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Cloning, in vitro expression and bioactivity of duck interleukin-2

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Abstract

In this report, the cDNA sequences of Shaoxing (SX) and Muscovy (MV) duck IL-2 were cloned, then recombinant duck IL-2 (rduIL-2) was produced in prokaryotic expression system. In vitro bioactivity of rduIL-2 was determined by lymphocyte proliferation assay and in vivo bioactivity of rduIL-2 was assessed by vaccine immunization. Monoclonal antibodies (mAbs) and polyclonal antibodies (pAbs) specific for rduIL-2 were generated and characterized by ELISA, Western blot and neutralizing assays. The cDNA contains an open reading frame (ORF) of 420-base pairs encoding a protein of 140 amino acids (aa) with a putative signal peptide of 21aa. The His-duIL-2 fusion protein was recognized in Western blot by mAb against chicken IL-2 (chIL-2), but not by mAbs against human IL-2 and mouse IL-2. Recombinant duIL-2 induces in vitro proliferation of Con A-stimulated duck splenocytes in MTT assay and strengthens duck immune responses induced by vaccinating the inactivated oil emulsion vaccine against avian influenza virus. Polyclonal antibodies and mAb 2B3 against rduIL-2 were shown to have effective neutralizing ability by inhibiting the biological activities of both recombinant duIL-2 and endogenous duIL-2. Despite the fact that duck and chicken IL-2s only share identity of 55.0–56.7% in amino acid sequence, duck and chicken IL-2 molecules displayed similar cross-priming activity in in vitro lymphocyte proliferation assays. The results, at the first time, indicated that rduIL-2 has the potential to be used as an immunoadjuvant for enhancing vaccine efficacy and an immunotherapeutic, and the mAbs against rduIL-2 further facilitate basic immunobiological studies of the role of IL-2 in avian immune system.

Keywords: Duck interleukin-2; In vitro expression; Monoclonal antibody; Lymphocyte proliferation; Neutralizing ability; In vivo bioactivity

1. Introduction

Interleukin-2 (IL-2) is a growth factor for variety of cell types and plays key roles in T cell growth and differentiation, functional B cell development and activation of NK cells following stimulation with antigens or mitogens (Merluzzi et al., 1983). IL-2 genes from more than 30 species have been identified since the human IL-2 (hIL-2) gene was first cloned and sequenced (Taniguchi et al., 1983). In 1997, a cDNA clone encoding chicken interleukin-2 (chIL-2) with homology to mammalian IL-2 has been described (Sundick and Gill-Dixon, 1997). Our previous studies revealed that some variations exist in the chIL-2 genes from different chicken breeds (Zhou et al., 2003).

The gene encoding hIL-2 consists of three introns and four exons with a promoter region containing a prototype 'TATA' sequence. It is mapped to chromosome 4, band q26-27 (Fujita et al., 1983; Seigel et al., 1984). The gene structure of chIL-2 resembles closely that of hIL-2 and maps to chicken chromosome 4 (Kaiser and Mariani, 1999). Based on these studies, recombinant hIL-2 (rhIL-2) and chIL-2 (rchIL-2) were successfully expressed in prokaryotic and eukaryotic systems. The monoclonal antibodies (mAbs) to these cytokines were produced and used to study the biochemical and biological activities of hIL-2 and chIL-2 (Brandt et al., 1986; Ide et al., 1987; Stepaniak et al., 1999; Kitamura et al., 1989; Miyamoto et al., 2001; Rothwell et al., 2001).

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In 1997, Bertram et al. (1997) found lymphokines released by duck peripheral blood lymphocytes and spleen cells stimulated with PHA, which was of similar functional homology to chIL-2 in maintaining the lymphocyte proliferation. Recently, a cDNA sequence of Mallard (ML) duck IL-2 (duIL-2) was deposited in GenBank (GenBank accession no. AF294323). Shaoxing layer (SX) duck is a Chinese layer breed originated from Zhejiang province. Muscovy (MV) duck is a foreign broiler breed, which has reared more than 250 years in China (Zheng et al., 1989). In this study, our objectives are to clone cDNA sequence of duIL-2 and to express the duIL-2 gene in prokaryotic expression system, to prepare monoclonal antibodies and polyclonal antibodies (pAbs) against rduIL-2, and to determine the bioactivity of the rduIL-2 protein and Abs against rduIL-2. It is the first report on the production and in vitro characterization of the recombinant duck IL-2 molecule as well as its Abs.

