Interleukin-35: Expanding Its Job Profile

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Counter-regulation afforded by specialized regulatory cell populations and immunosuppressive cytokines is critical for balancing immune outcome. The inhibitory potential of the established suppressive cytokines, IL-10 and TGF β , has been well elucidated in diverse inflammatory scenarios in conjunction with their key roles in Treg development and function. Despite the early predictions for an immunomodulatory role for the Ebi3/p35 heterodimer in placental trophoblasts, IL-35 biology remained elusive until 2007 when it was established as a Treg-restricted inhibitory cytokine. Since then, Treg-derived IL-35 has been shown to exhibit its suppressive activities in a range of autoimmune diseases and cancer models. Recent studies are beginning to explore other cellular sources of IL-35, such as Bregs and CD8⁺ Tregs. Despite these new cellular sources and targets, the mode of IL-35 suppression remains restricted to inhibition of proliferation and induction of an IL-35-producing induced regulatory T cell population referred to as iTr35. In this review, we explore the early beginnings, status quo, and future prospects of IL-35 biology. The unparalleled opportunity of targeting multiple immunosuppressive populations (Tregs, Bregs, CD8⁺ Tregs) through IL-35 is highly exciting and offers tremendous promise from a translational standpoint, particularly for cancer immunotherapies.

Cytokines and Immune Regulation

IMMUNE PROTECTION IS orchestrated as a balanced interplay of events without triggering aberrant responses to self or foreign antigens that underlie autoimmunity, allergies, chronic infections, and cancer. These varied responses are shaped largely by intercellular communication mediated by messenger molecules called cytokines. Cytokines are small soluble proteins secreted by immune cells in response to diverse external stimuli. Lymphocyte activation through receptor engagement (signal 1) and appropriate costimulation (signal 2) initiates the immune response and drives clonal expansion of antigen-specific cells. Cytokine signaling (signal 3) is critical for functional maturation of this response into appropriate effector lineages with helper, cytotoxic, memory, or antibody-secreting potential.

Cytokines are members of several distinct families based on their structure and receptor composition (hematopoietins, interleukins, interferons, TNF family, immunoglobulin supergene family, chemokines, and adipokines). They function in an autocrine or paracrine manner to coordinate a plethora of biological events ranging from embryonic development, cellular differentiation, migration, disease pathogenesis, and even cognitive functions and aging. Cytokine biology is extremely complex owing to the pleiotropic nature, functional redundancy, and also the growing addition of new members to an existing family of more than 100 cytokines and their receptors (Dinarello 2007; Yoshimoto and Yoshimoto 2013).

These cytokine families encompass both proinflammatory and suppressive members, and often the net effect of the cytokine milieu determines the immune outcome. Any trigger to the immune system elicits the release of proinflammatory cytokines and chemokines by the innate immune cells. This initial innate response holds the enemy at bay until adaptive immunity kicks in with its specialized armor of effector cells exhibiting distinct cytokine profiles and functions. These cytokine-driven cellular influxes and expansions promote inflammation that ultimately leads to the clearance of infection. Cytokine storms typically subside once the infection is eliminated or when the autoimmune response is curtailed by negative feedback circuits provided by suppressive cytokines (Banchereau and others 2012) and specialized regulatory cells (Tregs) (Sakaguchi and others 2010; Josefowicz and others 2012). Suppressive cytokines help restore the immune equilibrium and homeostasis with minimal collateral damage to the host (Banchereau and others 2012). A better understanding of the immune networks established by these positive and negative regulators will allow for effective cytokine modulation for therapeutic intervention.

Immune Modulation by Suppressive Cytokines

The established suppressive cytokines (IL-10 and TGF β) and the newcomers (IL-27 and IL-35) are critical constituents of the regulatory, negative feedback loops and tolerance-promoting pathways that are integral to the immune

system. These cytokines differ in their expression patterns, cellular sources, signaling circuits, and targets of suppression (Yoshimoto and Yoshimoto 2013). They typically act in concert for maximal suppressive potential, although different members may be more or less active under homeostatic or diverse inflammatory scenarios.

TGF β is highly expressed in most tissues under basal conditions (Li and others 2012). TGF β signaling is indispensable for limiting T-cell reactivity to self and maintenance of steady-state immune homeostasis and tolerance. Thus, mice with germ line TGF β deletion or T-cell-specific deficiency in the TGF β receptor develop spontaneous multifocal inflammatory disease associated with exuberant T-cell activation and Th1/Th2 cytokine release (Shull and others 1992; Li and Flavell 2008; Tran 2012). The same is true for patients with Sezary syndrome whose CD4⁺ T cells have reduced expression of TGF β receptor and consequently unrestrained T-cell proliferation (Capocasale and others 1995).

In contrast to TGF β , IL-10 is minimally expressed by unstimulated cells and often requires commensal- or pathogen-driven upregulation (Banchereau and others 2012). Although originally identified as a Th2 cytokine, it is now clear that many immune cell types can produce IL-10 (Moore and others 2001; Sabat and others 2010; Saraiva and O'Garra 2010). Whether activated through innate or adaptive pathways, IL-10 plays a major regulatory role in dampening most inflammatory responses. Indeed, mice that lack *Il10* are highly susceptible to acute inflammation, exhibit profound immune polarization, and succumb to colitis, similar to the early onset of IBD observed in humans that harbor mutations in the genes encoding IL-10 or its 2 receptor components (Glocker and others 2009, 2011).

Together, this established suppressive cytokine duo plays a dominant role in maintaining steady-state immune equilibrium and taming exuberant responses at environmental interfaces. While TGF β plays crucial roles in the induction of Foxp3 and the thymic development of Tregs (Xu and others 2010; Mahmud and others 2013), TGF β and IL-10 also harbor another unique trait that further aids their immunomodulatory properties. Both these cytokines can transmit their suppressive potential to effector T cells causing them to gain a regulatory phenotype as well as target dendritic cells to promote conversion of effector cells to potent suppressors. This propagation of the tolerance-promoting state between cells is referred to as infectious tolerance (Gershon and Kondo 1971; Qin and others 1993; Gravano and Vignali 2012). IL-10 and TGF β can drive naïve T-cell conversion to Tr1 and iTreg cells, respectively. Tr1 cells are identified by their ability to secrete large amounts of IL-10 and mild to moderate levels of TGF β (Roncarolo and others 2001, 2006), while Foxp3⁺ iTregs are induced in the periphery through many different mechanisms and contribute to immunity at mucosal interfaces (Chen and others 2003; Sun and others 2007; Yadav and others 2013).

