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Review Article

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Interleukin-18 Binding Protein (IL-18BP): A Long Journey From Discovery to Clinical Application

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AOSD, adult-onset still's disease; AS, ankylosing spondylitis; IFNaR2, type 1

ABSTRACT

IL-18 binding protein (IL-18BP) was originally discovered in 1999 while attempting to identify an IL-18 receptor ligand binding chain (also known as IL-18Rα) by subjecting concentrated human urine to an IL-18 ligand affinity column. The IL-18 ligand chromatography purified molecule was analyzed by protein microsequencing. The result revealed a novel 40 amino acid polypeptide. To isolate the complete open reading frame (ORF), various human and mouse cDNA libraries were screened using cDNA probe derived from the novel IL-18 affinity column bound molecule. The identified entire ORF gene was thought to be an IL-18Rα gene. However, IL-18BP has been proven to be a unique soluble antagonist that shares homology with a variety of viral proteins that are distinct from the IL-18Rα and IL-18Rβ chains. The IL-18BP cDNA was used to generate recombinant IL-18BP (rIL-18BP), which was indispensable for characterizing the role of IL-18BP *in vitro* and *in vivo*. Mammalian cell lines were used to produce rIL-18BP due to its glycosylation-dependent activity of IL-18BP (approximately 20 kDa). Various forms of rIL-18BP, intact, C-terminal his-tag, and Fc fusion proteins were produced for *in vitro* and *in vivo* experiments. Data showed potent neutralization of IL-18 activity, which seems promising for clinical application in immune diseases involving IL-18. However, it was a long journey from discovery to clinical use although there have been various clinical trials since IL-18BP was discovered in 1999. This review primarily covers the discovery of IL-18BP along with how basic research influences the clinical development of IL-18BP.

Keywords: Discovery of IL-18BP; Autoimmune diseases; rIL-18BP; Basic research; Clinical trials

DISCOVERY OF IL-18 BINDING PROTEIN (IL-18BP)

IL-18BP was discovered while endeavoring to find an IL-18 receptor alpha or ligand binding chain (IL-18Rα) [1]. As depicted in **Fig. 1A**, the first step was to isolate IL-18 interacting molecules from human concentrated urine using IL-18 affinity column (IL-18 affi-column). The IL-18 affi-column was constructed with 2.5 mg of recombinant IL-18 (rIL-18BP) protein immobilized to 0.5 ml of agarose beads Affigel-10. The 500 ml of a 1,000-fold concentrate crude urinary was passed on the IL-18 affi-column. The column was extensively washed with phosphate buffered (pH 7.4) containing 0.65 molar $NaCl_2$. The IL-18 interacting molecule was

interferon receptor 2; IL-18 affi-column, IL-18 affinity column; IL-18BP, IL-18 binding protein; IL-18Rα, IL-18 receptor alpha; IL-1Rrp, IL-1R related protein; MAS, macrophage activation syndrome; MS, mass spectrometry; NLRC4, NLR family CARD domain-containing protein 4; NUMA1, nuclear mitotic apparatus protein 1; ORF, open reading frame; PR3, proteinase 3; PVDF, polyvinylidene difluoride; RA, rheumatoid arthritis; rIL-18BP, recombinant IL-18BP; SLE, systemic lupus erythematosus; TM, transmembrane; TNFBP, TNF-α binding protein; XIAP, X-linked inhibitor of apoptosis protein.

Author Contributions

Conceptualization: Kim S; Funding acquisition: Kim S; Writing - original draft: Kim S; Writing - review & editing: Kim S, Yu H, Azam T, Dinarello CA.

