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Interleukin (IL)-35 is raising our expectations

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ABSTRACT

Purpose: *To elucidate and discuss the role of IL-35 in immunity to parasitic and bacterial infections as well as in autoimmunity in terms of its anti-inflammatory properties, we highlight significant findings on this novel member of the IL-12 family.* ***Methods:*** *Studies using genetically deficient mice have greatly enhanced our understanding of the biology of IL-35. On the basis of data derived from the analysis of these genetically deficient mice published by NIH, we focus on the key features of this heterodimeric cytokine, especially its relation to the other IL-12 family members, and discuss its potential relevance to the clinical usage.* ***Principal findings:*** *IL-35 is required for the $CD4^+CD25^+$ Treg cells-mediated immune regulation, the alleviation of some inflammatory responses, as well as the expansion of $CD4^+CD25^-$ Teff cells simultaneously. Moreover, administration or augmentation of IL-35 suppresses some diseases of autoimmune or allergic origin like collagen-induced arthritis or Helicobacter- induced colitis in animal models, demonstrating its potential in therapy of diseases mediated by inflammatory cytokines. However, some questions involving it are still unclear, including the composition of IL-35 receptor, IL-35-related cell signaling pathway, the different expression patterns of IL-35 between human and murine T cells, etc.* ***Conclusion:*** *As our understanding of the IL-35 is rapidly growing and changing, it will bring us more therapeutic strategies towards some intractable immune diseases such as Lupus Erythematosus.*

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Key words: *IL-12; IL-35; Treg cells; Teff cells.*

Las expectativas futuras de la interleukina (IL)-35

Esta es una revisión acerca del rol de IL-35, un nuevo miembro de la familia IL-12, en la respuesta inmunitaria contra infecciones parasitarias y bacterianas y de su rol beneficioso en reacciones auto inmunes, debido sus propiedades antiinflamatorias. Basándose en estudios de ratones genéticamente deficientes se ha determinado que se requiere IL-35 para la acción inmunoreguladora de las células T reguladoras $CD4^+CD25^+$, para mitigar algunos procesos inflamatorios y para expandir simultáneamente los clones de células T efectoras $CD4^+CD25^-$. Mas aún, la administración o estimulación de la acción de IL-35 en modelos animales, suprime algunas enfermedades de origen alérgico o autoinmune tales como la colitis colágena y la colitis inducida por Helicobacter. Estos experimentos demuestran el potencial terapéutico de IL-35 en enfermedades mediadas por citokinas inflamatorias. Sin embargo, algunos aspectos de la citokina aún no han sido dilucidados, tales como la

composición del receptor de IL-35, la vía de señalización celular asociada a IL-35 y los diversos patrones de expresión de la citokina en células humanas y de ratones. En la medida que aumente el conocimiento acerca de las acciones de IL-35, nos podrá proveer tratamientos para algunas enfermedades auto inmunes actualmente limitadas en su tratamiento, como el lupus eritematoso.

Several years ago, interleukin (IL)-12 was shown to be required for the differentiation of naive $CD4^+$ T cells into Th1 cells with the concomitant production of interferon (IFN)-γ[1-3]. The interleukin-12 (IL-12) cytokine family contains IL-12, IL-23, IL-27, and IL-35 today. These four members are all heterodimeric cytokines, which are composed of one chain (p19, p28, or p35) and the other chain (p40 or Epstein-Barr virus induced gene 3 (EBI3)), and then signal through unique pairings of five receptor chains (IL-12Rβ1, IL-12Rβ2, IL-23R, gp130, and WSX-1) respectively. The latest identified IL-35 consists of two subunits: p35 and EBI3[4,6]. It is known that, to function correctly, the immune system must discriminate between endogenous and exogenous substances. When this discrimination fails, the immune system will destroy cells and tissues of the body, thus resulting in autoimmune diseases[7]. Regulatory T cells (Treg cells) actively suppress activation of the immune system and prevent pathological self-destruction, i.e. autoimmune disease. The critical role of Treg cells in the immune system was documented by the severe autoimmune syndrome that resulted from a genetic deficiency of Treg cells. In murine Treg cells, the defects in either p35 or EBI3 often lead to the loss of host immune suppression and bring in subsequent deterioration of immune diseases[8-10]. In this review, we emphasize on the significant findings about IL-35. These findings have important implications for the design of new therapeutic approaches in some intractable immune diseases such as Lupus Erythematosus and Diabetes Type 1.