The recently discovered cytokines endowed with suppressive activity, IL-27 and IL-35, belong to the IL-12 family of cytokines. Unlike TGF β , but similar to IL-10, IL-27 and IL-35 are minimally expressed in human tissues and are mainly induced in inflammatory conditions (Li and others 2012). This is well supported by lack of lethal defects in mice with genetic deletions in IL-35 and IL-27 components (p35, Ebi3, and p28), unlike mice with TGF β 1 deficiency. Thus, TGF β functions as a housekeeping anti-inflammatory

cytokine, while IL-10, along with IL-27 and IL-35, can be categorized as inducible suppressive members (Li and others 2012).

IL-35: Latest Addition to the IL-12 Cytokine Family

The IL-12 cytokine family belongs to the IL-6 superfamily of type I cytokines. Unlike the IL-6 family members that are secreted as single-subunit monomers, the IL-12 family is characterized by heterodimeric members formed from chain sharing interactions of 3 alpha chains (p19, p28, p35), classic 4-helix bundle cytokines, and 2 beta chains (p40 and Ebi3), which resemble soluble versions of cytokine receptors (Jones and Vignali 2011; Vignali and Kuchroo 2012). This gives rise to the 4 current members: IL-12 (p35/p40), IL-23 (p19/p40), IL-27 (p28/Ebi3), and IL-35 (p35/Ebi3) (Fig. 1). IL-12 and IL-23 are predominantly proinflammatory, while IL-27 and IL-35 are primarily suppressive cytokines (Vignali and Kuchroo 2012). Additionally, the existence of monomers and homodimers has been reported, which function as natural antagonists of bioactive cytokine signaling (Jones and Vignali 2011; Yoshimoto and Yoshimoto 2013).

These cytokines signal through unique pairings of 5 receptor chains: IL-12R β 1, IL-12R β 2, IL-23R, IL-27R, and gp130 (Fig. 1). IL-12 signals through IL-12R β 1 and IL-12R β 2 and IL-23 through IL-23R and IL-12R β 1, while IL-27 utilizes gp130 and WSX-1. In T cells, IL-35 signals through IL-12R β 2 and gp130, although it can also signal through IL-12R β 2-IL-12R β 2 and gp130-gp130 homodimers (Collison and others 2012). Signal transduction through these receptor chains is mediated through the JAK-STAT pathway. The key Janus kinases mediating STAT phosphorylation for the IL-12 family members are JAK2 and either JAK1 or TYK2. STAT utilization varies between

FIG. 1. IL-12 family cytokines and their receptors. Four current members of the IL-12 cytokine family and their downstream signaling pathways: IL-12 (p35/p40) signals through IL-12R β 1 and IL-12R β 2, IL-23 (p19/p40) signals through IL-23R and IL-12R β 1, IL-27 (p28/Ebi3) signals through gp130 and WSX-1, and IL-35 (p35/Ebi3) utilizes IL-12R β 2 and gp130 heterodimers and homodimers, respectively.

receptor chains such that IL-12 signals primarily through pSTAT4, IL-23 through pSTAT3 and pSTAT4, and IL-27 through pSTAT1 and pSTAT3. IL-35 signaling through gp130 and IL-12R β 2 homodimers is mediated by pSTAT1 and pSTAT4, respectively (Collison and others 2012). These homodimeric receptors enable suppression of T-cell proliferation, but cannot induce iTr35 conversion (Fig. 2). In contrast, T-cell suppression and iTr35 induction can be mediated through an IL-12R β 2:gp130 heterodimeric receptor that drives formation of pSTAT1:pSTAT4 heterodimers, which in turn bind to unique sites in the *Ebi3* and *Il12a* promoters (Collison and others 2012). As discussed in detail below, recent studies have also suggested that IL-35 signaling in B cells is mediated through an IL-12R β 2:WSX-1 heterodimer and induces pSTAT1 and pSTAT3 (Shen and others 2014).

Despite the obvious structural similarities in the cytokine subunits, receptor components, and downstream signaling, the IL-12 family members display surprisingly diverse but balanced functional outcomes. IL-12, IL-23, and IL-27 are secreted by activated antigen-presenting cells (B cells, monocyte/macrophages, dendritic cells), in response to bacteria, bacterial products, or intracellular parasites (Ma and Trinchieri 2001; O'Shea and Paul 2002; Hunter 2005). IL-12 stimulates IFN- γ production from both NK and T cells, thereby bridging the innate and adaptive immune arms and concurrently downregulating IL-4, thus inhibiting Th2 propagation. IL-12 also enhances the cytolytic activity of macrophages, T cells, and NK cells. Similar to IL-12, IL-23 can also drive Th1 proliferation (Oppmann and others 2000). However, IL-23 has been mainly linked to inflammatory processes associated with Th17 differentiation (Aggarwal and others 2003). IL-23 potently enhances and stabilizes differentiation to the Th17 lineage, promotes memory T-cell proliferation, and can also aid in DC cytokine production and antigen presentation (Zeng and others 2013). Thus, IL-12 and IL-23 represent the strictly proin-

flammatory members of the IL-12 family with key roles in Th1 and Th17 development.

IL-27, first discovered in 2002, was suggested to be a proinflammatory IL-12 family member due to its role in supporting Th1 development and IFN- γ production, while inhibiting Th2 and Th17 differentiation programs (Pflanz and others 2002; Villarino and others 2006). However, recent studies have revealed a broader immunomodulatory role for this cytokine. IL-27 was demonstrated to inhibit Th17 differentiation by targeting the Th17 transcription factors, ROR γ t, ROR α , and RORc, as well as inhibiting IL-23-induced IL-17 production (Diveu and others 2009). IL-27 also promotes the generation of IL-10-producing Tr1 cells through induction of the transcription factor, cMaf, cytokine IL-21, and costimulatory receptor, ICOS, thus extending its ability to dampen inflammatory processes (Awasthi and others 2007; Fitzgerald and others 2007; Stumhofer and others 2007; Batten and others 2008; Pot and others 2009). These accumulating studies support a role for IL-27 in modulating immune responses.

