 alone was the main contributor in B cells protective role in RA [35]. Among genetic analysis of B cells, p35 and Ebi3 were showed to be expressed [36] upon activation by CD40 and TLR4 together through antigen engagement resulted in IL-35 secretion [11]. B cells activity involve IL-10 as well as IL-35 secretion which protect and suppress autoimmune diseases. As IL-35 is produced by nT_{Reg} and contributes to their suppressive activities, as it is produced by B cells and induces their conversion to B_{Reg} for suppressive activities [22,21]. Furthermore, IL-35 induces *in vivo* B_{Reg} conversion into another subset of $IL\text{-}35^+B_{Reg}$ [20].

Despite there is no observed unique markers that exclusively identified B_{Reg} [37], in autoimmune uveitis there was B_{Reg} phenotype, $CD1d^{hi}CD5^+$ B_{Reg}, that shown to suppress inflammation in contact manner [38,20]. In the same study, rIL-35 induced $B220^+$ B cells suppression and $CD19+CD5+B220_{low}$ B_{Reg} expansion *in vivo* [20]. $CD138^{hi}$ plasma cell subset that provide IL-35 and IL-10 during *Salmonella* infection, has been identified by Shen and colleagues [11]. Unlike iTr35 suppressive activity that is mediated by IL-35 without IL-10, $IL\text{-}35^+B_{Reg}$ requires the both IL-35 and IL-10 signals for suppressive functions [24,20].

IL-35 modes of action

In RA mouse model, Collagen-Induced Arthritis (CIA), a study confirming the possibility of IL-35 participation in autoimmune disease. Recombinant IL-35 (rIL-35) showed to expand $CD4^+CD25^+$ T_{Reg} cells, suppress the proliferation of effector T cells and attenuate T_H17 polarization and IL-17 production [23,15] (Figure 1). Kochetkova and colleagues found that rIL-35 administration resulted in the frequency of $CD4^+CD39^+T_{Reg}$, subset that express Foxp3 and IL-10, which promoted their proliferation. These cell subsets could protect CIA animal model in IL-10-dependent manner [15]. Active RA patients have a reduced level of T_{Reg} suppressive activity towards pro-inflammatory cytokines production by effector T cells [17,14]. A study concerned with the role of IL-35 in T cells of RA patients, Nakano et al. reported that IL-35 secretion was reduced in active RA compared with healthy controls. Also, this *in vitro* study revealed that recombinant human IL-35 enhanced nT_{Reg} function and suppress T_H17 in RA patients [16]. In addition, recombinant IL-35 clearly showed to increase antibodies titers [15], its ability to act on other subsets reflect its contribution to humoral immunity [33]. However, there is a decisive difference between IL-35 and IL-27, a member from the same family that share Ebi3 and has a suppressive activity [39], IL-35 was more efficient in CIA suppression while IL-27 could act at the disease onset only [23].

In contrast to IL-35-dependent suppression in inflammatory diseases like arthritis, tumor models showed that it contributes to tumor genesis through immune-directed and tumor-directed activities. IL-35 potentially promotes angiogenesis that induce tumor cell proliferation, however on the other hand, it may have anti-tumor activity by suppressing tumor cell infiltration [24,20]. Several diseases showed to be associated with high IL-35 levels like coronary artery disease and cancer e.g. acute myeloid leukemia [40]. This is might due to the decrease in immune response, in CIA model, rIL-35 attenuate the severity of arthritis and on the other hand, it showed to collapse the inflammatory response [15,23].

IL-10-DEPENDENT REGULATION

There are different B cell subsets showed to secret IL-10 in health and disease [Table 2]. Earlier, B-1 phenotype considered the major IL-10 producer [41] and upon IL-12 stimulation of B-1a cells ($CD5^+$B cells) [42]. Moreover, B cell types originated in the Marginal Zone (MZ B) and its Transitional Precursor (T2-MZP) with the phenotype $CD19^+CD23^+CD21^+CD1d^{hi}$ [38,43] are capable of producing IL-10 in

