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Interleukin 35: Critical Regulator of Immunity and Lymphocyte-mediated Diseases

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Abstract

Cytokines coordinate the activities of innate and adaptive immune systems and the Interleukin 12 (IL-12) family of cytokines has emerged as critical regulators of immunity in infectious and autoimmune diseases. While some members (IL-12 and IL-23) are associated with the pathogenesis of chronic inflammatory diseases, others (IL-27 and IL-35) mitigate autoimmune diseases. Unlike IL-12, IL-23 and IL-27 that are produced mainly by antigen presenting cells, IL-35 is predominantly secreted by regulatory B (i35-Bregs) and T (iTR35) cells. The discovery that IL-35 can induce the conversion or expansion of lymphocytes to regulatory B and T cells has considerable implications for therapeutic use of autologous regulatory B and T cells in human diseases. Although our current understanding of the immunobiology of IL-35 or its subunits (p35 and Ebi3) is still rudimentary, our goal in this review is to summarize what we know about this enigmatic cytokine and its potential clinical use, particularly in the treatment of CNS autoimmune diseases.

Keywords

Interleukin 35; IL-12 family cytokines; Autoimmune diseases; Cytokine therapy; Regulatory B cells; Breg; i35-Breg; iTR35; Adoptive B cell therapy

Introduction

Detection and eventual elimination of pathogens derive from productive interactions between the innate and adaptive immune systems. Cytokines produced by innate immune cells provide instructional signals for the differentiation of naïve lymphocytes into appropriate effector subsets while the differentiated effector lymphocytes in turn produce cytokines that orchestrate adaptive immune responses that eventually eliminate the pathogen

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and re-establish immune homeostasis. Cytokines are a broad group of soluble factors that function in an autocrine or paracrine manner. They play pivotal roles in coordinating activities of diverse immune cell types by coupling extracellular stimuli to intracellular signal transduction networks that mediate multiple physiological processes including differentiation, cell growth and development of target cells [1]. More than 100 cytokines have been described and classified into distinct families on the basis of their structure or receptor composition and include interleukins, interferons, hematopoietins, TNF family, adipokines and chemokines [1, 2]. In this review, our focus is on Interleukin 35 (IL-35), a member of the enigmatic IL-12 family of cytokines that have profound influence on cell-fate decision of differentiating lymphocytes and play crucial roles in shaping and regulating host immunity. In contrast to the other members of the family (IL-12, IL-23) that are pro-inflammatory, IL-35 is an immune-suppressive cytokine and a critical regulator of immunity during autoimmune and infectious diseases [3–5]. Here, we discuss the immunobiology of IL-35 and its considerable appeal as an important therapeutic target. We also highlight proof-of-principle studies which have established that IL-35 and IL-35-producing regulatory lymphocytes are effective in suppressing mouse CNS (Central Nervous System) autoimmune diseases and suggest that autologous i35-Breg therapy may be effective in the treatment of human autoimmune diseases such as uveitis and multiple sclerosis.

The Interleukin-12 (IL-12) family cytokines

Studies over the past decade have revealed that the quality and nature of the immune response is influenced by the predominant cytokines secreted by APC (antigen-presenting cells) and the cytokines found to play critical roles in lymphocyte differentiation and/or cell-fate decisions are mostly members of the Interleukin 12 (IL-12) family of heterodimeric cytokines.

