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Cloning, in vitro expression and bioactivity of goose interferon-α

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Abstract

The cDNA of goose interferon-α (goIFN-α) was amplified from PHA stimulated PBMCs of goose by RT-PCR. The cDNA encodes a 30-amino acid signal peptide and a 161-amino acid mature protein, respectively. Recombinant mature goIFN-α (rgoIFN-α) expressed by prokaryotic and eukaryotic system possessed antiviral activity that was neutralized by rabbit anti-rgoIFN-α antibody in vitro. On the other hand, rgoIFN-α lacks intrinsic macrophage activating factor (MAF) activity, peripheral blood leukocyte-derived macrophages (PBLMs) could not produce nitric oxide (NO) by stimulate with rgoIFN-α as compared to stimulate with recombinant mature goIFN-γ (rgoIFN-γ) that was a powerful NO stimulant in vitro.

Keywords: Goose; Interferon-α; Antiviral activity; Macrophage activating factor activity; In vitro

1. Introduction

IFNs constitute a large group of cytokines that are best known for their ability to induce cellular resistance to viral pathogens [5,10,14]. They also play a critical role in the response to microbial infections by modulating the innate and adaptive immune system. Furthermore, they are potent regulators of cell growth and have inflammatory and anti-inflammatory effects [3,15]. IFN are commonly grouped into tow types, type I and type II. Type II FNs form a still growing family of cytokines that comprises IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω, IFN-δ, IFN-τ and IFN-ξ (Limitin). Type III FN is also known as immune IFN (IFN-γ). Although IFN was discovered in 1957 during experimental work with embryonated chicken eggs, the molecular analysis of IFN from avian and other non-mammalian vertebrate species began only in 1994 [13] when most types of mammalian IFNs had already been characterized.

In this study, our objectives are to clone cDNA sequence of goIFN-α and to express the mature goIFN-α protein in *Escherichia coli* (designated as *ec*-rgoIFN-α) and the baculovirus system (designated as *bac*-rgoIFN-α), respectively, to prepare polyclonal antibodies (pAbs) against *ec*-rgoIFN-α, and to determine the bioactivity of the rgoIFN-α and pAbs against *ec*-rgoIFN-α by plaque reduction assays and nitric oxide (NO) assays.

2. Materials and methods

2.1. Animals, cells and viruses

The embryonated eggs of Dongbei White goose and Jinding duck were purchased from Zhengli poultry Co. Ltd., Heilong Jiang, China. New Zealand white rabbits (NZW rabbit) were purchased from Laboratory Animal Center of Harbin Medical University, Heilong Jiang, China. All laboratory animals and animal subjects used in this study have been approved by the Scientific Ethical Committee of the Northeast Agricultural University.

To prepare the peripheral blood leukocyte-derived macrophages (PBLMs), blood was collected by cardiac puncture from 90-day-old goose and PBLs were isolated by centrifugation on a Ficoll–Hypaque gradient at 200*g* for 30 min at room temperature (RT) as previously described [4]. PBLs were seeded at 5×10^7 cells/ml in RPMI-1640 complete

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medium in bottomed six-well plates, incubated for 24 h at 37 °C, in a humidified atmosphere with 5% CO_2, and nonadherent cells in the supernatant were removed [7].

Goose paramyxovirus (GPMV) strain JS/1/97/Go was kindly provided by Dr. Jiao Xinan. Recombinant vesicular stomatitis virus expressing enhanced green fluorescent protein (VSV-EGFP) strain was kindly provided by Dr. Bu Zhigao.

2.2. PBMC isolation and culture

PBMCs were isolated from whole blood of 60-day-old goose by centrifugation on a Ficoll–Hypaque gradient at 200*g* for 30 min at room temperature (RT) as previously described [4]. Briefly, PBMCs were collected and washed in RPMI-1640 (Gibco-BRL, Gaithersburg, USA). after centrifugation at 180*g* for 10 min, cell were suspended in 2 ml of fresh RPMI supplemented with 2 mM L-glutamine, 10% heat inactivated fetal calf serum (FCS, Gibco, Great Island, NY. USA) and plated at 1×10^7 cells/ml. PBMC suspensions was seeded in 6-well plates (Costar, Acton, MA, USA). Ten micrograms per milliliters of PHA (Sigma, St. Louis, MO) was added to the cultures and incubated for 18 h at 37 °C, in a humidified atmosphere with 5% CO_2.

