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Cloning and characterization of goose interleukin-17A cDNA

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ABSTRACT

Interleukin-17 (IL-17 or IL-17A) is a proinflammatory cytokine produced by activated T cells. IL-17A plays important roles in inflammation and host defense. In this study, the cDNA of the goose IL-17A (GoIL-17A) gene was cloned from thymocytes. Recombinant GoIL-17A (rGoIL-17A) was expressed using a baculovirus expression system and then biologically characterized. The complete open reading frame (ORF) of GoIL-17A contains 510 base pairs that encode 169 amino acid residues, including a 29-amino acid signal peptide and a single potential N-linked glycosylation site. This protein has a molecular weight of 18.9 kDa. The amino acid sequence showed 95.9%, 84.6%, 45.0% and 38.4% similarity with the corresponding duck, chicken, rat, and human IL-17A sequences, respectively. The six conserved cysteine residues were also observed in GoIL-17A. A recombinant, mature form of GoIL-17A was produced and its biological activities in goose embryonic fibroblasts were investigated. RT-PCR analysis revealed a marked up-regulation of IL-6 and IL-8 mRNA expression in goose embryonic fibroblasts treated with 1–50 μg of rGoIL-17A for 12 h. The GoIL-17A gene sequence and the biologically active recombinant protein may be useful for understanding the role of IL-17A in immune regulation.

1. Introduction

It has been almost two decades since the identification of interleukin (IL)-17 by Rouvier et al. (1993). IL-17 was cloned from a murine cytotoxic T lymphocyte hybridoma cDNA library as CTLA-8 (CTL antigen-8). Subsequently, CTLA-8 was confirmed to be a novel cytokine that binds to a novel cytokine receptor; the cytokine and receptor are now referred to as IL-17 and IL-17R, respectively (Yao et al., 1995a).

Classically, effector T helper cells have been classified as type 1 (Th1) or type 2 (Th2) based on their cytokine expression profiles and immune regulatory functions. A third subset of IL-17-producing effector T helper cells, Th17 cells, was discovered and characterized in 2005 (Harrington et al., 2005; Park et al., 2005). IL-17 has six family members (IL-17A to IL-17F). Although IL-17A and IL-17F share the highest amino acid sequence homology, they perform distinct functions; IL-17A is involved in the development of autoimmunity, inflammation, and tumours, and also plays important roles in the host defenses against bacterial and fungal infections, whereas IL-17F is mainly involved in mucosal host defense mechanisms.

IL-17 acts as a proinflammatory cytokine that can induce the release of certain chemokines, cytokines, matrix metalloproteinases (MMPs) and antimicrobial peptides. The release of these molecules leads to the expansion and accumulation of neutrophils during innate immune responses and links innate and adaptive immunity *in vivo*. Furthermore, increasing evidence indicates that the IL-17 and IL-17-producing cells are involved in the pathogenesis of various diseases, such as allergies, autoimmune diseases, allograft rejection and even malignancy (Xu and Cao, 2010). Moreover, it is becoming apparent that IL-17 plays protective roles against infectious diseases, especially in the mucosa (Dubin and Kolls, 2008). A critical characteristic of IL-17 in mucosal immunology is its ability to increase the production of granulocyte colony-stimulating factor (G-CSF) and CXC chemokines, resulting in the recruitment of neutrophils and contributing to bacterial and fungal clearance at mucosal sites. IL-17 also increases the expression of antimicrobial peptides and enhances epithelial repair functions that are important for controlling extracellular fungal pathogens. In the setting of vaccine-induced immunity, Th17 cells can induce the production of ligands for CXCR3 and enhance the recruitment of interferon-γ-producing Th1 cells to control the replication of intracellular pathogens (Khader et al., 2009).

The IL-17 genes of other species, including chickens IL-17A and IL-17F (Kim et al., 2012), pigs (Katoh et al., 2004), cows (Riollet

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et al., 2006), ducks (Yoo et al., 2009) and horses (Tompkins et al., 2010), have been cloned previously. This is the first report of the cloning of the goose IL-17A gene, its expression using a baculovirus system and the determination of its biological activities in primary cultures of goose embryonic fibroblasts (GEFs).The results of our experiments allow for a better understanding of the proinflammatory effect of goose IL-17A and provides the basis for further studies on its potential use as a mucosal vaccine adjuvant.

2. Materials and methods

2.1. RNA extraction and cDNA synthesis

Total RNA was isolated from 50 mg of goose thymus tissue using the E.Z.N.A.® HP Total RNA Isolation Kit (OMEGA Bio-Tek, Doraville, Georgia, USA). Its concentration and purity were determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Hudson, NH, USA). RNA was stored at −80 °C until required for cDNA synthesis. The cDNA was synthesized from the total RNA isolated from goose thymus tissue using SMART (Switching Mechanism at 5′ End of RNA Transcript) Reverse Transcriptase (Clontech, Palo Alto, CA, USA).

2.1.1. First-strand cDNA synthesis

One microliter of the First-dT_{20} primer (Table 1) was added to 3.5 μl (59 ng/μl) of total RNA. The tube was then mixed, spun briefly and then placed at 72 °C for 3 min, followed by incubation at 42 °C for 2 min. The cDNA synthesis was performed with a prepared mix of 1 μl of SMARTScribe Reverse Transcriptase (Clontech, Palo Alto, CA, USA) in the presence of 2 μl of 5× First-Strand Buffer, 0.25 μl of DTT (100 mM), 1 μl of dNTP Mix (10 mM), 0.25 μl of RNase Inhibitor (TaKaRa Bio, Otsu, Japan), 3G primer (Table 1) and deionized water to a final volume of 10 μl. This reaction mixture was incubated at 42 °C for 1.5 h.

