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Leading article

IL-35: A potential therapeutic target for controlling hepatitis B virus infection

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Interleukin (IL)-35, a recently identified cytokine of the IL-12 family, is a potent immunosuppressive cytokine secreted by regulatory T (Treg) cells and the newly reported regulatory B (Breg) cells. IL-35 functions as a crucial immunosuppressive factor in immune-mediated diseases, and the predominant mechanism of suppression is its ability to suppress T cell proliferation and effector functions. The pathogenic processes of the non-cytopathic hepatitis B virus (HBV) infection-related liver diseases are immune-mediated, including liver damage and viral control. It has been found that IL-35 is detectable in peripheral $CD4^+$ T cells in chronic HBV-infected patients, whereas it is undetectable in healthy individuals. There is growing evidence that cytokine-mediated immune responses play a pivotal role in determining the clinical outcome during HBV infection. It is particularly important to investigate the effects of IL-35 in the immunopathogenesis of chronic HBV infection. In this study, the recent understanding of this issue is discussed.

KEY WORDS: hepatitis B virus, human interleukin-35, immunotherapy, regulatory T lymphocytes, iTr35.

INTRODUCTION

Interleukin (IL)-35 is a recently identified heterodimeric cytokine that belongs to the IL-12 cytokine family, which is composed of the subunits of IL-27 β chain Epstein–Barr virus (EBV)-induced gene 3 (*Ebi3*) and IL-12 α chain p35.[1–3] IL-35 was first reported as Ebi3-p35 heterodimer at the 13th International Congress of Immunology in Rio de Janeiro, Brazil.[1,2] In this study, we focused on the studies investigating the characteristics of IL-35 and its association with chronic hepatitis B virus (HBV) infection.

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THE DISCOVERY AND ORIGIN OF IL-35

IL-12 cytokine family, including IL-12, IL-23, IL-27 and IL-35, is the only family consisting of heterodimeric cytokines.[1–4] An important characteristic of the IL-12 family is its chain-sharing of the cytokines and receptors.[4,5] The IL-12-related cytokines are heterodimeric proteins composed of two subunits, an α chain (p19, p28 or p35) and a β chain (p40 or Ebi3).[4,6] For example, IL-12 is composed of p35 and p40 subunits, whereas p40 binds to p19 to form IL-23 and Ebi3 binds to p28 to form IL-27.[7,8] The IL-12 family consists of five different subunits: IL-12 receptor β1 (IL-12Rβ1), IL-12 receptor β2 (IL-12Rβ2), IL-23 receptor (IL-23R), IL-27 receptor (IL-27R, also known as WSX-1) and gp130.[3,4,9,10] The receptors of IL-12 family also follow this chain-sharing character with several cytokines using the same receptor chains.[4,5,11] IL-12Rβ1 and IL-12Rβ2 form the receptor for IL-12, and that for IL-23 is composed of IL-12Rβ1 and IL-23R, while IL-27R and gp130 form the receptor for

IL-27, and the receptor for IL-35 is composed of gp130 and IL-12Rβ2.[4,9] Unusually, IL-35 also has another two receptors, that is, the homodimers of IL-12Rβ2–IL-12Rβ2 and gp130–gp130.[9] The Just Another Kinase (JAK)-Signal Transducers and Activator of Transcription (STAT) family consists of the downstream signal transduction pathway of these IL-12 receptors.[3,11]

Ebi3, which was first identified in EBV-infected B lymphoblastoid cell lines, is a homologue to IL-12 p40.[12] It has been reported that Ebi3 can bind the p35 subunit to form a heterodimeric hematopoietin by transfecting p35 and Ebi3 to cells *in vitro*.[13] In 2007, the Ebi3–p35 heterodimer was constructed, purified and identified as a cytokine *in vitro*,[1,2] and it was then officially named IL-35 by the International Union of Immunological Societies Subcommittee on Interleukin Nomenclature and the Human Genome Organisation (HUGO) Gene Nomenclature Committee.[14]

Subsequently, IL-35 was reported to be secreted by fork-head box protein 3 $(Foxp3)^+$ regulatory T (Treg) cells as an inhibitory cytokine.[1] Besides Treg cells, activated dendritic cells (DCs), macrophages, endothelial cells and aortic smooth muscle cells also express IL-35.[4,15–20] Since this discovery, biological function of IL-35 has been gradually illuminated during the recent years. IL-35 has been determined as a crucial cytokine of the IL-12 family.