The IL-12 family of cytokines is comprised of IL-12, IL-23, IL-27 and IL-35 and belongs to the Type 1 family of hematopoietic cytokines [6]. Unlike most cytokines that function as monomeric, homodimeric, or homotrimeric proteins, IL-12 cytokines are one of a few cytokines that function as heteromers. Each member comprises of heterodimeric subunits; an α -subunit with a helical structure similar to classic 4-helix bundle type 1 cytokines such as IL-6 and a β -subunit structurally related to the soluble IL-6 receptor (IL-6R α) [6]. As the three α -subunits (IL-12p35, IL-23p19 and IL-27p28) are structurally related, each can conceivably pair with either of the structurally homologous β subunits (IL-12p40 and Ebi3) and this is indeed the basis for the shared usage of IL-12p40 by IL-12 and IL-23 and similar sharing of Ebi3 by IL-27 and IL-35 [7, 8]. Although there are currently four known members in the family, the predictable range of combinations is six and it is conceivable that additional IL-12 members would soon be discovered. It is however interesting that pairing of an alpha chain with IL-12p40 appears to generate IL-12 cytokines that promote inflammation while dimerization with Ebi3 gives rise to members that suppress inflammation and autoimmune diseases (Figure 1). Mechanistically, IL-12 cytokines mediate their biological activities through high-affinity receptors composed of heterodimeric or homodimeric subunits, each characterized by presence of cytokine-receptor homology domains, fibronectin-like domains and immunoglobulin-like domains [6, 9]. Upon engaging their cognate receptor, receptor-associated Janus kinases (JAKs) are activated by

transphosphorylation, providing phosphotyrosine-docking sites that recruit specific members of the STAT (signal transducers and activators of transcription) family of transcription factors [6, 9]. STATs recruited to the receptor complex are phosphorylated at critical tyrosine residues present at the transcription activation domain, form homo- or hetero-dimers and translocate into the nucleus where they bind to specific DNA sequences and modulate gene expression [10, 11].

Interleukin 12 (IL-12) was the first member of the family described and was identified and purified from the cell culture media of Epstein–Barr virus (EBV)-transformed B lymphoblastoid cell lines [12]. It is comprised of the IL-12p35 and IL-12p40 subunits [6] and several transcription factors including IFN-regulatory factor-1 (IRF-1) and IRF-8 have been implicated in regulation of the gene coding for IL-12p35 or IL-12p40, respectively [13, 14]. IL-12p35 is ubiquitously expressed, while IL-12p40 expression is inducible in some hematopoietic cell types and co-expression of both subunits in the same cell is required to produce the disulfide-linked bioactive IL-12p70 cytokine [15, 16]. Although it can be secreted by a variety of hematopoietic cell types and EBV-transformed B cells, the major physiological producers of the IL-12 cytokine are dendritic cells (DCs) and macrophages. High-affinity IL-12 receptor (IL-12R), comprised of IL-12R β 1 and IL-12R β 2 subunits, is expressed mainly by activated T cells, NK cells and detectable on DCs and EBV-transformed B cells lines. It is however, undetectable on most resting T cells. Naïve T cells are unresponsive to IL-12 signal because they express only the IL-12R β 1 subunit while antigen-stimulation Th1 cells express both subunits and are responsive to IL-12. The IL-12 signal is transduced by TYK2 (tyrosine kinase 2) that constitutively associates with IL-12R β 1 subunit and JAK2 with the IL-12R β 2 subunit [6, 17]. Although STAT1, STAT3 and STAT4 are all activated to varying extents by IL-12 *in vitro*, physiological responses of IL-12 are mediated mainly through activation of STAT4 [6, 17, 18].

Interleukin-23 (IL-23) was discovered in 2003 and shares the IL-12p40 subunit with IL-12 but differs from IL-12 because of its unique IL-23p19 subunit [19–21]. Similar to IL-12, co-expression of IL-12p40 and IL-23p19 subunits in the same cell is required to secrete the disulfide-linked bioactive IL-23 cytokine. Sharing the IL-12p40 subunit enables IL-12 and IL-23 to interact with the IL-12R β 1 receptor subunit. The high affinity IL-23 receptor derives from the combination of IL-12R β 1 with a unique IL-23 receptor subunit (IL-23R) and biological effects of IL-23 on its target cells are mediated through activation of TYK2, JAK2, STAT3 and STAT4 [20, 21]. Many innate immune cells including DCs, macrophages, B cells and endothelial cells produce IL-23 and the high affinity IL-23 receptor is expressed on activated T cells and other immune cells including Th17 cells, $\gamma\delta$ T cells, natural killer T (NKT) cells and innate lymphoid cells (ILCs) [19]. IL-23 prolongs the expression of type 17 signature cytokines (such as IL-17, IL-22 and GM-CSF) that induce tissue pathology and mediate chronic inflammation by promoting the survival and maintenance of Type 17 cells [19, 20].