The structure and receptor for IL-35

At first, the structure of IL-12 family member will only concisely be described here (Figure 1)[11]. 1) IL-12p70 is one type of heterodimeric cytokine consisting of p40 subunit and p35 subunit. While p35 subunit is structurally associated with type I cytokines, p40 subunit is homologous to the α-chain of soluble IL-6 receptor. The IL-12p70 receptor comprises IL-12Rβ1 chain and IL12-Rβ2 chain. p40 is also secreted as monomers or homodimers (IL-12p80) which then signals through IL-12Rβ1. 2) p19 protein was identified in terms of its homology with IL-6 or IL-12p35[12] and it was shown to interact with IL-12p40 subunit to form another type of heterodimeric cytokine known as IL-23. The IL-23 receptor consists of IL-12Rβ1 chain and another subunit termed IL-23R. 3) EBI3 has been identified as an IL-12p40 homologue, which was found to interact non-covalently with p28, another IL-12p35 homologue, thereby taking the shape of IL-27. The IL-27 receptor consists of WSX1 and gp130 (the latter is also a subunit of IL-6 receptor complex). 4) Recently, EBI3 was reported to interact with IL-12p35 to form the novel cytokine, termed IL-35, but the receptor for this heterodimeric cytokine remains unclear[13]. In terms of the homology of IL-35 with other IL-12 family members, some predictions about its receptor structure will be addressed here. 1) For IL-12, IL-12Rβ2 is expressed on activated T cells, whose expression could be stimulated by the agonists that promote the development of Th1 cells, or inhibited by the ones that promote the development of Th2 cells. Upon the interaction with its ligands, IL-12Rβ2 becomes tyrosine phosphorylated and provides binding sites for kinases such as Tyk2 and Jak2, which are important in activating critical transcription factors such as Stat4 in T cells or NK cells[14]. However, IL-12Rβ1 contains neither the N-terminal Ig-like activation domain, nor the cytoplasmic tyrosine residues required for docking and activating Stat4[15]. Thus, IL-12Rβ1 is mainly responsible for ligand interaction, not like IL-12Rβ2 as a signal transducer. 2) For IL-23, Jak2 and Stat3 transcription factors physically interact with IL-23R in a ligand-dependent manner and IL-23R itself could be tyrosine-phosphorylated upon the interaction with IL-23. Therefore, like IL-12Rβ2, IL-23R is mainly responsible for IL-23 signal transduction[16,17]. 3) For IL-27, WSX1 directly binds to IL-27, but it cannot activate any signaling