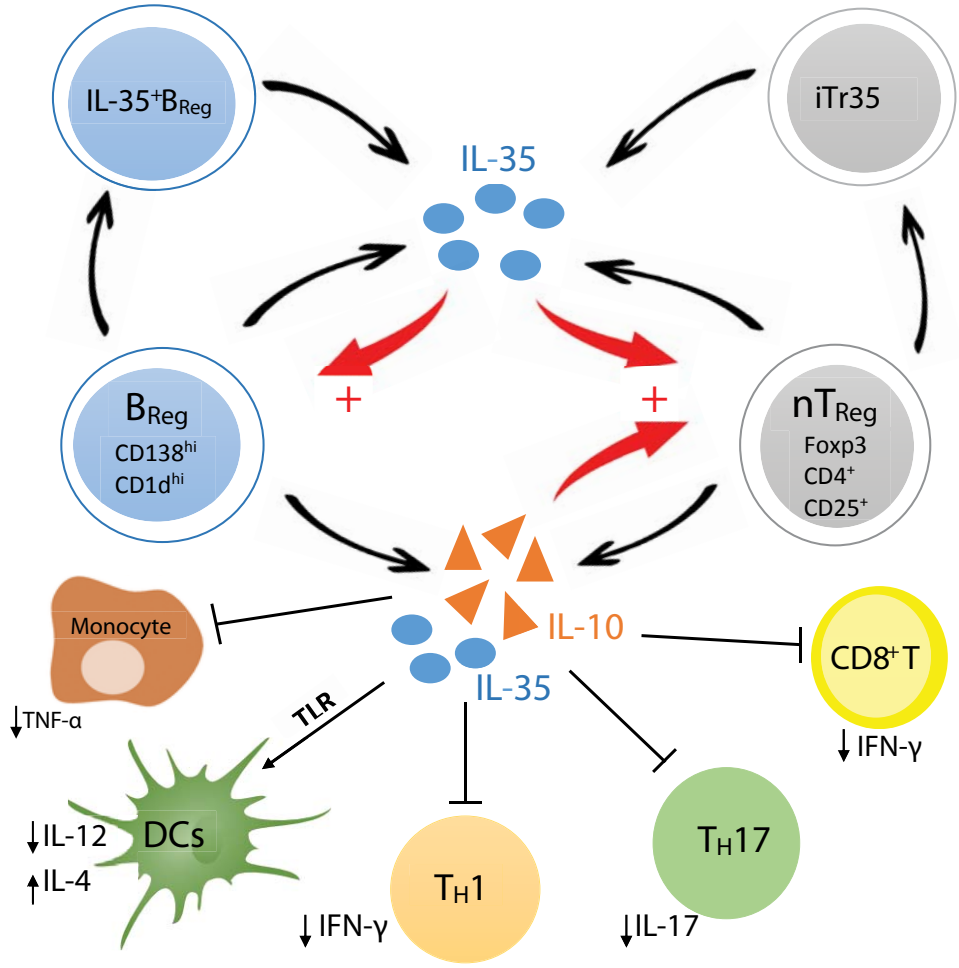

Figure 1: Immunoregulatory functions of IL-35 and IL-10. The possibility by which IL-35 and IL-10 modulate immune responses may include the following: Promote T_{Reg} expansion, suppress T_H1, T_H17, CD8+ cytotoxic T cells and monocytes differentiation and can Dampen Dendritic Cells (DCs) activation.

Table 2: IL-10 producing cells subsets.

Cell type	Phenotype	Features	Reference
T_{Reg}	$CD4^+CD39^+$	- IL-35-dependent stimulation	[15]
Immature B cells	$CD19^+CD24^{hi}CD38^{hi}$	- Induce T_{Reg} cells - Suppress T_H1 and T_H17	[45]
B10	$CD24^{hi}CD27^+$ (Human)	- Suppress effector T cells - Suppress DCs	[46-48]
B10	$CD1d^{hi}CD5^+$ (Mouse)	- Suppress effector T cells - Suppress DCs	[38]
B-1a	$CD5^+$	- IL-12-dependent stimulation	[42]
T2-MZP	$CD19^+CD23^+CD21^+CD1d^{hi}$	- Induce T_{Reg} cells - Suppress effector T cells	[43,38]
MZ	$CD19^+CD21^{hi}CD23^-$	- Induce T_{Reg} cells - Suppress effector T cells	[43,57,58,]
Plasma cells	$CD138^{hi}B220^+$	- Suppress effector T cells - Suppress NK cells	[11]
B_{Reg}	$CD19^+CD5^+B220^{lo}$	- IL-35-dependent stimulation	[20]

lupus [44] and arthritis [43], respectively. Another IL-10-producing B cell phenotype was identified as $CD1d^{hi}CD5^+$ B10 that shares typical markers with B-1a, MZ B and T2-MZP cells [38]. Immature B cells showed a response toward CD40 stimulation and converted into IL-10-producing phenotype, $CD19^+CD24^{hi}CD38^{hi}$ B cells in the peripheral of healthy individuals [45]. Although CD27 is a phenotypic marker of human memory B cells, $CD27^+$ B cells could expand and act as a biomarker in autoimmune diseases e.g. $CD24^{hi}$ $CD27^+$ B10 in RA [46-48].