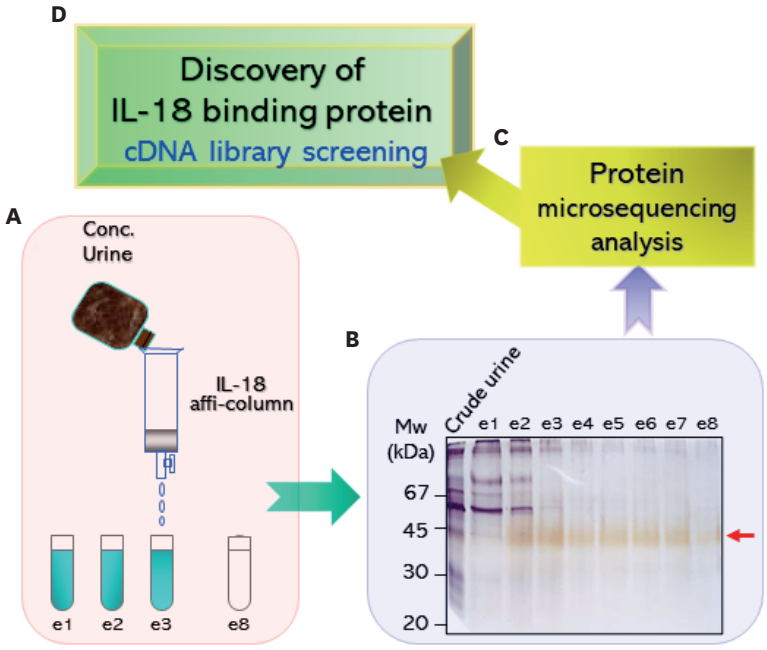

Figure 1. Schematic drawing of IL-18 affi-column to isolate IL-18BP from concentrated human urine. (A) Concentrated human urine was passed through IL-18 affi-column then eluted bound molecules. (B) The eluted fraction was visualized by silver staining. (C) Edman degradation protein microsequencing revealed a novel 40 amino acid sequence. (D) The screening cDNA libraries discover full ORF of four human and two mouse IL-18BP isoforms.

eluted with acidic solution (pH 2.2) then each fraction was neutralized immediately with an alkaline buffer to stabilized eluted molecules in the tube. IL-18 affi-column bound molecules were separated by 10% SDS-PAGE then visualized by silver staining **Fig. 1B** (1).

Besides multiband in elution 1 and 2, approximately 40 kDa molecule with gold color (red arrow) appeared from elution 2 then become a dominant band from elution 3 through elution 8 (**Fig. 1B**). Currently mass spectrometry (MS) analysis allows to use material excised directly from 10% SDS-PAGE silver-stained. However, only protein microsequencing was available as described in 1997 (2). The 10% SDS-PAGE was blotted onto polyvinylidene difluoride (PVDF) membrane and stained with Coomassie blue to analyze the 40 kDa molecule. Approximately the 40 kDa band was excised from PVDF membrane and sent for protein microsequencing analysis that revealed a novel 40 amino acid sequence (**Fig. 1C**). The robust experiment of ligand affinity chromatography discovered clinically important molecules such as TNF-α binding protein (TNFBP), type 1 IFN receptor 2 (IFNaR2), and IL-32 binding protein (also known as neutrophil proteinase 3, PR3) (3-5).

The novel 40 amino acid sequence information could be good enough to identify full open reading frame (ORF) in National Center for Biotechnology Information (NCBI) gene bank (https://www.ncbi.nlm.nih.gov/) by blasting since 2003. However, the whole human genome in NCBI gene bank was not available in 1997. Therefore, the information of novel polypeptide sequence was used to search a sequence homology in a databank that identified a RNA sequence sharing homology with a viral protein (6). We used the RNA sequence information to design a pair of sense and reverse primer to perform RT-PCR experiment using total RNA from human Jurkat cells (ATCC; https://www.atcc.org) (**Fig. 1D**). The PCR product was cloned into a plasmid vector pGEM-T easy TA cloning vector (Promega, Madison, WI, USA). Several positive plasmid clones were sequenced to examine whether DNA sequence corresponds to