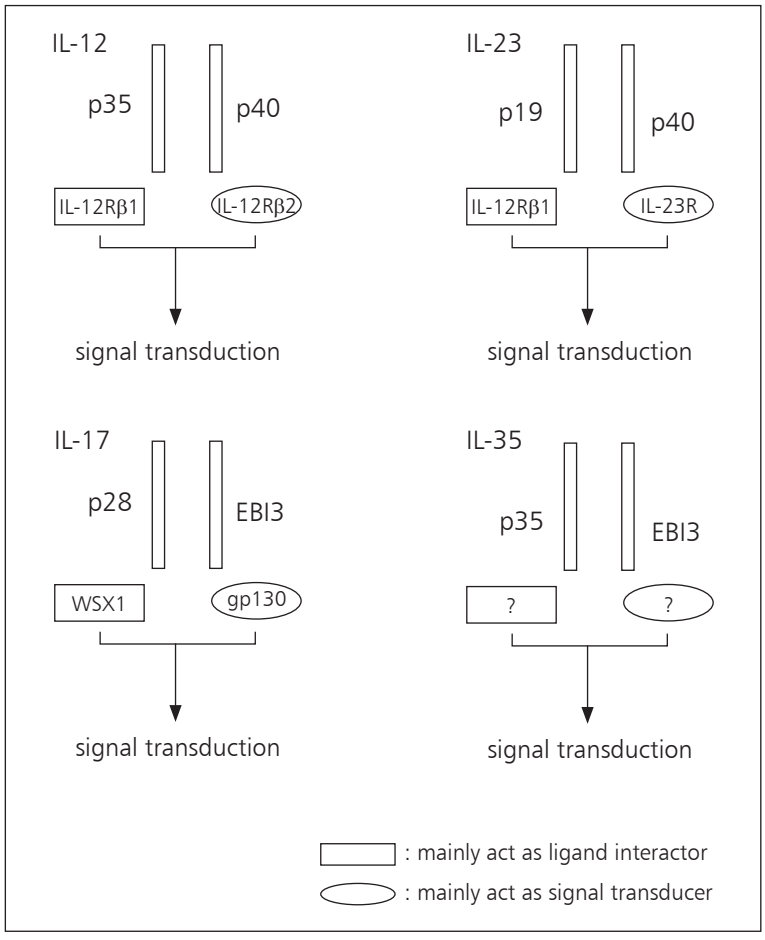

Figure 1. IL-12 family members and their receptors, A: IL-12 and its receptor; B: IL-23 and its receptor; C: IL-27 and its receptor; D: IL-35 and its predicted receptor.

pathways as monomers[18]. Surprisingly, WSX1 actually owns one tyrosine-based phosphorylation motif in the cytoplasmic domain, and the sequence of this domain closely resembles the Stat1 binding motif in the cytoplasmic region of IFN-γR, which selectively activates tyrosine phosphorylation of Stat1[19]. Contrasted with WSX1, gp130 leads to the intracellular activation of Src, Jak and Stat family members[20,21,22]. Based on the structural and functional similarity between IL-35 and other IL-12 family members, it is predicted that IL-35 receptor probably shares common features with the receptors for other IL-12 family members, in another word, the IL-35 receptor might consist of two subunits as well, one subunit mainly act as ligand interactor, and the other one mainly act as signal transducer. However, this remains to be further verified.

The expression of IL-35

The expression of EBI3 is restricted in peripheral $CD4^+CD45RB^{lo}CD25^+$ Treg cells versus naive $CD4^+CD45RB^{hi}CD25^-$ Teff cells sorted from C57BL/6 mice, or in $Foxp3^+$ Treg cells versus $Foxp3^-$ Teff cells sorted from GFP-Foxp3 knock-in mice[13]. Lauren W. collision, et al then investigated whether IL-12α, p19 or p28 was expressed in peripheral Treg cells, and then ensured that IL-12α was the unique one expressed in Treg cells. Further analysis confirmed that Teff cells ($CD45RB^{hi}$ or $CD45RB^{lo}$) expressed little amount of EBI3 or IL-12α mRNA, thus being distinguished from $Foxp3^+$ Treg amongst $CD4^+$ T cells. Immunoblot analysis verified that EBI3 was co-immuno-precipitated with IL-12α in supernatants from resting Treg cells, but neither Teff nor $EBI3^{-/-}$ Treg cells, demonstra-