THE BIOLOGICAL ACTIVITY OF IL-35 AND OTHER IL-12 CYTOKINES

Despite the common structures of the cytokines and receptors as well as the downstream signaling components, the biological activities of the IL-12 family members are, to some extent, quite diverse.[3,21] A consensus has been reached that IL-12 and IL-23 are mainly proinflammatory and pro-stimulatory cytokines that play vital roles in the differentiation of T helper (Th) 1 and Th17 cells, respectively.[22–24] And IL-27 is an immune-regulatory cytokine with both proinflammatory and anti-inflammatory effects.[3,4,21,22,24–26] However, biological activity of the newly identified IL-35 is distinctive. Strictly speaking, IL-35 is a potential immunosuppressive cytokine produced by Treg cells and the recently reported regulatory B (Breg) cells.[3,4,9,10,18,21,27–30]

IL-35-INDUCED Treg AND Breg CELLS

Treg cells are a special subset of T cells that are indispensable in regulating immune-mediated diseases such as autoinflammatory diseases, tumorigenesis and certain infections. The naturally occurring thymus-derived $CD4^+$ Treg cells are named as natural Treg (nTreg) cells.[31–33] The small population of nTreg cells in the subgroup of $CD4^+$ T cells have the ability to maintain immune homeostasis through *in vivo* conversion of non-Treg cells to suppressive cells, a process called 'infectious tolerance'.[18,34] $Foxp3^+$ Treg cells are important regulators for infectious tolerance via their ability to convert conventional $CD4^+Foxp3^-$ T cells into induced Treg (iTreg) cells directly by the production of suppressive cytokines, such as IL-10, transforming growth factor (TGF)-β or IL-35, or via DCs indirectly.[18,34–38] Type 3 Treg (Tr3) and type 1 Treg (Tr1) cells have already been reported according to the cytokines that induce them with potently suppressive biological activity both *in vitro* and *in vivo*.[34,39–42]

Conventional human or mouse T cells treated with the inhibitory cytokine IL-35 can generate a new type of iTreg cells that are different from TGF-β- or IL-10-induced iTreg cells. IL-35-induced Treg cells are named iTr35 cells, which mediates the immunosuppressive function via IL-35 but not via inhibitory TGF-β or IL-10.[18] Unusually, the transcription factor Foxp3 is not expressed or required by the iTr35 cells.[18,43] The iTr35 cells are stable *in vivo* with a strongly suppressive ability. This new subset of Treg cells are significant regulators of infectious tolerance and are involved in Treg cells-mediated tumor progression.[3,18,34] IL-35 and iTr35 cells develop a positive feedback loop to interact with each other: iTr35 cells generation can be induced by IL-35, while more IL-35 is further secreted by iTr35 cells.[18]

Most recently, Shen *et al*.[27] have found that in autoimmune and infectious diseases, IL-35-secreting B cells function as crucial mediators in the negative regulation of immunity, and mice lacking IL-35 expression in B cells did not recover from experimental autoimmune encephalomyelitis only, which is a T cell-mediated demyelinating autoimmune disease. However, these mice showed obviously improved resistance to infection with intracellular bacterial pathogen (*Salmonella enterica* serovar Typhimurium) compared with the mice with wild-type B cells. Wang *et al*.[28] have demonstrated that IL-35 can induce the formation of Breg cells and facilitate their conversion to a new subgroup of Breg cells that secrete IL-35 and IL-10. This study also showed that mice treated with IL-35 were protected from experimental autoimmune uveitis, and mice lacking IL-35 ($p35^{-/-}$) or those defective in IL-35 signaling ($IL\text{-}12R\beta2^{-/-}$) produced

endogenously fewer Breg cells or after treated with IL-35, resulted in severe uveitis. IL-35 functions as both an inducer and a mediator of Breg cells,[29] it can switch off inflammation in rodent models of autoimmunity, whereas its absence in B cells enhanced their survival after infected with pathogens, indicating the potential therapeutic value of IL-35 targeting human autoimmune and infectious diseases.[29] The two studies have now taken the investigation of IL-35 to a new height.