2. Materials and methods

2.1. Animals

SX and MV duck embryonated eggs were purchased from Shaoxing Duck Breeding Co. Ltd. and Yuyao Shengnong poultry Co. Ltd., Zhejiang, China. Eggs were hatched in our laboratory, and baby ducks were kept in a special room with unlimited access to feed and water. Thirty-day-old specific pathogen-free (SPF) Leghorn chickens were obtained from Beijing Merial Vital Laboratory Animal Technology Co. Ltd., Beijing, China. Four-week-old SPF BALB/c mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China. New Zealand white rabbits (NZW rabbit) were purchased from Laboratory Animal Center of Zhejiang Academy of Medicine, Zhejiang, China. All laboratory animals and animal subjects used in this study have been approved by the scientific ethical committee of the Zhejiang University.

2.2. Isolation of splenic mononuclear cells (SMC) and preparation of endogenous duIL-2 protein

Ducks at 30 days old were sacrificed by intravenous inoculation of barbiturate. Spleens were collected aseptically and rinsed with Ca^{2+}- and Mg^{2+}-free PBS (717 mM K_2HPO_4, 283 mM KH_2PO_4, pH 7.2), minced with a pair of scissors and then passed through a stainless steel screen to obtain a homogeneous cell suspension. The spleen cells were collected after centrifugation at 250 × *g* for 10 min at 4 °C and resuspended in Hank's balanced salt solution (HBSS). Cell suspension was then overlaid on equal volume of Histopaque-1077 (Sigma Chemical Co., St. Louis, USA). An interface rich in mononuclear cells was recovered after centrifugation at 500 × *g* for 30 min at 4 °C. Cells were washed twice in serum-free RPMI 1640 (GIBCO BRL, Gaithersburg, USA) and planted in six-well cell culture plates in complete RPMI 1640 medium at a concentration of 1 × 107 cells/ml. The cells were stimulated with Con A at a final concentration of 10 μg/ml at 40 °C in 5% CO_2 for 0 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h and 96 h. The cell culture supernatants were harvested at each time point by centrifugation. The preparation of chicken SMC for following bioactivity assay was processed the same way as that of duck SMC.

For each sample of cell supernatants, the residual Con A in the cell supernatant was removed by incubating with 0.1 M a-methyl D-mannoside for 30 min. Ammonium sulfate was added to each supernatant with 90% saturation and stirred overnight at 4 °C. The precipitate was collected after centrifugation at 25,000 × *g* for 30 min at 4 °C, dissolved in PBS and dialyzed in PBS. Finally the purified proteins were sterilized by passing through a filter (0.2 μm), and stored at −70 °C after further concentration by using polyethylene glycol 6000 method.

2.3. RNA isolation, RT-PCR and sequencing

A pair of the specific oligonucleotide primers amplifying 748 bp in length were designed and synthesized according to ML duIL-2 cDNA (GenBank accession no. AF294323): the upstream primer 5′-GCGGATCCAACACTGACAAGATGTGC-3′ (matching the nucleotide sites from 34 to 64) and the downstream primer 5′-GCGGATCCGTAGGTTACTGAAATTTA-3′ (matching the nucleotide sites from 728 to 748). Both primers contain a single *Bam*HI restriction site. SMC stimulated with Con A 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 a special oligonucleotide primer (5′-GCGGATCCGTAGGTTACTGAAATTTA-3′). SX and MV duIL-2 nucleic acid sequences were then amplified by PCR. The PCR assay was carried out with conditions consisting of 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 56 °C for 45 s and DNA extension at 72 °C for 2 min. Lastly, the reaction was carried out at 72 °C for 10 min. RT-PCR products were purified by a QIAquick PCR Purification Kit (Qiagen, California, USA) and sequenced. Signal peptide of duIL-2 protein was predicted by SignalP version 2.0 (SignalP-NN and SignalP-HMM software) of SignalP World Wide Web server (Nielsen et al., 1997). The nucleotide sequence of duIL-2 open reading frame (ORF), along with that of avian IL-2 from Genebank, was analyzed by DNASTAR 5.0 software (DNASTAR Inc., Madison, WI, USA).

2.4. Construction of plasmid pBAD/His B-duIL-2

A cDNA sequence encoding the mature duck IL-2 molecule was generated by PCR using the following primers: forward primer 5′-CGAATTCGCACCTCTATCAGAGAAA-3′ contained the *Eco*RI site, and reverse primer 5′-