IL-10 induction signals

However, there is no distinct studies concerning identification of specific surface markers in human or mouse B_{Reg} [7], EAE model suggested that TLRs as a part of the microenvironment are involved in modulation B_{Reg} function [49]. Combined together, TLR stimulation, BCR and CD40 ligation result in further amplification of IL-10-producing cell subsets for further IL-10 production and an effective suppression [49]. In human B cells, Bouaziz and colleagues reported that IL-10 production depends on TLR9 signaling which is a CpG (synthetic TLR9 agonist) receptor [50]. Other signals that might contribute in IL-10 production by B cells include B-Cell Activating Factor (BAFF) which could induce $CD1d^{hi}CD5^+$B cells to secret IL-10 [51], as well as *in vivo* induction of $CD4^+Foxp3^+$T cells to suppress

effector T cell in B cell-dependent manner [52]. Either, apoptotic cells act as endogenous IL-10 inducer that further induces T cells to secret IL-10 in CIA model [53]. In 2012, Qian, et al. [54] investigated that dendritic cells have a crucial regulatory role as B cell inducer to produce IL-10.

IL-10 modes of action

Naturally expressed IL-10 by B_{Reg} is very low while it could be expanded *in vitro* for further immune suppression. IL-10 has shown to suppress T cell proliferation as well as inflammatory cytokines production by T_H1 [38,43,51] [Figure 1]. Also, it has been demonstrated that IL-10 inhibits T_H17 through decreasing STAT3 phosphorylation and RORγt reduction [55]. Dendritic cells could be promoted to secret IL-4 and reduce IL-12 resulted in T_H1/ T_H2 imbalance in IL-10-dependent manner [56]. Either, B_{Reg} produced IL-10 promotes $CD4^+CD25^+Foxp3$ T_{Reg} infiltration to suppress inflamed airway [53,57, 58].

RECENT APPLICATIONS

The accumulation of the Mesenchymal Stem Cells (MSCs) in the inflammation sites have shown that they can inhibit immune responses as well as induction of immune components *in vitro* and *in vivo* [59,60]. Regarding these characteristics, MSCs could be immersed as a vehicle for drugs and genes delivery, a cell-based immunotherapy [61]. Previous studies reported the efficacy of MSCs for IL-10 [62] and Foxp3 [63] overexpression. Recently, a transfected MSCs with a lent virus vector for overexpression of murine IL-35 for therapeutic immunosuppressive function investigated their ability to suppress $CD4^+$ T cells [64]. There are further applications for prospective immunotherapies techniques concerning more future studies among IL-10 and IL-35 regulatory mechanisms' components and pathways.

CONCLUSION

Nowadays, there is a great eager for the evaluation of new immunotherapies. IL-35 and IL-10 are potent immune regulatory cytokines in autoimmune and infections. They perform their roles through expanding the suppressive functions of T and B lymphocytes. IL-35 is produced by nT_{Reg} and activated B_{Reg}; and by the STATs signals, it has shown to auto regulate its precursors conversion into IL-35-producing cells, iTr35 and $IL\text{-}35^+$ B_{Reg}. This results in further expansion and IL-35 production for maintaining more suppressive activities. IL-35 restrict T_H1 and T_H17 differentiation in RA models and other autoimmune and inflammatory diseases. Although only a few human studies are available showing IL-10-dependent immune suppression in RA, mouse models confirm the therapeutic role of IL-10 in CIA. IL-10 is produced naturally by different B and T cells phenotypes as well as it could be induced *in vitro* for further suppressive functions. Also, IL-10 inhibits T_H17 proliferation through STATs signals and induces T_{Reg} expansion. Depending on these findings and the unknown causes of rheumatoid arthritis, there must be further future focusing on the dynamics and process of generation of regulatory cells and cytokines.

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