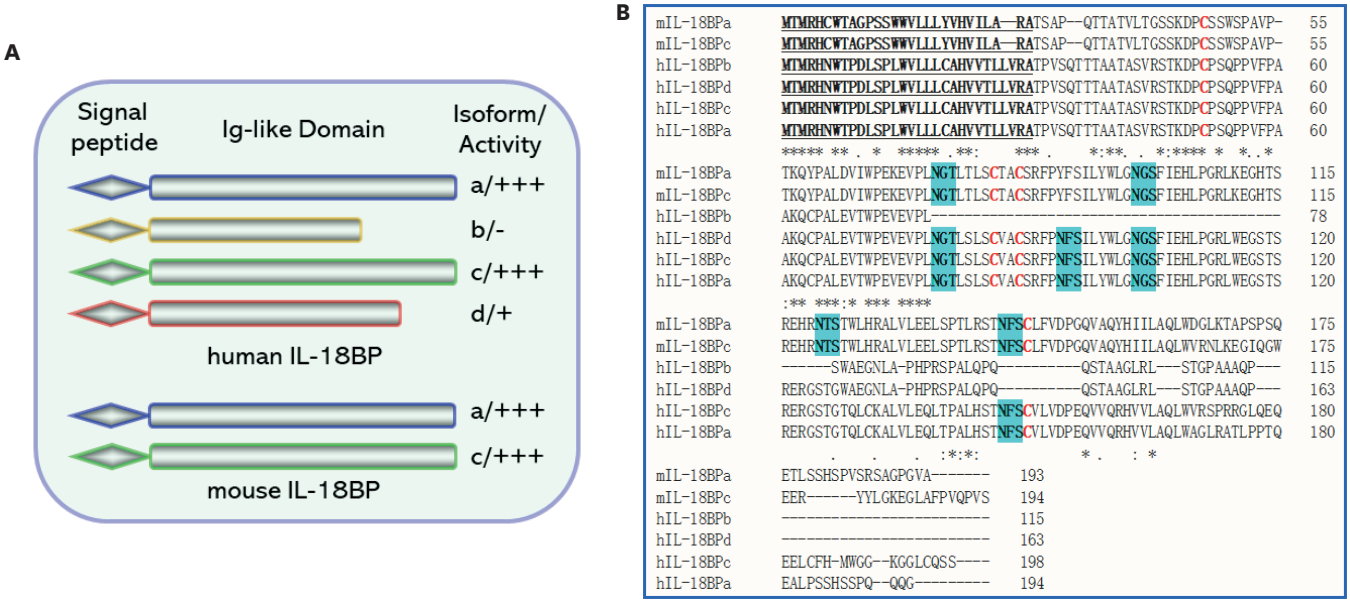

Figure 2. Amino acid alignment of four human and two mouse IL-18BP isoforms. (A) Human IL-18BPa and IL-18BPc possess a complete Ig domain with distinct C-terminal sequence. Human IL-18BP b and d possess incomplete Ig domain resulting in losing activity. Both mouse IL-18BPa (former mouse IL-18BPd) and c have complete Ig domain. Mouse IL-18BPa and IL-18BPc correspond to human IL-18BPa and IL-18BPc, respectively. The biological activity of 6 IL-18BP isoforms was exhibited on the right. (B) The amino acid sequence of 6 IL-18BP isoforms was aligned.

the novel molecule isolated from concentrated human urine. The sequenced cDNA clones were identical to the novel 40 amino acid polypeptide as well as RNA clones in databank.

The fragment of cDNA corresponding to the novel polypeptide was released by using DNA restriction enzyme. The cDNA insert was ^{32}P-labeled by random priming then used for screening various human cDNA libraries. The first publication was delayed due to searching for a typical transmembrane (TM) domain among the identified several dozen cDNA clones from various human and mouse cDNA libraries. However, there was not a single clone possessing the TM domain that is responsible for transmitting signal transduction upon IL-18 ligand binding (1). Further experiments were extended to screen several mouse cDNA libraries to identify an unknown single TM domain of typical interleukin receptor because it was believed that the IL-18 affinity purified molecule is a ligand binding chain of IL-18Rα containing a single TM domain. For all that six IL-18BP mRNA splice variants, four human and two mouse IL-18BP isoforms, do not possess the expected typical TM domain (**Fig. 2A**). Eventually, it was decided to analyze intron/exon structure as well as to perform Northern blot representing the size of transcripts that could finalize the existence of TM domain form.

The screening human genomic library and sequencing individual clone revealed that there was not an exon presenting a hydrophobic polypeptide stretch for potential TM domain prior to adjacent gene, nuclear mitotic apparatus protein 1 (NUMA1) at 3' end. In addition to this, the Northern blot of human and mouse showed that a dominant transcript just below 2 kb, which represents the most abundant isoform IL-18BPa in both human and mouse (1). Later, the known orpan IL-1R related protein (IL-1Rrp, also known as IL-1R5) was characterized as IL-18Rα ligand binding chain (7). Mouse isoform IL-18BPd is renamed as isoform IL-18BPa because it shares the highest homology with human isoform IL-18BPa rather than human isoform IL-18BPd. The large transcript in human and mouse represents isoform IL-18BPc in both species (1).

BIOLOGICAL ACTIVITIES OF 6 IL-18BP ISOFORMS

rIL-18BP was expressed in mammalian cells since *Escherichia coli* rIL-18BP was inactive (8). Approximately 20 kDa of heavy glycosylation influences its biological activity. The potential N- and O-glycosylation sites were highlighted by blue color (**Fig. 2B**). IL-18BP has a single Ig like domain with 4 conserved cysteine residues indicated by red letters. However, human IL-18BPb and d have incomplete Ig like domain. The human IL-18BPd with 3 cysteine residues loses a partial activity. The human IL-18BPb with a single cysteine residue completely loses its biological activity on suppressing IL-12 plus IL-18-induced IFN-γ in Th1 immune response (**Fig. 2A and B**). These experiments were performed with different forms of rIL-18BP, intact from human urine as well as C-terminal his-tag and Fc fusion from Chinese Hamster Ovary and African green monkey kidney fibroblast-like cells (8).