Interleukin 27 (IL-27) was first identified in 1996 and it is comprised of EBV-induced gene 3 (Ebi3) and IL-27p28 subunits [22]. The Ebi3 subunit is a soluble type I cytokine receptor-like molecule that shares homologies with IL-12p40 and CNTFR while IL-27p28 is similar to the IL-12p35 or IL-23p19 subunits of IL-12 and IL-23 cytokines, respectively [21, 23]. In

contrast to IL-12 and IL-23, IL-27p28 and Ebi3 are not secreted as disulfide-linked dimer and the nature of the association between IL-27p28 and Ebi3 *in vivo* is uncertain [24]. Thus, co-expression of Ebi3 and IL-27p28 subunits in the same cell may not be required for production of the bioactive IL-27 cytokine and may instead be secreted independently by various cell types [22]. The IL-27R is comprised of the ubiquitously expressed gp130 protein and the WSX-1/TCCR subunit that is abundantly expressed on naïve T cells and NK cells and to a lesser extent on endothelial cells, mast cells, activated B cells, monocytes, Langerhan's cells, activated dendritic cells, and polarized T-helper (Th) cells [23, 25]. Upon ligation of its receptor, IL-27 has been shown to activate both JAK/STAT and MAPK signaling pathways, although its activation of p38 MAPK and ERK1/2 has not been extensively characterized [26, 27]. Nonetheless, the biologic effects of IL-27 are primarily mediated through activation of a heterogeneous JAK/STAT signaling cascade that includes phosphorylation of JAK1, STAT1, STAT3, STAT4 and STAT5 in T cells, STAT1/STAT3 in monocytes, and STAT3 in mast cells [21, 27]. In terms of its role in host immunity, a number of reports indicate that IL-27 limits autoimmune encephalomyelitis by suppressing the development of Th17 cells and inducing the expansion of a population of IL-10-secreting T cells [28–30]. In the immune privileged ocular tissues, IL-27 produced by retinal cells has also been shown to suppress uveitis and contribute to mechanisms of ocular immune privilege by inducing IL-10 and complement factor H [31, 32].

Discovery of Interleukin 35 (IL-35)

Soon after the identification and purification of IL-12 from cell culture media of Epstein-Barr virus (EBV)-transformed B cell lines [12], Devergne and others discovered the Epstein-Barr virus-induced gene 3 (Ebi3), a 34-kDa glycoprotein related to the IL-12p40 with 27% amino acid identity to the IL-12 p40 subunit [33]. In the quest to identify potential pairing partners for the Ebi3 subunit, co-expression of IL-12p35 and Ebi3 led to the discovery of the novel IL-12p35/Ebi3 heterodimer now named IL-35 [34, 35]. Reciprocal co-immunoprecipitations in COS7 cells confirmed that Ebi3 associates with the IL-12p35 subunit to form a secreted non-covalent heterodimeric cytokine and although Ebi3 specifically associates with IL-12p35 in culture media, the association is more extensive when both proteins are expressed in the same cell [34]. In fact, Ebi3 and IL-12p35 tend to accumulate in immature forms in the endoplasmic reticulum when expressed alone, whereas co-expression of Ebi3 and IL-12p35 facilitates their efficient secretion. [34]. Ebi3 has only 3 methionine and 4 cysteine residues, while IL-12p35 has 10 methionine and 7 cysteine residues. Because it contains only the two pairs of conserved cysteine predicted to mediate intra-molecular disulfide linkages in hematopoietin receptors it is posited that Ebi3 is not disulfide-linked to IL-12p35 [5, 34, 35].