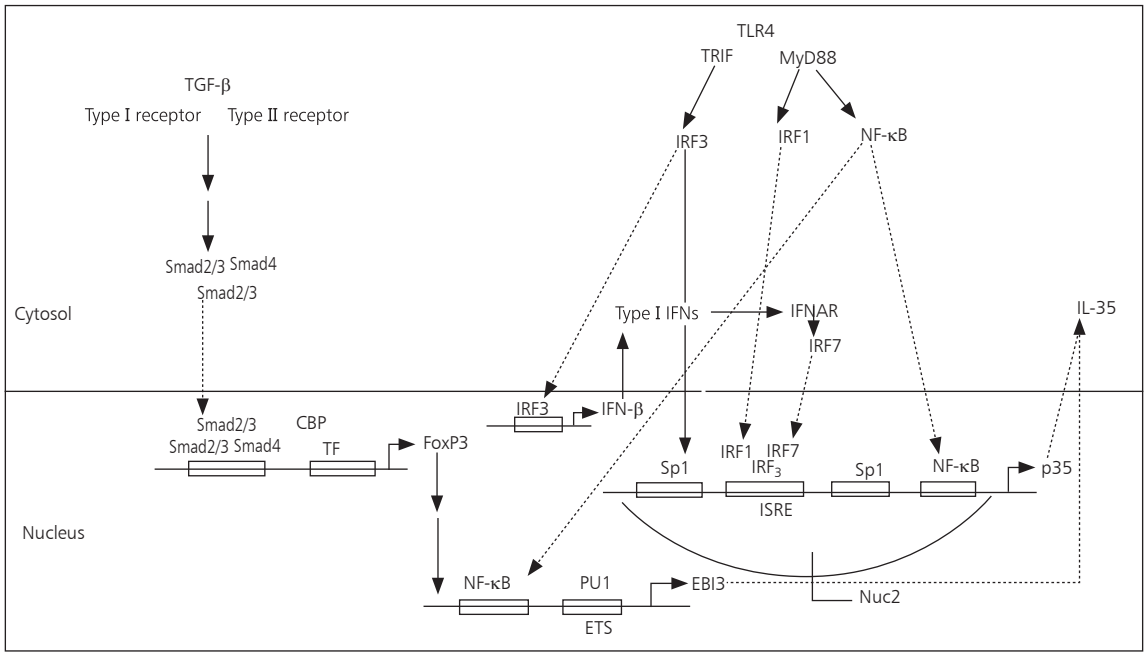

Figure 2. The expression of IL-35.

ting the preferential secretion of EBI3-IL-12α (IL-35) by T cells. However, additional analysis of other haematopoietic populations suggested that there was still a significant but low expression of EBI3 or IL-12α in αδ and $CD8^+$ T cells.

Myeloid differentiation primary-response gene 88 (MyD88), which is coupled to Toll-like receptor 4 (TLR4), leads to the activation of nuclear factor-κB (NF-κB) (Figure 2), so as to induce the expression of relevant genes that encode interleukin-12 (IL-12) family members[23, 24]. Since IL-35 consists of p35 and EBI3, the expression of each one will be addressed here respectively. The expression of p35 could be regulated by multiple routes, as follows: Firstly, the interaction of MyD88 with IRF1 expands the expression of p35 gene [25]. Secondly, the TIR-domain-containing adaptor protein inducing IFN-β (TRIF) triggers the nuclear translocation of IRF3, which is then recruited to IFN-stimulated response element (ISRE) sites in the promoter regions of both p35 gene and IFN-β gene[26]. Next, IFN-β binds to the type I IFN receptor (IFNAR) and leads to the activation of IRF-7 that induces the expression of p35 gene[27]. Thirdly, upon the activation of TRIF-related pathway, IRF1 also enhances the expression of p35 gene[28,29]. Moreover, the activation of p35 gene also requires the selective remodeling of nucleosomes to release it from the transcriptional suppression by Nuc2, because Nuc2 within the p35 promoter masks crucial ISRE–IRF-E and SP1 binding sites, like Nuc1 in the p40 promoter masks the transcription initiation site and CCAAT/ enhancer binding protein (C/EBP) binding site[30-32]. On the other hand, EBI3 is regulated via MyD88 and NF-κB like p35, and the PU1 binding to ETS cis-regulatory element is another necessity for activating EBI3 promoter[33]. Moreover, some reports have already documented the induction of Foxp3 by stimulating conventional T cells in the presence of TGF-β[34-36]. Ethan M. Shevach, et al discovered that the induction of Foxp3 almost completely relied on the TGF-β present in the serum, which was markedly inhibited by the addition of anti-TGF-β antibodies to the cell cultures[37]. Besides this, as a downstream target of Foxp3, the expression of EBI3 was considerably increased in Foxp3-transduced Teff cells compared with control group, whereas Foxp3 only induced a limited expression of p35[38]. However, microarray analysis uncovered that Foxp3 regulated EBI3 indirectly, because it was not discovered within the Foxp3 direct target gene set[39].