IL-35, the newest IL-12 family member and focus of this review, is unique in its expression pattern and function. Unlike the other members, IL-35 is predominantly secreted by regulatory T cells, has been shown to suppress T-cell proliferation and function in a number of *in vitro* and *in vivo* disease models, and appears to be required for maximal suppressor function of mouse and human Tregs (Collison and others 2007, 2009; Pillai and others 2011). As recently reported, it can also be produced by regulatory B cells (Shen and others 2014; Wang and others 2014). Like IL-10 and TGF β , IL-35 can also induce the conversion of naïve T cells into an IL-35-dependent regulatory population referred to as iTr35 cells (Collison and others 2010). iTr35 cells function independently of Foxp3, IL-10, and TGF β and have been demonstrated to exhibit tolerance-promoting abilities in mouse models of parasite infection and tumors. Since its discovery in 2007, IL-35 has been shown to contribute to the

FIG. 2. IL-35 signaling in T and B cells. Production of IL-35 by Foxp3⁺ T cells and CD19⁺CD138^{hi} B cells inhibits T- and B-cell proliferation. Signaling through gp130 and IL12R β 2 homodimers inhibits T-cell proliferation, while formation of the gp130/IL12R β 2 heterodimer is required for both inhibition of T-cell proliferation and generation of iTr35 cells (*left panel*). B cells respond to IL-35 through the IL27R α /IL12R β 2 heterodimer leading to expansion of IL-10 and IL-35-producing Bregs that function to inhibit cellular proliferation (*right panel*).

regulatory milieu in a wide variety of disease states by several groups, thereby warranting detailed characterization of its cellular source, targets of suppression, and mechanism of action. Interestingly, thus far, it only seems to have a suppressive function, suggesting that it has a relatively restricted pattern of activity.

Early Beginnings of IL-35 Biology and Its Addition to the Treg Arsenal

IL-12, the archetypal member of the IL-12 family, was identified and purified from cell culture media of Epstein-Barr virus (EBV)-transformed B cell lines (Devergne and others 1997). Macrophages and EBV-transformed B cells could produce this 70 kDa p35/p40 heterodimer and typically an excess of p40. IL-12 α /p35 expression was ubiquitous, while p40 seemed restricted and inducible. This dissociation between p35 and p40 gene regulation had hinted at the possibility that either or both of these subunits might have additional partners. Thus, the discovery of the other p40-related beta chain (Ebi3) in EBV-infected B cells in 1996 initiated experiments to determine if it was an alternate binding partner for p35 (Devergne and others 1996; Niedobitek and others 2002). This led to the first demonstration in 1997 that Ebi3 can heterodimerize with p35 to form a novel potentially naturally occurring hematopoietin (Devergne and others 1997). As Ebi3 is expressed in EBV-transformed B lymphocytes, tonsil, spleen, and placental trophoblasts and the Ebi3/p35 heterodimer was also coimmunoprecipitated from trophoblasts of a human full-term placenta (Devergne and others 2001), it was proposed that this Ebi3/p35 heterodimer might be an important immune modulator. However, its function and physiological relevance were unknown.

Despite these early observations, it was not until 2007 that a name and function were given to this p35/Ebi3 heterodimer when it was shown by the Vignali laboratory that IL-35 was produced by regulatory T cells and was required for their maximal suppressive function (Collison and others 2007). This rather came as a surprise to the field since both the IL-35 subunits are constituents of the IL-12 family of cytokines that were thought to be exclusively produced by APCs (Collison and Vignali 2008). Ebi3 was also noted as a candidate gene upregulated in Treg cell-specific gene arrays and was proposed as a potential Foxp3 target (Knoechel and others 2006; Collison and others 2007; Hill and others 2007; Zheng and others 2007). IL-35 appeared to be constitutively secreted by mouse Foxp3⁺ Tregs, but not effector T cells, and its expression was further boosted following coculture of Tregs with effector T cells (Collison and others 2007, 2009). Importantly, Treg cells deficient in either IL-35 subunit (*Ebi3*^{-/-} or *Il12a*^{-/-}), stimulated alone or following coculture with effector T cells, displayed reduced suppressive function *in vitro* and failed to control homeostatic proliferation and colitis *in vivo* (Collison and others 2007). Furthermore, recombinant IL-35 was able to suppress T-cell proliferation and its ectopic expression conferred suppressive potential to naïve T cells (Collison and others 2007). These studies were the first to delineate a defined function for this long-known Ebi3/p35 heterodimer as a Treg-restricted suppressive cytokine needed for maximal function, joining ranks with IL-10 and TGF β as soluble, cell contact-independent mediators of suppression.

Around the same time, another group generated an Ebi3 and p35 Fc fusion protein and also demonstrated that this heterodimer suppressed CD4⁺CD25⁻ effector T-cell proliferation and IFN- γ production (Niedbala and others 2007). It also inhibited Th17 cell differentiation and was noted to alleviate development of collagen-induced arthritis, a mouse model of Th1/Th17 inflammation. Interestingly, this group reported that this Ebi3:p35:Fc fusion protein could expand CD4⁺CD25⁺ Treg cells without induction of additional Foxp3 expression while retaining their suppressive function (Niedbala and others 2007). Taken together, these initial studies validated the immunomodulatory potential of IL-35 and paved the beginning for an exciting series of future studies to assess the physiological and pathophysiological roles of IL-35 in health and disease.

Regulatory Potential of Treg-Derived IL-35

The addition of IL-35 to the Treg cell arsenal in 2007 sparked tremendous enthusiasm in the Treg field as there has been continued interest in identifying novel mechanisms of Treg suppression that can be optimally channeled to either boost or inhibit Treg activity. While Treg suppression through inhibitory receptor expression (PD1, Lag3), cytotoxicity (Granzyme, Perforin), and metabolic disruption (IL-2 deprivation, adenosine) have all been documented, release of suppressive cytokines has prevailed as a major cell contact-independent mechanism of suppression. The inclusion of IL-35 to this important category of Treg suppressive mechanisms was particularly fascinating as IL-35 was the only known inhibitory mediator that appeared to be exclusively secreted by Treg cells alone, unlike IL-10 and TGF β that are also secreted by other immune cells in addition to Tregs. Although this view has begun to change with the recent identification of new cellular sources of IL-35, this critical difference brought IL-35 at the forefront in this category and triggered a series of investigations into its mechanism of suppression.

Thus far, IL-35 has only been shown to exhibit suppressive activities and mechanisms. (Collison and others 2007; Gravano and Vignali 2012; Vignali and Kuchroo 2012). Regarding its suppressive activity, this is supported by experiments demonstrating the failure of Ebi3- and IL-12 α -deficient Tregs to suppress T-cell proliferation *in vitro* and in a wide variety of inflammatory scenarios *in vivo* (Collison and others 2007; Wirtz and others 2011; Bettini and others 2012). This has also been validated by gain-of-function approaches using recombinant IL-35 in cell culture systems or ectopically expressed single-chain Ebi3:p35:Fc fusion proteins. In all these settings, IL-35 decreased T-cell proliferation. IL-35 appears to induce cell cycle arrest, but how this is mediated remains unknown. This has been confirmed in NOD transgenic mice with islet-specific expression of IL-35 (Bettini and others 2012). These mice exhibit substantially reduced diabetes incidence and inflammatory pathology owing to reductions in islet-resident CD4⁺ and CD8⁺ T cells. Even though the proportion of islet-infiltrating T cells in the G1 phase was comparable, beta cell-secreted IL-35 blocked their progression at the G1:S transition.