Recombinant IL-35

A major impediment to the understanding of the immunobiology of IL-35, particularly its role in regulation of autoimmune disease, carcinogenesis, sterilizing immunity and infectious diseases is the availability of highly purified heterodimeric IL-35 cytokine. Similar to IL-27, IL-35 is not secreted as a disulfide-linked heterodimer as Ebi3 associates non-covalently with the IL-12p35 [3, 34]. Based on *in vitro* overexpression studies in COS7

cells, only about 4% of the secreted Ebi3 co-precipitated with the IL-12p35 [34], providing an explanation for difficulty of isolating the native Ebi3/p35 heterodimer *in vivo*. Moreover, high level of Ebi3 expression is restricted to a few cell types (interfollicular cells in tonsils, perifollicular cells in spleens, and placental trophoblasts) but in the absence of IL-12p35, substantial amount of the Ebi3 associates with the endoplasmic reticulum (ER) membrane molecular chaperon, Calnexin, with the most nascent Ebi3 glycoprotein degraded in the ER [33, 34]. This thus reduces the bioavailability of Ebi3, further contributing to the low *in vivo* levels of IL-35. We and others generated recombinant mouse IL-35 (rIL-35) using a bicistronic vector containing IRES (internal ribosomal entry site) that allowed stoichiometric expression of the Ebi3 and IL-12p35 [3, 35]. Crude preparations of the insect cell supernatant contained substantial amounts of homodimers (p35:p35 and Ebi3:Ebi3) and monomers (Ebi3 and IL-12p35) and less than 20% of the preparation was the Ebi3/p35 heterodimer (Figure 2). Use of the crude IL-35 preparation is therefore not recommended because the biological activity of rIL-35 is influenced by the relative amounts of contaminating monomers and homodimers in the preparation. Surprisingly the homodimers are extremely stable even under reducing conditions and the preferential formation Ebi3:Ebi3 and p35:p35 complexes may account for the difficulty of producing significant amounts of IL-35 with reproducible biological activity (Egwuagu et al.; unpublished data). In fact, after more than 3 cycles of FPLC purification the yield of p35/Ebi3 heterodimer barely exceeds 2–5% of the starting protein extract [3]. Another approach that has been used is to construct a heterodimeric protein covalently linking Ebi3 and IL-12p35. However, while the Ebi3-p35 fusion protein expressed in mammalian cells was biologically active and had therapeutic effects against collagen-induced arthritis [36], another Ebi3-p35 fusion protein produced in bacteria was biologically inactive and failed to induce signal transduction in Ba/F3 cells expressing IL-12R β 2 and gp130 [37].

IL-35-producing regulatory T cells (iTR35)

More than a decade after the identification of Ebi3 in B cells and the suggestion that Ebi3/p35 heterodimer might be an important immune modulator [33, 34], Ebi3 was found to be one of the genes up-regulated in Treg cells [38, 39]. This was subsequently confirmed in a functional genomics screen showing significant up-regulation of Ebi3 in mouse Tregs (CD4⁺CD25⁺) in comparison to naïve effector T cells (CD4⁺CD25⁻) [35]. Furthermore, among the IL-12 cytokine family α -chain genes (Il12a, Il23a, Il27a), Il12a (coding for IL-12p35) was expressed in Treg cells and immunoprecipitation/immunoblot analysis confirmed that the Ebi3-p35 heterodimer is constitutively secreted by Treg but not resting or activated effector CD4⁺ effector cells [35]. Also, ectopic expression of IL-35 was shown to confer regulatory activity on naïve T cells and rIL-35 suppresses T-cell proliferation, suggesting that IL-35 is specifically produced by Treg cells and required for its maximal suppressive activity. Subsequent studies showed that the treatment of naïve human or mouse T cells with supernatants of HEK293T cells transfected with IL-35-expressing vector induced a regulatory population whose suppressive effects were mediated by IL-35 but not IL-10 nor TGF- β [40]. This provided compelling evidence that mouse IL-35 can convert conventional T cells into the IL-35-producing regulatory T cell population now known as iTR35 (Figure 3) [40]. Around the same time, an Ebi3-p35 Fc fusion protein was also