The biological functions of IL-35

IL-35 is required for the function of Treg cells

Some earlier reports documented that Treg cells exerted the immuno-suppressive function through their T-cell antigen receptors (TCR)[15, 16]. Next, researchers assessed how EBI3 and IL-12α mRNA levels in activated Treg cells were changed in the presence or absence of Teff cells[38]. Their results showed that both EBI3 and IL-12α mRNA were reduced significantly after the co-stimulation of anti-CD3 and anti-CD28, but were markedly upregulated together in Treg cells recovered from an in vitro Treg assay, which was coincided with the process of active suppression. While the loss of negative regulatory EBI3-IL-12α (IL-35) could be reversed by the loss of pro-inflammatory cytokines such as IL-27 and IL-12 in either EBI3-/- or IL-12α-/- mice respectively, those EBI3-/- mice were indeed more susceptible to leishmaniasis[18]. Likewise, those IL-12α-/- mice, differing from IL-12β-/- mice, were more susceptible to Helicobacter-induced colitis, Leishmania major infection, experimental autoimmune encephalomyelitis and collagen-induced arthritis[17,19-21]. Treg cells have already been proven to control the homeostatic expansion of Teff cells in a lymphopenic, recombination activating gene 1 (Rag1)-/- environment[5,22]. Moreover, researchers also assessed the ability of EBI3-/- or IL-12α-/- Treg cells to suppress the proliferation of wild-type Teff cells in vitro, and found that the immuno-suppressive capacity of EBI3-/- or IL-12α-/- Treg cells was markedly reduced, regardless of the population of Teff cells[38]. When purified wild-type Teff cells, either alone or in the presence of wild-type, EBI3-/- or IL-12α-/- Treg cells, were adoptively transferred into Rag1-/- mice by scientists, which subsequently confirmed that the expansion of wild-type Teff cells was hardly reduced in the presence of either EBI3-/- or IL-12α-/- Treg cells compared with that in the presence of wild-type Teff cells, indicating that EBI3, together with p35 as a heterodimeric cytokine of IL-35, exerted the immuno-suppression synergistically. However, the latest report demonstrated that unlike murine Treg cells, ex vivo human Treg cells didn't express significant EBI3 mRNA, and p35 mRNA wasn't different between ex vivo Treg and Teff cells either, in other words, neither EBI3 nor p35 mRNA was affected by the over-expression of Foxp3 in human Treg cells, suggesting that IL-35 might not contribute to the suppressive mechanism of human Treg cells, contrasted with the results in murine counterparts[40].

IL-35 is required for the anti-inflammatory responses

Due to the clinical importance of Treg in the control of rheumatoid arthritis (CIA), Wanda Niedbala, et al investigated the effects of IL-35 in the CIA model of DBA/1 mice[41-43]. Control group treated with PBS undoubtedly developed the expected disease progression. In contrast, mice treated with IL-35 displayed a significant reduction in the incidence and number of arthritic paws. Histological analysis showed that mice treated with PBS displayed mononuclear and poly-morphonuclear cell infiltration into the joint compartment, synovial hyperplasia, adjacent cartilage and bone erosion, but which were obviously reversed in the mice treated with IL-35, demonstrating that IL-35 potently suppressed the development of CIA, and such activity can prevent the progression of articular damage as well[44]. Serum of IL-35-treated mice contained significantly higher concentrations of IL-10 compared to the control group, and IL-10 could prevent NF-κB from initiating the transcription of relative genes that encode pro-inflammatory cytokines by inhibiting the activation of IKK and NF-κB's DNA binding capacities[45]. However, Lauren W et al discovered that Teff-derived IL-10 might also contribute to this regulatory milieu, because Treg co-cultures with IL-10$^{-/-}$Teff exhibited reduced suppression compared with wild type Teff[46]. On the other hand, severe IBD pathology, including loss of goblet cells mucus secretion, mucosal hyperplasia, extensive ulceration or infiltration of CD3$^+$ T cells, remarkable transmural lymphohistiocytic inflammation, and destruction of the normal physiological functions by the inflammatory infiltration, was observed in the Treg-deleted recipients, but there was such a significant alleviation of the inflammation and CD3$^+$ T-cell infiltration, as well as the regeneration of goblet cells and mucus secretion in wild-type Treg recipients[39,47,48]. Likewise, among IL-12 family members, IL-27 owns the similar immunosuppressive function requiring the activation of Stat1 and its downstream effector Socs3[49]. However, the anti-inflammatory mechanisms of IL-35 are still unclear.