2.1.2. cDNA PCR amplification reaction

Two microliters of cDNA from the previous reaction was amplified with 1 μl of PrimeSTAR™ HS DNA Polymerase (TaKaRa Bio, Otsu, Japan) in the presence of 20 μl of 5× PrimeSTAR Buffer, 2 μl of dNTP Mix (2.5 mM), 1 μl each of the 5′ PCR primer and the 3′ PCR primer (Table 1) and deionized water to a final volume of 100 μl. The reaction contents were then mixed. The PCR was completed using a Mastercycler ep Gradient thermocycler (Eppendorf, Hamburg, Germany) with the following program: 95 °C for 3 min followed by 30 cycles of 98 °C for 30 s, 65 °C for 30 s, and 72 °C for 6 min. The dsDNA product was stored at −20 °C until use.

2.2. Cloning of Go-IL17A

GoIL-17A-specific primers (Table 1) were designed based on the sequence of chicken IL-17A (GenBank ID: NM_204460.1) and were used to acquire the actual sequence of goose IL-17A. Touchdown PCR was performed as follows: an initial step at 94 °C for 5 min, followed by 30 cycles each of denaturation at 94 °C for 1 min, annealing at a variable temperature (65–50 °C) for 30 s, and extension at 72 °C for 1 min. For the first cycle, the annealing temperature was set to 65 °C. For each of the 29 subsequent cycles, the annealing temperature was decreased by 0.5 °C. These 30 cycles were followed by 10 cycles of 94 °C for 1 min, 50 °C for 30 s, and 72 °C for 1 min. Amplified fragments were inserted into the pEASY-Blunt vector (TransGen Biotech, Beijing, China). DNA sequencing was performed using the dideoxy chain termination method. Sequences were initially analyzed using a BLAST search to confirm that the correct gene had been cloned. The CLUSTALW (Larkin et al., 2007) program was used to align the sequences, and ESPript (Gouet et al., 1999) was used to format the multiple sequence alignments in a single postscript file.

2.3. Expression of GoIL-17A in Escherichia coli and baculovirus-infected insect cells

To subclone the GoIL-17A cDNA without the signal peptide region, sense and antisense primers were designed that included *Bam*HI and *Hin*dIII restriction sites at the 5′-ends of the primers. After digestion with *Bam*HI and *Hin*dIII, the PCR fragment was ligated into both the pET32a (Novagen) expression vector and the pFastBac HTB donor vector, which is part of the baculovirus expression system (Invitrogen).

The pET32a expression vector containing the GoIL-17A gene was transformed into the *E. coli* Rosetta (DE3) pLysS strain (Promega). Transformants were selected for on LB-ampicillin agar plates. Log phase cultures (approximate $OD_{600} = 0.6$) were induced at 37 °C for 4 h by adding IPTG (Sigma) to a final concentration of 1 mM. The cells were harvested by centrifugation (5000*g* for 15 min) and suspended in PBS buffer. The cells were disrupted by sonication, and the insoluble material was collected by centrifugation (5000*g* for 20 min).

The baculovirus donor vector pFastBac HTB (Invitrogen) was sequenced to confirm the insertion of the GoIL-17A gene. The recombinant vector was then transformed into DH10BAC bacterial cells (Invitrogen) for recombination of the GoIL-17A cDNA with the genetically modified baculovirus genome (bacmid). Positive recombinant bacmids were transfected into Sf9 (Invitrogen) insect cells. All procedures were performed according to the manufacturer's protocols (Bac-to-Bac, Invitrogen). The recombinant baculovirus was submitted to four rounds of amplification (72 h each) by infecting Sf9 monolayers to generate a high titer of recombinant virus. The virus stocks were protected from light at +4 °C or −80 °C. Protein expression was analyzed by 12% SDS–PAGE.

2.4. Purification and renaturation

The prokaryotic rGoIL-17A protein was dissolved in 2 ml of denaturing buffer (100 mM NaH_2PO_4, 10 mM Tris–Cl, 8 M urea, 10 mM imidazole, pH 8.0), sonicated for 15 min in an ice bath, and then centrifuged at 5000*g* for 10 min. The supernatant was

Table 1
Oligonucleotide primers used to amplify cDNAs for Goose IL-17.

Gene name	Primer name	Primer sequence	Ann *T* (°C)
cDNA	First-dT_{20}	TCTAGAGTCGACCTGCACATTTTTTTTTTTTTTTTTTGC	65
	3G primer	GAGCTCGAATTCACTTAGTATAGCGCGCGGG	65
ds cDNA	5′PCR primer	TCTAGAGTCGACCTGCACAT	52.5
	3′PCR primer	CTCGAATTCACTTAGTATAGCG	52.5
Goose IL-17	IL-17 S	GGGTCGCCCAGCACAAGCA	62.2
	IL-17 A	ACTCCTGTGCTGTGGGCTCCCT	61.9
Ex IL-17	mIL-17 S	CGCGGATCCATGAAGGTGATACGGCCC	65
	mIL-17 A	CCCAAGCTTTTAAGCCTGGTGCTGGATCAA	65