generated and shown to suppress the proliferation of CD4⁺CD25⁻ T cells and differentiation of Th17 cells while inducing the expansion of IL-10-producing Foxp3⁺ regulatory T cells [36]. Taken together, these initial studies validated the potent immune-suppressive effects of IL-35 and suggest that rIL-35 and ex-vivo generated iTReg35 might have therapeutic utility.

IL-35-producing regulatory B cells (i35-Bregs)

The reports showing that IL-35 is produced by natural Treg (nTreg) cells and contributes to their suppressive activities spurred the interest to know whether other lymphoid and myeloid cell types also produce IL-35. A screen of a wide variety of hematopoietic and lymphoid cells led to the discovery that while IL-35 inhibits the proliferation of CD19⁺B220^{hi}CD5⁻ B cells, it induces the expansion of IL-10-producing CD5⁺CD19⁺B220^{lo} regulatory B cells (i10-Bregs) [3]. Although there is no unique marker, or set of markers, that exclusively identifies the IL-10-producing B cells (Bregs), i10-Breg induced by IL-35 is phenotypically similar to the B10 Breg population described by Tedder's lab [41]. Given the close relationship between B and T cells, we speculated that IL-35 might also have a role not only in inducing i10-Bregs but also IL-35 producing regulatory B cells. The physiological relevance of the induction of i10-Breg by rIL-35 was validated *in vivo* in mice injected with LPS and/or rIL-35. About 8% of the i10-Bregs induced by rIL-35 in the mouse spleen also produced IL-35 and ex-vivo stimulation of the i10-Bregs with rIL-35 increased the level of the IL-35-producing B cells (i35-Bregs) to ~35%, with ~18% of these cells co-producing IL-10 and IL-35 [3]. Interestingly, prolonged propagation of the cells *in vitro* led to progressive loss of B cells co-producing IL-10 and IL-35 and expansion IL-35-producing B cells (i35-Bregs), suggesting that B cells that co-produce IL-10 and IL-35 and i35-Bregs may be overlapping i35-Breg subsets or i35-Breg cells at different stages of development. These studies provided direct evidence that IL-35 can induce the conversion of conventional B cells or i10-Breg cells into the novel IL-35-producing B cells, named i35-Breg (Figure 3) [3]. However, it is unclear whether IL-35 directly induces the differentiation of B cells into i35-Bregs or merely expands an extremely rare i35-Breg population. Exploiting the fact that B cells require activation by Toll-like receptors (TLR) to exert their suppressive activity, a complementary and independent study using mice lacking single TLRs in B cells, identified TLR4 and CD40L co-stimulation as potential driver of B cell differentiation into i35-Breg [4].

IL-35 Signaling in T and B cells

Mechanism of how IL-35 and other IL-12 cytokines initiate signaling is poorly understood. In the absence of X-ray crystallographic data for any of the IL-12 family cytokines, predictions of IL-35 extracellular signaling complex have been inferred from the 'site 1-2-3' architectural paradigm originally established for IL-6 and based on the hexameric structure and assembly of the interleukin-6/IL-6 α -receptor/gp130 complex [42]. Structural models of cytokine-receptor complexes formed by IL-12 cytokines predict that "site 1" might mediate interaction between the four-helix bundle α -chains (p19, p28, p35) and the soluble receptor β -chains (Ebi3, p40) and further suggest that distinct interactions between the different IL-12 members with sites 2 and 3 regions might activate divergent signaling pathways that form the basis for their unique effects in the immune system [9]. However, interpretation of