In addition to suppressing proliferation, IL-35 has also been shown to suppress differentiation to effector T cell lineages. An Ebi3:p35:Fc fusion protein was shown to suppress Th17 differentiation and attenuate collagen-induced arthritis

(Niedbala and others 2007). Mice lacking Ebi3 also exhibit increased Th17 responses (Yang and others 2008; Liu and others 2012). In addition, there have been several studies demonstrating that recombinant IL-35 administration reduces Th17 differentiation and function. IL-35 also limited collagen-induced arthritis in another study by suppressing both Th1 and Th17 cells (Niedbala and others 2007; Wirtz and others 2011). Furthermore, intratracheal delivery of single-chain recombinant IL-35 significantly attenuated allergic airway inflammation by reducing the Th2 effector and humoral responses (Huang and others 2011). Thus, IL-35 can modulate effector T-cell differentiation pathways depending upon the nature of the inflammatory insult.

Another proposed mode of IL-35 action is through Treg expansion. Exposure to an Ebi3:p35:Fc fusion protein was shown to induce the proliferation of a population of CD4⁺ CD25⁺ Foxp3⁺ T cells (Niedbala and others 2007). These IL-35-expanded Tregs expressed IL-10 and were able to suppress effector T-cell proliferation, potentially contributing to its inhibitory effects in a mouse model of arthritis. Similarly, in another report, IL-35 treatment expanded Foxp3⁺CD39⁺ CD4⁺ T cells that were dependent on IL-10 for mediating autoimmune protection following adoptive transfer in a collagen-induced arthritis model (Kochetkova and others 2010). However, it remains to be determined if IL-35 mediates expansion of Tregs in a physiological setting.

Last, similar to IL-10 and TGF β , IL-35 seems to mediate infectious tolerance (Belkaid and Chen 2010; Gravano and Vignali 2012). Thus, naïve T cells stimulated in the presence of IL-35 are not only suppressed but also converted into an IL-35-producing regulatory population termed iTr35 cells. The promotion of infectious tolerance by IL-35 also derived from studies by the Vignali group who demonstrated that Treg cell-mediated suppression induces, through the concerted actions of IL-10 and IL-35, a distinct regulatory population characterized by IL-35 secretion that they termed iTr35 cells (Collison and others 2010). Notably, these cells are phenotypically and functionally distinct from the other known induced regulatory populations (TGF β -induced Tregs [iTregs] and IL-10-induced Tregs [Tr1]), in that they do not express Foxp3, and suppress exclusively through IL-35 and independently of IL-10, TGF β , CTLA-4, or any other known Treg suppressive mediators. Transcription profiling suggested that these iTr35 cells exhibit a highly restricted gene signature (CD4⁺Foxp3⁻Ebi3⁺p35⁺IL-10⁻TGF β ⁻), indicative of limited rather than global changes following IL-35 treatment of T cells. Mouse iTr35 cells exhibited potent suppressive potential in 5 distinct disease models. Importantly, inflammatory milieu generated following parasite infection (*T. muris*) and tumor inoculation (B16 and MC38) induced iTr35 cells, which seemed to provide a substantial physiological contribution to the regulatory milieu established by nTregs in the B16 melanoma model. Even naïve human CD4 T cells could be induced to develop into iTr35 cells in the presence of IL-35 (Collison and others 2010) or viral-exposed DCs (Seyler and others 2010), and these human iTr35 cells also suppressed primary human T-cell proliferation through IL-35. One of the more exciting features of this induced regulatory population was its demonstrated stability and efficiency relative to TGF β -iTr and IL-10-iTr cells. iTr35 cells were almost as efficient as nTregs in restoring homeostasis and preventing autoimmunity in *Foxp3*^{-/-} mice, limiting T-cell proliferation in

lymphopenic settings, curtailing inflammation in experimental autoimmune encephalitis (EAE) and colitis models, and promoting tumor growth in the B16 melanoma model. iTr35 cells also displayed increased stability and retained suppressor potential relative to TGF β -induced counterparts following recovery 3 weeks post-transfer into immune-competent hosts. These intriguing aspects of the IL-35-induced regulatory population make these cells a safe and attractive therapeutic candidate considering the complexities associated with generation, short life span, and stability of *ex vivo*-generated TGF β -iTr and IL-10-iTr cells (Collison and others 2010). However, further studies are warranted to fully elucidate the contribution of iTr35 cells in diverse disease scenarios, and the availability of purified rIL-35 remains a major limitation.

Thus, inhibition of effector T-cell proliferation through cell cycle arrest, modulating T-cell differentiation programs, expansion of regulatory T cells, and induction of iTr35 cells emerge as the key mechanisms for IL-35-mediated regulatory functions. As new cellular targets and sources of IL-35 will be deciphered, more information will likely emerge, expanding its scope of influence in immune regulation.

Identification of a Regulatory B Cell Subset Secreting IL-35

Analogous to the regulatory T cell (Treg) counterpart, the existence of regulatory B cells (Bregs) and their role in control of autoimmune diseases (EAE, CIA) has been described by several groups (Fillatreau and others 2002; Mauri and Ehrenstein 2008; Yanaba and others 2008; Barr and others 2012; Mauri and Bosma 2012). A key focus of the Breg cell field is to identify phenotypic markers to better characterize the subsets that have been identified thus far [CD1d^{hi}CD5^{hi} B10 cells (Yanaba and others 2008), CD21^{hi}CD23^{hi}CD24^{hi} transitional type 2 marginal zone precursors (Evans and others 2007), Tim1-expressing B cells (Ding and others 2011), and CD138-expressing plasma cells (Neves and others 2010)]. Despite the lack of a unifying signature, IL-10 has accounted for most of the regulatory function ascribed to these regulatory B cell subsets (Fillatreau and others 2002; Banchereau and others 2012; Kallampokis and others 2013). In fact, IL-10-producing Breg cells have also been identified in humans, and numerical and/or functional perturbations in these IL-10-secreting Breg cell subsets have been reported in human autoimmune diseases (multiple sclerosis and type 1 diabetes) (Blair and others 2010; Mauri and Nistala 2014).