IL-35 is required for the expression of $CD4^+CD25^+$ Teff cells

Wanda Niedbala, et al purified $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells from spleen and lymph nodes of BALB/c mice and cultured them upon the stimulation with plate-bound anti-CD3 and anti-CD28 antibodies, to trigger maximal T cell activation. It was demonstrated that IL-35 markedly expanded the proliferation of $CD4^+CD25^+$ Treg cells with the elevated synthesis of IL-10, but also induced marked proliferation of $CD4^+CD25^-$ Teff cells with the elevated concentration of IFN-γ in culture supernatant, which was surprisingly paradoxical to the immuno-suppressive functions of IL-35 restricted in Treg cells[44]. Moreover, Lund, et al. reported their similar findings that induction of the early antiviral immune response at a site of infection was actually promoted by Treg cells, in which the expansion of both Treg and Teff cells were synchronized, even that their ratio remained relatively constant[50,51]. Consequently, researchers found that the ablation of Treg cells caused the overaccumulation of chemokines (CCL2, CXCL9, and CXCL10) released by dendritic cells, natural killer cells, and stromal cells in the lymph node, which further attracted surrounding dendritic cells, natural killer cells, and T cells to the lymph node, thus amplifying the immune response there, leading to the abrogation of antiviral responses at those actual sites of infection, thus providing virus with enough time to aggravate the infection[52,53]. In another word, Treg cells can expand and drive immune Teff cells out of the lymph node into the sites of viral infection, in which EBI3-p35 (IL-35) is probably required for the immuno-stimulatory activity of Treg cells because EBI3 could induce macrophages to synthesize the MIP-1α, which further attracted B and T lymphocytes to the sites of inflammation[54]. However, Treg cells and Teff cells differ in their T cell antigen receptors, thus how Treg cells respond to foreign antigens and whether IL-35 is playing an important role here require further research[55,56]. In addition, there is one possible explanation that Treg cells could be synchronously triggered by IL-2 released from Teff cells[57].

IL-35 suppressed the differentiation of Th17 cells

Recently, it was demonstrated that IL-35 markedly suppressed the differentiation of Th17 cells compared to the ones in medium alone[44]. Mangan group and Bettelli group discovered that TGF-β could promote pro-inflammatory responses through accelerating the differentiation of Th17 cells[58,59]. Some researchers speculated that IFN-γ might inhibit the phosphorylation of TGF-β receptor's downstream effector Smad3, so as to block the TGF-β-induced differentiation of Th17 cells, while IFN-γ could be upregulated by IL-35[44,60,61]. Moreover, Th17 cells are characterized by the secretion of IL-17 and IL-22, in which IL-17 was regarded to be involved in host defense and protective immunity[62]; IL-22 was considered to mediate IL-23-induced Th17 cell generation[63]; and RORγt was found to be the key transcription factor for the differentiation of Th17 cells[64]. Meanwhile, the deletion of EBI3 could also upregulate the expression of IL-17, IL-22 and RORγt, thus confirming the immuno-suppressive effect of IL-35 on the differentiation of Th17 cells as well[65]. However, the precise mechanism hidden behind this phenomenon requires further verification.

Conclusion and prospect

On the basis of present research, it was demonstrated that IL-35, as a novel member of interleukins, was required for the immunological capacity of Treg cells, and concomitant anti-inflammatory responses; it expanded not only $CD4^+CD25^+$ cells but also $CD4^+CD25^-$ cells declaring its function in early anti-viral immune reactions; besides these, it could also abrogate the differentiation of Th17 cells like its homologue IL-27. However, French scientists published their surprising discovery not long ago; they reported that although CD3/CD28 stimulation induced low levels of EBI3 in various human $CD4^+$ T cell subsets, no EBI3 could be detected in CD3/CD28-stimulated human Treg cells[66]; furthermore, whereas p35 mRNA were detected in both Teff and Treg cells, EBI3 mRNA were detected only in activated human Teff cells, but not in resting or activated human Treg cells, which contrasted with their murine counterpart, indicating the unknown role of IL-35 in human T cells. Thus, the composition of IL-35 receptor, IL-35-related cell signaling pathway, the different expression patterns of IL-35 between human and murine T cells, as well as the molecular mechanisms hidden

behind its more biological activities requires to be further investigated. As our understanding of the IL-35 is rapidly growing and changing, it will bring us more therapeutic strategies towards some intractable immune diseases such as Diabetes Type 1 and Lupus Erythematosus.

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