these models based on the site 1-2-3' paradigm is confounded by the fact that IL-6 family proteins like IL-6, CNTF, OSM and LIF function as monomeric or homodimeric proteins while IL-12 cytokines are heterodimeric. On the other hand, given the relatedness of IL-12 α -subunits to IL-6, it is plausible that some of the biological activities of IL-35 or IL-27 might be mediated by trans-signaling mechanisms through interaction of soluble IL-12p35 or IL-27p28 with Ebi3 and gp130, akin to IL-6 trans signaling [43]. Studies using cells deficient in IL-12 family receptor chains revealed that IL-35 signals through unconventional receptors comprised of IL-12R β 2/gp130, IL-12R β 2/IL-12R β 2 or gp130/gp130 [44]. Interestingly, while IL-12R β 2 and gp130 heterodimers activate signal transducer and activator of transcription 1 (STAT1) and STAT4, IL-12R β 2 or gp130 homodimers activate only STAT4 or only STAT1, respectively. However, capacity to up-regulate Ebi3 and IL-12p35 expression requires the IL-35 heterodimeric receptor. It is however not clear which of these receptor combinations is the high affinity IL-35 receptor [9, 44]. Interestingly, in B cells IL-35 activates STAT1 and STAT3 via heterodimeric receptors comprising of IL-12R β 2 and IL-27R α [3]. However, the analysis of IL-35 receptor usage in B cells did not examine whether IL-12R β 2/IL-12R β 2 or IL-27R α /IL-27R α homodimers are also utilized and additional studies using gp130^{-/-} B cells are needed to confirm that IL-35 signaling does not require gp130 homodimers [3]. It is intriguing that gp130 homodimerization, as a functional receptor signaling pathway, has only been observed in viral IL-6 signaling or during IL-6 trans-signaling via IL-6:IL6R:gp130 complexes [45, 46]. In the latter scenarios both STAT1 and STAT3 are activated in stark contrast to the pattern of STAT1 or STAT4 activation by IL-35 noted in T cells [47, 48]. Nevertheless, it is still unclear whether the differential receptor and STAT utilization in response to IL-35 derives from intrinsic differences between IL-35 signaling mechanisms of T and B cells or due to differences in IL-35 preparations used for the analyses.

IL-35 Therapy

In view of the immune-suppressive effect of rIL-35 *in vitro* and *in vivo*, we investigated whether rIL-35 can be used to treat an organ-specific autoimmune disease such as the CNS autoimmune disease, Uveitis. Uveitis is a diverse group of potentially sight-threatening intraocular inflammatory diseases that account for more than 10% of severe visual handicaps and includes sympathetic ophthalmia, birdshot retinochoroidopathy, Behcet's disease, Vogt-Koyanagi-Harada syndrome, pars planitis, ocular sarcoidosis [49, 50]. Experimental autoimmune uveitis (EAU) is the animal model of human uveitis. It is induced in susceptible animal species by immunization with retinal proteins in CFA and is characterized by the development of papilledema, retinal vasculitis, retinal folds, substantial infiltration of inflammatory cells into the vitreous and chorio-retinal infiltrates [50, 51]. Treatment of EAU mice with rIL-35 (100ng/mouse) suppressed ocular inflammation and ameliorated symptoms of severe uveitis by inhibiting the proliferation of CD4⁺CD25⁻ effector T cells and suppressing the differentiation of Th17 cells while inducing the expansion of Tregs and regulatory B cells [3]. Mice that lack IL-35 or defective in IL-35-signaling develop an exacerbated uveitis with reduced capacity to produce i35-Breg and IL-10-producing Bregs, further underscoring the critical roles of Bregs in regulating intraocular inflammation. In line with the immune suppressive function of IL-35, Ebi3^{-/-}

and $Il12a^{-/-}$ Treg cells are functionally defective in regulatory activity and are unable to cure inflammatory bowel disease [35]. Recombinant IL-35 fusion protein has also been shown to suppress collagen-induced arthritis by inducing the expansion of regulatory T cells and suppressing Th17 cells [36] and collectively, these and other studies have provided compelling evidence that IL-35 is therapeutically effective in suppressing and ameliorating autoimmune diseases, at least in mice.