However, B cells have also been shown to mediate regulatory effects independent of IL-10 secretion (Wilson and others 2010; Su and others 2011). B cells can also induce Tregs in an IL-10-independent manner (Flores-Borja and others 2013). It is therefore not too hard to conceive that, similar to Tregs, Bregs may also harbor a range of suppressive mediators that can account for their broader involvement in immune regulation. Two recent studies have focused on elucidating other regulatory mediators with the potential to induce Bregs, drive B cell IL-10 secretion, and act independently or synergistically with IL-10 to elicit maximal Breg function *in vivo* (Shen and others 2014; Wang and others 2014). Interestingly, Ebi3, the beta chain of the Treg inhibitory cytokine, IL-35, emerged as one of the differentially regulated genes in a transcriptome analysis of

B cells with TLR4 and CD40 costimulation relative to TLR4 priming alone (Shen and others 2014). Analysis of B cell-specific expression of other IL-12 family constituents showed that B cells also constitutively express the IL-35 alpha chain, p35, but do not express p40. Thus, BCR engagement along with TLR4 and CD40 stimulation results in increased Ebi3 and p35 transcription and IL-35 secretion, identifying B cells as a novel source of IL-35.

To test the *in vivo* functional relevance of B-cell-derived IL-35, bone marrow chimeric mice were generated with B cells lacking either the p35 or Ebi3 chain of IL-35. These B-cell-restricted IL-35-deficient mice developed exacerbated EAE and were protected from *S. typhimurium* (Salmonella)-induced sepsis relative to controls (Shen and others 2014). These responses were largely accounted for the enhanced antigen-presenting potential of IL-35-deficient B cells (associated with higher expression of MHCII, CD80, CD86), thereby stimulating higher proliferation and inflammatory cytokine production by their cognate CD4⁺ T cells. Thus, the suppressive effects of B-cell-derived IL-35 mainly stemmed from its ability to regulate the APC function of B cells, restraining inflammatory cell infiltration (T cells, macrophages) and thus limiting autoimmunity or mediating enhanced pathogen clearance.

The regulatory potential of B-cell-derived IL-35 was further confirmed and complemented by another independent study that showed that recombinant IL-35 treatment induced IL-10-secreting Bregs and these Bregs efficiently suppressed proliferation of CD19⁺ B cells (Wang and others 2014). Furthermore, IL-35 also mediated conversion of B cells into a Breg cell population that secreted IL-35 (IL-35⁺ Bregs). Administration of recombinant IL-35 or IL-35⁺ Breg cells efficiently controlled initial and established stages of experimental autoimmune uveitis (EAU). This IL-35⁺ Breg-mediated protection was dependent on increased induction and expansion of endogenous Bregs and Foxp3⁺ Tregs and inhibition of pathogenic Th1 and Th17 effector cells (Wang and others 2014). Thus, these 2 studies provide support for the existence of comparable regulatory modules within the T and B cell lineages, with IL-35 and IL-10 serving as a common link.

To assess the relationship between IL-35- and IL-10-producing B cells, *Ebi3* and *Il10* transcripts were quantified between CD19⁺CD138⁻ B cells and CD138^{hi} plasma cells. This analysis revealed that CD138^{hi} plasma cells were the major source of IL-10 and IL-35 *in vivo*, although very few plasma cells cotranscribed *Il10*, *Ebi3*, and *Il12a* together, suggesting distinct regulatory plasma cell subsets secreting IL-10 and IL-35 (Shen and others 2014). Both studies demonstrated IL-35 enrichment within CD1d- and Tim1-expressing B cells (Shen and others 2014; Wang and others 2014). One of the major differences between the 2 studies was the relative importance of IL-10 or IL-35 to the regulatory function of B cells. At the peak of Breg expansion, almost 3% and 12% of the CD138^{hi} plasma cells secreted IL-35 and IL-10, respectively. Despite this difference, deletion of either p35 or IL-10 resulted in similar exacerbations in EAE severity, and animals capable of producing either cytokine, but not both, had similar disease scores as wild-type animals, indicating that the cytokines functioned independent of each other (Shen and others 2014). In complete contrast, when B cells deficient in IL-12 α , IL-12R β 2, and IL-10 were cultured in the presence of rIL-35

and then tested for their capacity to control autoimmune uveitis (EAU), loss of IL-10 completely abrogated the suppressive effects of IL-35, suggesting that IL-10 is the final mediator of suppression of IL-35⁺ Bregs (Wang and others 2014). This was compatible with their *in vitro* studies demonstrating that addition of the neutralizing IL-10 antibody abrogated the suppressive effects of rIL-35.

The finding that IL-35 treatment of human B cells also induces IL-10 expression while inhibiting B-cell proliferation, suggests that IL-35 may hold promise for autologous Breg induction in the near future in conjunction with IL-21, which is currently used for large-scale B10 cell expansion *ex vivo* (Tedder and Leonard 2014).

IL-35 Signaling in T and B Cells

Considering that IL-35 shares its subunits with IL-12 and IL-27 and the fact that chain sharing of the IL-12 family also extends to the receptor chains and downstream signaling, it was likely that the IL-35 receptor and STAT utilization would involve components of the IL-12 and/or IL-27 signaling cascades. However, since the biological activity of IL-35 contrasts with its other siblings, utilization of a completely novel signal transduction mechanism was also possible. Using cells deficient in the IL-12 family receptor chains, it was demonstrated that IL-35 signals through gp130 and IL12R β 2 in T cells (Collison and others 2012). Interestingly, while cells deficient for both receptor chains were completely resistant to the suppressive effects of IL-35, activated *Il6st*^{-/-} (gp130-deficient) or *Il12rb2*^{-/-} T cells were only partially resistant to IL-35 or iTr35 cell-mediated suppression. Thus, unlike IL-12 and IL-27, IL-35 was capable of signaling through a single receptor chain (Garbers and others 2012; Vignali and Kuchroo 2012). However, its capacity to upregulate Ebi3 and p35 expression and iTr35 conversion required the IL-35 heterodimeric receptor (IL-12R β 2: gp130). IL-35 was demonstrated to signal through 3 receptor combinations in T cells: gp130 homodimers and IL-12R β 2 homodimers, which are sufficient to mediate suppression, and gp130:IL-12R β 2 heterodimers, which are essential for iTr35 conversion and required for maximal suppression. A unique STAT1:STAT4 heterodimer was identified as the major downstream mediator of IL-35 signaling and bound to many sites in the *Il12a* and *Ebi3* promoters to initiate transcription. It is intriguing that the STAT1:STAT4 heterodimer predominates only after IL-35 stimulation and not after simultaneous stimulation with IFN- γ and IL-12 (activators of STAT1 and STAT4, respectively), which in contrast induces a proinflammatory Th1 response.