2.3. RNA isolation, RT-PCR and sequencing

A pair of the specific oligonucleotide primers amplifying 615 bp in length were designed and synthesized according to DuIFN-α cDNA (GenBank Accession No. X84764), including the upstream primer 5′-GCACAACCCAGGATCCACCA-3′ containing the *Bam*HI site and the downstream primer 5′-GTGCGCGTGTGGGGTACCT-3′ containing the *Kpn*I site. PBMCs stimulated with PHA were harvested by centrifugation at 500*g* for 5 min at 4 °C and followed by washing three times with PBS. Total cellular mRNA was extracted with Trizol reagent (Gibco-BRL). The cDNAs were synthesized from total cellular RNA using the downstream primer. goIFN-α nucleic acid sequences were then amplified by PCR. The PCR was performed according to 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 62.4 °C for 45 s and DNA extension at 72 °C for 1 min. RT-PCR products were purified by a QIAquick PCR Purification Kit (Qiagen, CA, USA) and followed by sequencing. Signal peptide of goIFN-α protein was predicted by Signal P version 2.0 (Signal P-NN and Signal P-HMM software) of Signal P World Wide Web server [9]. The nucleotide sequence of goIFN-α open reading frame (ORF), along with that of avian IFN-αs from GenBank, was aligned using DNASTAR 5.0 software. Phylogenetic analysis was utilized DNAMAN 5.2.2 software.

2.4. Expression of goIFN-α in bacteria

For bacterial expression, we used a vector pET30a (Invitrogen Corporation, CA, USA) coding for a N-terminal detection and purification epitope (poly histidine tag). Accordingly, the 5′ goose IFN-α secretion signals was not amplified by PCR that using the following primers: forward primer 5′-GGATCCTGCAGCCCCCTGC-3′ containing the *Bam*HI site, and reverse primer 5′-GTGCGCGTGTGGGGTACCT-3′ containing the *Kpn*I site. IFN-specific expression clones were grown at 37 °C in LB medium (kanamycin) to optical density of 0.5 (620 nm). Then IPTG (1 mM) was added to induce protein production for 4 h followed by centrifugation (20 min; 12,000 rpm; Sorvall centrifuge) of a bacterial pellet. The inclusion bodies were solubilized in 6 M guanidium lysis buffer. Proteins were purified on Nickel chelated column according to the manufacturer's instructions under hybrid conditions. After elution, the proteins were dialysed against PBS and filtered for bioassays.

2.5. Expression of goIFN-α in Sf9 cells

The sequence encoding the mature goose IFN-α molecule was subcloned into transfer vector pMelBacA. The recombinant transfer vector was transfected into Sf9 cells with infectious AcMNPV DNA by using Lipofection. The cultures were harvested, plaque-assayed with X-gal, and blue plaques were selected as recombinant viruses. In order to optimize protein expression levels, the resulting virus was inoculated into Sf9 cells at multiplicity of 5 or 10 plaque forming unit (pfu)/cell and the infected culture cells were harvested every 24 h post-inoculation, respectively.

2.6. Production of rabbit antiserum against recombinant goIFN-α

Antiserum against *ec*-rgoIFN-α (pAb) was generated by biweekly immunization of NZW rabbits with the purified *ec*-rgoIFN-α (100 μg of *ec*-rgoIFN-α per rabbit) emulsed with complete Freund's adjuvant (CFA, Sigma). Immunized rabbits were bled 14 days after last injection and serum was collected.

2.7. SDS–PAGE and Western blot analysis

The recombinant protein was analyzed by SDS–PAGE and visualized with Coomassie blue. Proteins were transferred onto nitrocellulose and the membranes were blocked for 2 h at 37 °C with TBST (25 mM Tris–HCl, 125 mM NaCl, 0.1% Tween 20, pH 8.0) containing 5% skimmed milk. The membranes were then incubated shaking overnight at room temperature with the following antibodies diluted in TBST containing 5% skimmed milk as listed: rabbit polyclonal antibodies (diluted 1:5) against *ec*-rgoIFN-α; mouse monoclonal antibody (diluted 1:400) to hexa-histidine was used. Alkaline phosphatase-conjugated secondary antibodies against polyclonal and monoclonal antibodies were anti-rabbit IgG (diluted 1:2000) and anti-mouse IgG (diluted 1:5000), respectively (Southern