Adoptive i35-Breg Therapy

Demonstration that rIL-35 can induce ex-vivo conversion of mouse or human B cells into IL-35 and IL-10-producing Breg cells [3], begged the question whether *ex-vivo* generated Bregs and i35-Bregs can be used to treat an ongoing autoimmune disease. In a proof-of-principle study, highly enriched Bregs (>93% IL-10⁺ B-cells) and IL-10^{negative} B-cells (<1% IL-10⁺ B-cells) were generated from LN and spleen of EAU mice, activated *in vitro* and then adoptively transferred into mice with EAU. Mice treated with ex-vivo generated Bregs suppressed ocular inflammation and conferred protection from ocular pathology by inducing the expansion of Bregs, Tregs and i35-Breg in the spleen and lymph nodes and inhibiting pathogenic Th17 cells [3]. The expansion of i35-Bregs during EAU raises the possibility that production of IL-35 by i35-Breg in lymphoid tissues or the retina may orchestrate a positive feedback loop that further increases the levels of Bregs and Tregs, thereby contributing to the suppression of uveitis. Thus, effective suppression of EAU may require the combined actions of Breg, i35-Breg and Tregs. IL-35-producing B cells have also been shown to ameliorate experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis [4]. EAE was induced in wild type (WT) or bone marrow chimeric mice reconstituted with IL-12p35^{-/-}, Ebi3^{-/-}, IL-12p40^{-/-} or IL-27p28^{-/-} B cells. The mice reconstituted with IL-12p35^{-/-} or Ebi3^{-/-} B cells developed exacerbated EAE, indicating that provision of IL-35 and i35-Breg is required for recovery from EAE [4]. In addition, mice with loss of IL-35 expression in the B cell compartment could not recover from EAE and mice lacking IL-35 or defective in IL-35-signaling were more resistant to bacteria infection [4]. The immunomodulatory effects observed in the EAE and bacteria infection studies were attributed to the expansion of IL-35- and IL-10-producing plasma cells exhibiting IgM⁺CD138^{hi}TACI⁺CXCR4⁺CD1d^{int}Tim1^{int} phenotype [4]. Similar to i35-Bregs, IL-35-producing T cells (iTR35) have potent regulatory effects *in vivo*. As noted above, IL-35 induces regulatory (iTR35) T cell population that mediates immune suppression independent of IL-10 or transforming growth factor- β (TGF- β). Physiological relevance of iTR35 derived from the finding that Tregs induced iTR35 generation *in vivo* under inflammatory conditions and within tumor microenvironments, where they contributed to the regulatory milieu [40]. Thus, iTR35 cells may be an important mediator of infectious tolerance and targeting iTR35 may be exploited to inhibit tumor progression. It is however important to bear in mind that it is still unclear the extent to which the anti-inflammatory effects of IL-35 derive from intrinsic effects of i35-Bregs and iTR35.

Conclusion

The discovery that IL-35 induces the expansion of IL-35-producing B cells (i35-Bregs) and T cells (iTR35) and that these regulatory lymphocytes exert potent anti-inflammatory effects

in vivo, suggests that i35-Bregs and iTR35 can be exploited therapeutically. In fact, several proof-of-principle studies have established that IL-35 and adoptive i35-Breg therapy are effective in mouse, suggesting that autologous i35-Breg therapy may also be effective in the treatment of human diseases. Interestingly, IL-12p35 and Ebi3 also inhibit lymphocyte proliferation but cannot induce Breg or Treg cells [3]. This suggests that single chain subunits of IL-35 have functions independent of IL-35 and can be exploited to modulate host defense without inducing Breg cells that may interfere with sterilizing immunity. A better understanding of the immunobiology and factors that regulate IL-35, Ebi3, IL-12p35, i35-Bregs and iTR35 *in vivo* is needed to fully exploit the therapeutic potential of these biologics.