The recent Breg studies have proposed a new composition for the functional IL-35 receptor on B cells (Wang and others 2014). Unlike its T cell counterparts, silencing of gp130 through siRNA-mediated knockdown or using neutralizing antibodies did not affect IL-35-mediated inhibition of B-cell proliferation or IL-10 induction. In contrast, silencing of IL-12R β 2 and IL-27R α completely abrogated the inhibitory effects of IL-35 in B cells. Further analysis revealed that IL-35 signals through a receptor comprising IL-12R β 2 and IL-27R α and activates STAT1 and STAT3 in B cells (Wang and others 2014). These findings indicate that IL-35 may mediate its distinct biological activities in diverse cell types through differential receptor and STAT utilization (Fig. 2).

The composition of IL-35 receptor sheds some light on its potential cellular targets. Although gp130 is ubiquitous in its expression, IL-12R β 2 is fairly restricted (namely activated T cells, NK cells, B cells, and dendritic cells) (Presky and others 1996; Grohmann and others 1998). IL-12R β 2 is undetectable on most resting T cells, but gets rapidly induced in the presence of cytokines, such as IL-2, IFN- γ , IL-12, IL-27, and TNF α (Collison and others 2012). Indeed, pretreatment of activated T cells with IL-2 or IL-27 increases their sensitivity to IL-35-mediated suppression (Collison and others 2012). It is thus possible that IL-27-mediated upregulation of IL-12R β 2 may prime T cells to the suppressive effects of IL-35, which may underlie the perceived inhibitory role of IL-27 (Vignali and Kuchroo 2012). The expression of IL-27R α , a component of the IL-35 receptor on B cells, is also primarily restricted to immune cells (T, B, NK, mast cells, monocytes, neutrophils) (Pflanz and others 2004). Since gp130 is ubiquitous and has a broad expression pattern, gp130 homodimers may facilitate IL-35-mediated suppression of a broad array of cell types. Thus, the ability of IL-35 to signal through multiple receptor combinations expands its sphere of influence and also varies the suppressive impact and consequences it has on different cell types.

Regulatory Role of IL-35 in Inflammation and Tumors

The regulatory potential of IL-35 has been highlighted in multiple disease models. Earlier studies were mainly performed using mouse strains deficient in p35, p40, and Ebi3 (Collison and Vignali 2008). However, the mice that lacked p40 were deficient in both IL-12 and IL-23 and thus any observation made could be attributed to effects from either cytokine. This was evident in the different susceptibilities of *Il12a*^{-/-} and *Il12b*^{-/-} mouse strains in EAE and CIA models. With the discovery of p19, some of the inexplicable differences were attributed to the proinflammatory role of IL-23 following reevaluation of the phenotypic differences in *Il12a*^{-/-}, *Il12b*^{-/-}, and *Il23a*^{-/-} mouse strains. The discovery of IL-35 suggested even more complexity as *Il12a*^{-/-} mice lack IL-12 and IL-35, while *Ebi3*^{-/-} mice lack IL-27 and IL-35. These observations warranted reassessment of the phenotypes attributed to either IL-12 or IL-27, which, in fact, could be features attributed to IL-35 (Collison and Vignali 2008).

Despite these complications, studies utilizing *Il12a*^{-/-} and *Ebi3*^{-/-} mouse strains provided substantial evidence confirming a role for IL-35 in ameliorating inflammation in various autoimmune and chronic inflammatory settings. Thus, IL-35 mediates suppressive effects in models of inflammatory bowel disease, EAE, collagen-induced arthritis, myocarditis, and liver fibrosis to name a few (Collison and others 2007; Niedbala and others 2007; Kochetkova and others 2010; Tirotta and others 2013). Several recent studies have complemented these genetic deletion approaches with gene therapy-based tools utilizing either plasmid DNA encoding single-chain IL-35 fusion protein, intraperitoneal injections of recombinant IL-35 or IL-35 transgenic mice. Ectopic IL-35 expression through these approaches conferred protection in mouse models of allergic airway inflammation, colitis, and diabetes (Huang and others 2011; Wirtz and others 2011; Bettini and others 2012). More recent studies have further expanded the protective effects of

IL-35 overexpression to murine acute graft-versus-host disease, viral myocarditis, and atherosclerosis (Huang and others 2013; Hu and others 2014; Liu and others 2014).

Involvement of IL-35 in tumor progression and tumor immune surveillance can be traced back to earlier observations demonstrating increased Ebi3 expression in EBV-associated Hodgkin lymphoma, diffuse large B-cell lymphoma, nasopharyngeal carcinoma, and acute myeloid leukemia cells (Niedobitek and others 2002). The IL-12 p35 subunit, but not IL-27p28, was associated with Ebi3⁺ tumor cells, providing early implications for a role for IL-35 in the tumor microenvironment (Niedobitek and others 2002; Larousserie and others 2005). Tregs, inhibitory cytokines, and induced regulatory populations are highly prevalent in peripheral blood and the tumor microenvironment in both mouse tumor models and cancer patients (Liyanage and others 2002; Wolf and others 2005). Furthermore, recent studies have suggested a role for Treg-derived IL-35 in limiting antitumor immunity (Collison and others 2010). The IL-35-induced regulatory population, iTTr35 cells, was shown to contribute significantly to the regulatory milieu in the tumor microenvironment, thereby confirming a critical role for the regulatory triad of IL-35, Tregs, and iTTr35 cells in tumor progression (Collison and others 2010). In addition to Tregs and induced regulatory populations, tumor-infiltrating dendritic cells have also been shown to express Ebi3, although IL-35 production by tolerogenic dendritic cells remains to be explored (Niedobitek and others 2002; Larousserie and others 2005). Stable Ebi3 expression in lung cancer cells promoted tumor progression, while siRNA-mediated Ebi3 gene silencing inhibited cancer cell proliferation (Nishino and others 2011). In fact, high Ebi3 expression in lung cancer cells was shown to be associated with poor prognosis (Nishino and others 2011). Similarly, Ebi3-deficient mice are protected from lung metastasis upon intravenous injection of the B16-F10 tumor cell line and this is mainly associated with induction of CD11c⁺B220⁺NK1.1⁺Gr1⁻ (IFN- γ -releasing) killer dendritic cells (IK-DCs) in the lungs that augment the local CD8⁺ T-cell antitumor response (Sauer and others 2008). Another recent report demonstrated a role for tumor-derived IL-35 in promoting tumor growth through the accumulation of suppressive myeloid cell populations (MDSCs) and increasing angiogenesis (Wang and others 2013). IL-35-producing cancer cell lines considerably increased tumorigenesis in both immune-competent and immune-deficient mice in 2 tumor models (J558 plasmacytoma and B16.F10 melanoma). Treatment with an IL-35-neutralizing antibody abrogated tumor enhancement, confirming the specificity of these effects to IL-35. IL-35⁺ tumors showed significant infiltration with CD11b⁺Gr1⁺ MDSCs that have been linked to promoting tumor angiogenesis through production of vascular endothelial growth factor and other proangiogenesis factors. MDSCs also mediate immune suppression by inhibiting cytotoxic T-cell responses. In line with this, IL-35⁺ tumors had reduced cytotoxic T lymphocyte (CTL) responses, although IL-35 did not directly affect CTL activation and effector function. Indeed, depletion of MDSCs with anti-Gr1 mAb abrogated tumor progression, confirming a critical role for MDSC accumulation in the tumorigenesis mediated by IL-35 (Wang and others 2013). IL-35 produced by tumor cells was also shown to play a role in making the tumor cells resistant to CTL-mediated destruction through

