



## Molecular cloning, characterization, and bioactivity analysis of interleukin 18 in giant panda (*Ailuropoda melanoleuca*)

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**ABSTRACT.** Interleukin 18 (IL-18), as a member of IL-1 superfamily, is an important pleiotropic cytokine that modulates Th1 immune responses. In this report, we cloned and identified a homolog of IL-18 in giant panda (*Ailuropoda melanoleuca*) (designated as AmIL-18) from peripheral blood mononuclear cells stimulated with lipopolysaccharide. The open reading frame of *AmIL-18* cDNA is 579 bp encoding a deduced protein of 192 amino acids. *AmIL-18* gDNA fragments contained 5 exons and 4 introns. The amino acid sequence of AmIL-18 shared 23.9 to 87.0% identity with other species. To evaluate the effects of AmIL-18 on the immune response, we expressed the recombinant AmIL-18 in *Escherichia coli* BL21 (DE3). The fusion protein PET-AmIL-18 was purified by nickel affinity column chromatography and verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blot analysis. The biological function of purified PET-AmIL-18 was determined

on mouse splenocytes by quantitative real-time polymerase chain reaction. INF- $\gamma$  and other cytokines were increased when stimulated by PET-AmIL-18, particularly when combined with recombinant human interleukin 12, while a Th2-type cytokine, interleukin-4, was strikingly suppressed. These results will provide information for the potential use of recombinant proteins to manipulate the immune response in giant pandas and facilitate the study to protect this treasured species.

**Key words:** Giant panda; Interleukin 18; Molecular cloning; Bioactivity analysis