Nonetheless, medications induce clinical improvement in less than two-thirds of patients with RA who relapse after drug discontinuation and current RA therapies have been causing serious side effects such as infections and lymphomas (9). A recent review article described the importance of TLR2 in autoimmune diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ankylosing spondylitis (AS) (10). Therefore, investigation on the basic biological function of IL-18BP in TLR2-mediated various Th1 autoimmune diseases could help determine whether IL-18BP is useful for a particular subset of those autoimmune diseases. Heretofore, most biological activities of IL-18BP were focused on suppressing Th1 immunity to treat Th1 autoimmune diseases. However, it is necessary to investigate the role of IL-18BP in Th2 autoimmune disease since recent study suggested the potential role of NLR family CARD domain-containing protein 4 (NLRC4), which is known for IL-18 processing enzyme, mediating a variety of eosinophilic Th2 immune response (11). In addition to this, alarmins induce a Th2 immune response that triggers the alarm of IL-33, IL-25, and TSLP was first discovered in allergies and infectious diseases (12).

IN VIVO EXPERIMENTS INFLUENCE IL-18BP CLINICAL TRIALS

It is an ongoing odyssey from IL-18BP discovery to its clinical application. IL-18BP clinical trial was initiated in early 2000 however the progression of IL-18BP clinical trial was delayed due to the absence of exact IL-18BP function *in vivo* as well as the lack of clear follow up and evaluation in clinical application, which prohibited the progression of the trials (13-15). For example, Nowarski et al. (13), suggested deletion of the *IL-18BP* gene results in severe colitis associated with loss of mature goblet cells. This result was obtained from a specific experimental design using *IL-18BP* deficiency mouse. This study suffers from the lack of detailed characterization of *IL-18BP* gene deletion. They confirmed the *IL-18BP* deletion using RT-PCR alone. There were no further experiments charactering the *IL-18BP* gene deletion using immunostaining, Western blot, or ELISA at protein level.

Recently active IL-18BP clinical trials were pursued, for examples X-linked inhibitor of apoptosis protein (XIAP) deficiency (also known as NLRC4-macrophage activation syndrome [MAS]: NCT03512314 & NCT03113760 phase III) and adult-onset still's disease (AOSD: NCT02398435 phase II). The AOSD was completed in July 2016 (16) and the result was not posted on clinical trials.gov for this study. Since then, no additional study was undertaken, meaning the clinical trial did not attain sufficient data to continue IL-18BP on

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AOSD clinical phase III. Recently GSK developed a monoclonal antibody against IL-18 to treat various autoimmune disease such inflammatory bowel diseases (NCT01035645 phase I), kidney transplantation (NCT02723786 phase II) with other molecule, atopic dermatitis (NCT04975438 phase I), Crohn disease (NCT03681067 phase II), and Behcet's disease (NCT03522662 phase II). The clinical trial of kidney transplantation ends with lack of efficacy and the clinical trial of Behcet's disease has not been verified in more than 2 years.

Eventually, the clinical trial Phase III of XIAP deficiency (also known as NLRC4-MAS) is expected to be completed in October 2023 (NCT03512314) with 15 patients as well as in January 2024 with 10 patients (https://clinicaltrials.gov/study/NCT03113760). The XIAP deficiency (also known as NLRC4-MAS) is caused by the gain of IL-18 function due to NLRC4 mutation (17-19). This study remarks on final U.S. Food and Drug Administration approval whether IL-18BP appropriates for treating XIAP deficiency (also known as NLRC4-MAS) patients (20).

CONCLUSION

Not all molecules are used to treat patients in the clinic although their function is important for sustaining life. In most cases, molecules have been used clinically due to their gain or loss of function caused diseases. Recombinant protein and antibody therapies have been used to treat patients for more than four decades. There have been several clinical trials since the discovery of IL-18BP in 1999, but the trials were delayed as the results were invalidated. Finally, the phase III clinical trial of IL-18BP for XIAP deficiency (also known as NLRC4-MAS) will provide answers to the relevance of IL-18BP for treating life-threatening MAS in clinical use.

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