upregulation of gp130 expression on tumor cells. Importantly, gp130 signaling has been associated with cancer cell resistance to chemotherapy. IL-35 overexpression in tumor cells can also induce cell cycle arrest at the G1 phase and sensitize tumor cells and other infiltrating cells to serum starvation-induced apoptosis (Long and others 2013). Thus, through varied mechanisms, IL-35 may actually help the tumor cells evade immune surveillance and establish long-term tolerance, thereby promoting tumorigenesis. Since IL-35 can be produced by tumor cells as well as the tumor-infiltrating Tregs, induced regulatory cells, and possibly other populations in the tumor microenvironment, a better understanding of the cross talk between these populations would allow for effective targeting of the IL-35-mediated regulatory milieu for therapeutic purposes.

Serum IL-35 as a Diagnostic Biomarker for Human Cancers and Chronic Inflammation

While there is considerable literature highlighting the suppressive potential of IL-35 in various mouse models, comparable validation for the human counterpart is limited. Earlier studies failed to detect IL-35 production by human Tregs, both direct *ex vivo* and following T cell receptor (TCR) stimulation (Bardel and others 2008). Ectopic Foxp3 ex-

pression could not induce Ebi3 and p35 expression in human T cells (Allan and others 2008). These differences could be partially explained by the observations from a data mining study suggesting that IL-35 is not constitutively expressed in human tissues unlike TGF β , but may get induced in certain tissues and cell types in response to various inflammatory stimuli (Li and others 2012). This may indeed be true since it has been shown that human Tregs in the face of strong inflammatory stimuli (eg, following rhinovirus and hepatitis C virus infection) secrete IL-35, suggesting that IL-35 induction maybe a novel activation program in human T cells responding to viral infections (Langhans and others 2010; Seyerl and others 2010). Human CD4⁺ T cells exposed to IL-35 also express and mediate suppressive effects through IL-35 analogous to mouse iTr35 counterparts (Collison and others 2010). In addition, a human CD8⁺ regulatory T cell population expressing CTLA-4 and secreting IL-35 has been shown to regulate the antitumor immune response in prostate cancer (Olson and others 2012).

Interestingly, there seems to be an increasing number of reports documenting increased or decreased IL-35 levels in human samples from a variety of disease states (Table 1). Although warranting rigorous assessment in terms of the reagents and controls used for IL-35 detection, the overall trend suggests that increased serum IL-35 levels correlate

TABLE 1. SERUM IL-35 AS A DIAGNOSTIC BIOMARKER FOR CANCER AND AUTOIMMUNE DISEASES

Disease/model	IL-35 expression	References
Elevated circulating IL-35 levels in human cancer		
Colorectal cancer	Serum IL-35 levels are positively correlated with Treg numbers in the peripheral blood of colorectal cancer patients and reduction in serum IL-35 following tumor resection	Zeng and others (2013)
Pancreatic ductal adenocarcinoma	Increased plasma IL-35 levels associated with lymph node metastasis and late-stage tumor	Jin and others (2014)
Acute myeloid leukemia	Increased plasma IL-35 concentration has also been correlative in newly diagnosed acute myeloid leukemia patients, one of the most common hematological malignancy in adults	Wu and others (2012)
Reduced circulating IL-35 levels in human autoimmune diseases		
Multiple sclerosis (MS)	Serum IL-35 levels were lower in MS patients and treatment with interferon β , methylprednisolone, or combination of both offered beneficial effects through the upregulation of IL-35 production	Jafarzadeh and others (2015)
Asthma and chronic obstructive pulmonary disease (COPD)	Expression of IL-35 mRNA and protein were both downregulated in asthmatic children, and serum IL-35 level was inversely related to serum IL-4 and positively correlated with serum IFN- γ levels	Chen and others (2014) and Ma and others (2014)
Allergic rhinitis	Serum IL-35 and Ebi3 expression was also significantly reduced in allergic rhinitis patients relative to normal controls	Wan and others (2014)
Ulcerative colitis (UC) and Crohn's disease (CD)	Serum IL-35 levels were also significantly reduced in ulcerative colitis (UC) and Crohn's disease (CD) patients suggestive of insufficient anti-inflammatory activity <i>in vivo</i> .	Li and others (2014)
Immune thrombocytopenia (ITC)	Reduced plasma IL-35 levels in active ITC patients than those in remission	Yang and others (2014)
Fetomaternal tolerance, recurrent pregnancy loss (RPL), and preeclampsia (PE)	Decreased plasma IL-35 levels were noted in females with RPL and PE	Ozkan and others (2014, 2015)
Atherosclerosis and coronary artery diseases (CAD)	Plasma IL-35 levels were significantly decreased in patients with stable and unstable angina pectoris (SAP and UAP) and acute myocardial infarction (AMI)	Lin and others (2012)

FIG. 3. Proposed model for the role of IL-35 in health and disease. In the tumor microenvironment, replete with IL-35, the increased induction of IL-35⁺ populations (Tregs, iTr35, Bregs, CD8⁺ Tregs) impairs the antitumor immune response (CD8 effector, CD4 effector, and B cells), resulting in tumorigenesis (*left panel*). On the other end of the spectrum lie autoimmunity and chronic inflammation, wherein IL-35 deficiency results in activation of autoreactive cells, leading to the exacerbated inflammation associated with experimental autoimmune encephalitis (EAE), colitis, arthritis, and experimental autoimmune uveitis (EAU) (*right panel*).

with the severity of malignancy and the clinical stage of the tumor (Wu and others 2012; Zeng and others 2013; Jin and others 2014). In contrast to observations made with human malignancies, reduced IL-35 levels seem to be associated with human autoimmunity and chronic infections (Lin and others 2012; Mao and others 2013; Chen and others 2014; Jafarzadeh and others 2015; Li and others 2014; Ma and others 2014; Ozkan and others 2014, 2015; Wan and others 2014; Yang and others 2014).