Some of the unresolved questions that need to be addressed include: (i) Why are the α/β subunits of the two pro-inflammatory IL-12 family members (IL-12 and IL-23) secreted as disulfide-linked heterodimeric proteins while the two immunosuppressive members (IL-27 and IL-35) are secreted as non-covalently associated heterodimers? (ii) What factors regulate the stability of the non-covalently linked IL-12p35/Ebi3 heterodimer or regulate their dissociation to allow termination of their inhibitory activities? (iii) What are the physiological inducers of i35-Bregs and iTR35 and are they generated in response to distinct physiological cues? (iv) Do these factors induce the differentiation or conversion of conventional B and T cells into the regulatory B and T cell phenotypes, respectively or do they merely expand pre-existing i35-Bregs or iTR35 populations? (v) Are i35-Breg and i10-Breg cells distinct Breg subsets or are they overlapping populations at different stages of B cell development? It is likely that answers to these questions would open exciting new avenues of research leading to the development of therapeutics based on the enigmatic IL-12 family of cytokines. Nevertheless, the discovery that IL-35 induces the expansion of human Bregs holds out the promise of potential use of IL-35 and/or Breg/i35-Bregs to treat human uveitis and other CNS autoimmune diseases, including multiple sclerosis.

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Biographies

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Dr. Renxi Wang received a Doctor of Philosophy (Ph.D) degree in Immunology at Beijing Institute of Basic Medical Sciences, Beijing, China in 2006 and after obtaining his degree was appointed Assistant Professor (2006–2010). In 2010, he moved to USA for a 1-year Postdoctoral Research Fellowship at University of Nebraska Medical Center and a 2-year Postdoctoral training at the National Institutes of Health (NIH), Bethesda, Maryland. In 2012 he returned to China and become a group leader at the Beijing Institute of Basic Medical Sciences and was promoted to Associate Professor of Immunology in 2014. His current research interest is centered on molecular mechanisms underlying lymphocyte development and differentiation, and treatment of immune-related diseases such as type I diabetes, systemic lupus erythematosus (SLE) multiple sclerosis (MS) and sepsis.

Highlights

- Update on current understanding of the immunobiology of IL-35 and subunits (p35 and Ebi3)
- IL-35 induces expansion of lymphocytes into regulatory B (i35-Breg) and T (iT35) cells
- Autologous i35-Breg is effective therapy for experimental uveitis.
- IL-35 suppresses experimental uveitis and collagen-induced arthritis

Figure 1.

The IL-12 family of heterodimeric cytokines. Each member is comprised of an α -subunit (IL-12p19, IL-12 p35, IL-27p28) homologous to classic 4-helix bundle type 1 cytokines (e.g. IL-6) and a β -subunit (IL-12p40, Ebi3) structurally related to the soluble IL-6 receptor (IL-6R α). Upon binding to cognate receptors (IL-12R β 1, IL-12R β 2, IL-23R, IL-27R α , or gp130), receptor-associated Janus kinases (Jak1, Jak2, Tyk2) are activated, providing phosphotyrosine-docking sites that recruit specific members of the STAT (signal transducers and activators of transcription) family of transcription factors.

Figure 2.

Analysis of FPLC fractions of supernatant from insect cells expressing mouse recombinant IL-35 (rIL-35) by SDS-PAGE. Numbers (16 to 33) indicate fractions collected during size exclusion chromatography. The arrows indicate the presence of p35/Ebi3 (rIL-35), p35:p35 and Ebi3:Ebi3 homodimers as well as p35 and Ebi3 monomers in the preparation after passage through the first gel filtration column. For all functional and animal studies, the rIL-35 was purified by affinity chromatography using Ni-NTA followed by at least two consecutive cycles of gel filtration using Sephacryl S-200 HR Hiprep 16/60 and Superose-6 HR 10/30.

Figure 3.
Role of IL-35-producing B (i35-Breg) and T (iTR35) cells in the regulation of hematopoietic cells.