POTENTIAL ROLE OF IL-35 IN HBV INFECTION

Chronic HBV infection is a leading cause of liver fibrosis, cirrhosis and even hepatocellular carcinoma, contributing to a major health threat and major economic burden in the world, despite the significant progress in vaccinations and antiviral therapy during the past decades.[44] It is estimated that two billion people worldwide have been infected, and 350–400 million are chronic HBV carriers.[45] In the 2010 Global Burden of Disease study, HBV infection ranked in the top health priorities in the world and was the 15th leading cause of death (786 000 deaths per year).[46]

As HBV is a non-cytopathic virus, both HBV-related liver damage and viral control are immune-mediated. The natural history of chronic hepatitis B is generally divided into four phases: the immune tolerant phase, the immune clearance phase, the low replicative or inactive carrier stage and the reactivation phase.[47–49] There is growing evidence that cytokine-mediated immune responses play an important role in determining clinical outcomes during HBV infection.[44,50–54]

As an immunosuppressive cytokine, the predominant mechanism of suppression associated with the activity of IL-35 is its ability to inhibit T cell differentiation and effector functions. IL-35 plays a critical role in a variety of diseases, such as inflammatory bowel disease,[55–57] T cell-dependent colitis,[58] central nervous system demyelination,[59] smoking-related lung inflammation,[60] autoimmune diabetes,[61] coronary artery diseases,[62] autoimmune encephalomyelitis,[63] human prostate tumor,[64] atherosclerosis,[65] pregnancy,[66] arthritis,[67–69] coronavirus-induced encephalomyelitis,[70] colorectal cancer,[71] asthma,[72–74] multiple sclerosis,[75] pancreatic ductal adenocarcinoma,[76] pancreas cancer,[77] leukemia[78,79] and systemic lupus erythematous.[80]

Based on the abovementioned studies on other immune-mediated diseases, there is abundant reason to believe that IL-35 might play a pivotal role in the immunopathogenesis of HBV infection and its related liver diseases, especially in the development of the immune tolerant phase and persistent chronic HBV infection. However, few studies have focused on the association between IL-35 and HBV infection so far. Thus, more studies are needed to elucidate the roles of IL-35 in HBV infection and its related lesions.

In the field of viral hepatitis and liver diseases, Langhans *et al*.[81] have reported that the core antigen of hepatitis C virus (HCV) could induce the generation of a relatively high level of HCV core-specific Treg cells in patients with chronic hepatitis C. These HCV core-specific Treg cells could suppress the reporter T cells via the production of IL-35 and IL-10,[81] indicating a potential ability of IL-35 to inhibit virus-specific T cell responses that lead to persistent chronic HCV infection. Liu *et al*.[82] have provided the first research data that IL-35 is detectable in circulating $CD4^+$ T cells in chronic HBV-infected patients, whereas it is undetectable in healthy controls. Liu *et al*. have showed that both the mRNA expression of Ebi3/p35 and the protein secretion of IL-35 is detectable in circulating $CD4^+$ T cells in patients with chronic hepatitis B, suggesting that HBV could also act as an inducer for the $CD4^+$ T cells to initiate IL-35 secretion.[82] In a study on primary biliary cirrhosis, Tsuda *et al*.[83] deleted the gene encoding the IL-12p35 subunit from dominant-negative TGF-β receptor type II (dnTGF-βRII) mice, which resulted in an IL-12p35(-/-) dnTGFβRII strain. They found that there was a strikingly high frequency (>50%) of liver fibrosis in the $p35^{-/-}$ dnTGF-βRII mice, suggesting a possible correlation between IL-35 and liver fibrosis. Wang *et al*.[84] investigated the changes in the expression of IL-35 in patients with liver cirrhosis-related portal hypertension complicated with esophageal variceal bleeding and splenomegaly before and after phased joint intervention (surgical intervention for treating the complications of decompensated liver cirrhosis), showing that the circulating concentrations of IL-35 were obviously reduced the efficient surgical intervention, and serum IL-35 levels were positively correlated with total bilirubin levels and international normalized ratio but was negatively correlated with albumin levels. This indicated that IL-35 might be correlated with the deterioration of liver cirrhosis.

In conclusion, IL-35, a recently identified cytokine of the IL-12 family, can induce the generation of iTr35 cells and Breg cells, which function as an immunosuppressive factor in immune-mediated diseases such as

autoimmune and infectious diseases. Data on the roles of IL-35 in HBV infection, especially the effects of IL-35 in immunopathogenesis during chronic HBV infection, are still limited. Future investigations are needed to fully elucidate the interrelationship between IL-35 and persistent HBV infection and its related liver deterioration. IL-35 might play a crucial role in the disorder of immune regulation during HBV infection. With further advances in the understanding of IL-35 and its immunosuppressive mechanisms, it will be possible to design targeted immunotherapies and antivirus approaches to modulate the immune response for controlling persistent chronic HBV infection or deterioration in liver diseases.

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