These different correlative studies offer a consistent theme—increased IL-35 expression in tumors and malignancies and decreased IL-35 expression in autoimmune and chronic inflammatory conditions (Fig. 3). These observations can be reconciled to highlight critical information about this inhibitory cytokine. First, IL-35 levels may correlate with the degree of Treg infiltration in these different diseases. Thus, Tregs and other induced regulatory populations heavily infiltrate or are induced in the tumor microenvironment, impeding tumor immune surveillance, while autoimmunity and chronic inflammation are associated with reduced Treg numbers. Second, IL-35 expression may be associated with maximal Treg function or may mark highly suppressive Tregs. This can explain the high functionality of tumor-associated Tregs, while Tregs from autoimmune and inflammatory environments have impaired suppressor potential. Third, IL-35 levels may be highly indicative of the degree of Treg stability across a spectrum of immunological diseases. High IL-35 expression or increased IL-35⁺ Tregs in tumors may be an indication of highly stable Tregs im-

pairing the antitumor immune response. On the other hand, reduced IL-35 levels or decreased proportion of IL-35⁺ Tregs may underlie the basis for development of autoimmunity and chronic inflammation. While these observations are encouraging, more rigorous assessments of the functional scope of human IL-35 will be required to fully evaluate its therapeutic potential through blockade in cancer or administration in autoimmune and inflammatory diseases.

Concluding Model for IL-35-Driven Regulatory Milieu and Infectious Tolerance

The recent studies on B-cell-derived IL-35 have expanded the regulatory scope of IL-35 to other cellular lineages apart from Tregs. Given that the Breg field focused entirely on IL-10 as the sole mechanism of suppression over past several years, this discovery that Breg cells can also suppress through IL-35 secretion marks significant progress. In addition, given recent observations that IL-35 expression is inducible in many human tissues under conditions promoting inflammation, it is reasonable to predict that new cellular sources and targets of IL-35 may be identified, expanding the range of cells and tissues that are susceptible to IL-35-dependent immune regulation.

Importantly, IL-35⁺ Breg cells can induce Tregs while Treg-derived IL-35 can also sustain Breg cells. Thus, in inflammatory scenarios that elicit the IL-35-dependent regulatory milieu, these different IL-35-producing populations may promote reciprocal activity through a positive

feed-forward mechanism, resulting in induction of iTr35 cells and IL-35⁺ Bregs through infectious tolerance (Fig. 3). This may be particularly relevant in tumor settings that are heavily infiltrated with IL-35-producing Tregs, Bregs, and other suppressive populations. If this is true, approaches that boost or target IL-35 may result in recruitment or blockade, respectively, of both the B and T cell arms of the immune response, offering broader therapeutic benefits.

Perspectives, Challenges, and Future Directions

There are 7 key challenges that need to be resolved and questions that need to be addressed before a greater understanding of the physiological importance and therapeutic promise of IL-35 can be established.

- (1) Progress in the IL-35 field has been relatively slow due to significant challenges related to its unique biology. Given that IL-35 and the various IL-35 receptors share components with other IL-12 family cytokines and their receptors, dissecting and determining the true contributions of IL-35 has been challenging.
- (2) A major hindrance to progress in the field has been the lack of critical reagents. The lack of mutant mouse models to conditionally and temporally delete Ebi3, p35, and its receptor chains has been a major limitation. It would also be highly desirable to have reporter and Cre lines to further assess the expression and fate of IL-35, IL35R, and cells that express them. Another major limitation has been the lack of purified recombinant mouse and human IL-35 and the questionable activity and purity of the recombinant versions that are available. Last, the lack of availability of good antibodies for IL-35, and in particular the IL-35 receptor, has also been a significant limitation. The availability and quality of these key reagents will dictate the pace of future progress.
- (3) It will be important to determine the physiological relevance and contribution of IL-35 *in vivo* from the known cellular sources, especially Tregs, iTr35 cells, and Bregs. It will also be important to determine if there are any other cellular sources of physiological significance.
- (4) Given the apparent complexity of IL-35 receptor utilization, further studies are needed to clearly establish whether all these combinations are physiologically relevant and required. We also need to gain a greater understanding of the distribution of the IL-35 receptor and the cells that are impacted by IL-35.
- (5) The intracellular mechanism of action of IL-35 remains largely unknown. How does IL-35 inhibit cell cycle progression? How can a receptor that induces STAT1, STAT4, and/or STAT3 in different cell types mediate suppression and IL-35 (or IL-10) induction, while the same STATs can mediate proinflammatory events when activated by different cytokine receptors? In addition, are these receptors and signaling mechanisms really different in T versus B cells? Simply put, we need to know how IL-35 works.
- (6) The importance of IL-35 in humans also needs to be fully and rigorously established. We also need to determine if its mechanism of action is the same in human cells as it is in mouse cells.
- (7) Several clinically relevant questions remain. Is IL-35 a viable therapeutic target in cancer? Could recombinant IL-35 be used to treat autoimmunity and inflammatory diseases? Last, there needs to be a more rigorous assessment of IL-35 expression by different tissues and cell types in disease settings to determine the utility of IL-35 as a diagnostic biomarker.

Overall, while the expanded job profile of IL-35 generates enthusiasm, it also raises significant questions and challenges that need to be resolved. With expansion in its sources and targets, new roles have been ascribed to IL-35 beyond inhibition of proliferation and infectious tolerance, namely modulating T-cell differentiation programs, expansion of Tregs, and regulating APC function of B cells, thus far. Nevertheless, IL-35 remains an inhibitory cytokine and does not seem to have the opposing and pleiotropic activities of many other cytokines. More in-depth studies are warranted using recombinant IL-35 and specialized mouse models proposed above in appropriate cellular lineages to fully appreciate the physiological impact of IL-35. Understanding the cross talk between the various IL-35⁺ cell populations (Tregs, Bregs, CD8⁺ Tregs) in diverse disease settings and the overall regulatory network established by this unique suppressive cytokine will greatly aid in effective manipulation of IL-35 for translational benefit.

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Author Disclosure Statement

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