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cDNA cloning and functional analysis of goose interleukin-2

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Abstract

cDNA encoding goose IL-2 (GoIL-2) was cloned from Con A-stimulated goose splenic mononuclear cells (SMC) using oligonucleotide primers based on the conserved sequence of duck (DuIL-2), chicken (ChIL-2) and turkey IL-2s (TuIL-2). The GoIL-2 cDNA is 718 nt long, which contains an open reading frame (ORF) of 423 base pairs encoding a protein of 141 aa. The GoIL-2 shows, respectively, 79%, 82–85%, and 91–92% identities with TuIL-2, ChIL-2 and DuIL-2 in cDNA, and also shows, respectively, 63%, 63–64%, and 82–85% identities with TuIL-2, ChIL-2 and DuIL-2 in amino acid sequence. Recombinant GoIL-2 (rGoIL-2) protein expressed in *Escherichia coli* has an approximate molecular weight of 18 kDa. The rGoIL-2 has biological effect on goose and duck as well as chicken lymphocytes in a dose-dependent manner, though the effect on duck and chicken lymphocytes has been found to be relatively weak. In addition, rGoIL-2 also strengthens goose immune responses induced by vaccinating the inactivated oil emulsion vaccine against avian influenza virus. The monoclonal antibodies (mAb) to rGoIL-2 recognized the binding epitopes of nature GoIL-2 protein expressed in vero cells. Antiserum and mAb 5B10 to rGoIL-2 can inhibit the biological activity of rGoIL-2 and endogenous GoIL-2. The results, at the first time, indicated that goose IL-2 reserves species-specialties in the biological functions and can be used as a potential immunoadjuvant for goose vaccination and immunotherapeutic purposes. Finally, the mAbs to rGoIL-2 also provide a useful tool for further immunobiological studies of IL-2 in avian immune systems.
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1. Introduction

IL-2 is a glycoprotein produced principally by activated T cells and some B cells and a cytokine that regulates lymphocyte function. IL-2 controls the amplification of native T cells by initially stimulating growth following antigen activation, and also has effects on NK cells, monocytes/macrophages and neutrophils [1–4]. IL-2 has been developed as an immunotherapeutic agent in chronic microbial infection and cancer, and as an enhancing agent in vaccine [5–7]. Previous reports also found that IL-2 exerts its effects by binding to the high affinity IL-2 receptors (IL-2R) present on those cells [8]. IL-2R is composed of three distinct subunits: IL-2Rα, IL-2Rβ and IL-2Rγ which are encoded, respectively, by distinct and structurally unrelated genes [9]. Resting T cells express low levels of IL-2Rα-subunit (Tac antigen) that binds to IL-2 with a low affinity. IL-2Rβ-subunit alone or in combination with α or γ produce a receptor with intermediate affinity, and the γ-subunit alone does not bind IL-2, while a receptor comprised of three subunits offers the highest affinity for IL-2 [8].

Up to now, in addition to human IL-2, IL-2 genes for more than 30 animal species also have been cloned and identified [10]. Recently, IL-2 gene for chicken (ChIL-2) and turkey (TuIL-2) have been cloned and sequenced

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successfully [11–13]. The ChIL-2 gene is mapped to chromosome 4, linked to annexin V with synteny with mouse chromosome 3 and human chromosome 4 [12]. Our previous study revealed that some variations exist in the open reading frame (ORF) of ChIL-2 from different chicken breeds [14]. The cDNA coding for ChIL-2 protein was introduced into several prokaryotic and eukaryotic expression systems to produce biologically active recombinant cytokines [13,15,16]. ChIL-2 plays an important role as an immunoregulatory molecule identical to mammalian IL-2 and has been shown to stimulate T lymphoblast cells, increase the proportion of both $CD4^+$ and $CD8^+$ peripheral blood T cells [17], enhance NK cells activity and vaccine response to *Eimeria* parasites [18], and strengthen the protective immunity against infectious bursal disease virus [19], indicating that ChIL-2 has practical importance in potentially enhancing immune responses to vaccines and infectious agents in poultry. Furthermore, mAbs to ChIL-2 have also been produced, and used to study the biological activities of ChIL-2 [20,21]. Comparatively, little work has been performed on avian IL-2s and their receptors. Only a mAb designated INN-CH-16 can react with a 48–50 kDa antigen expressed on activated chicken T lymphocytes and inhibited the proliferation of Con A-stimulated blasts in response to supernatants of stimulated chicken T cells. These features led to the hypothesis that INN-CH-16 may recognize the ChIL-2R [22,23].

As for waterfowl cytokines in 1997, Bertram et al. [24] found lymphokines released by duck peripheral blood lymphocytes and splenocytes stimulated with PHA, which was of similar functional homology to ChIL-2 in maintaining the lymphocyte proliferation. Recently, the cloning and functional characterization of duck IL-2 have been reported [25–27]. However, no information was available on the goose cytokines. Hereby, the goal of the present study was to molecularly clone the cDNA fragment of goose IL-2 (GoIL-2), to express the GoIL-2 open reading frame in vitro expression system, to prepare mAbs to GoIL-2, and to detect the bioactivity of the recombinant GoIL-2 (rGoIL-2) protein in vitro and in vivo.

2. Results

2.1. Molecular cloning and analysis of goose IL-2 cDNA

The mRNA was isolated from goose SMC stimulated with 10 μg/ml Con A at 0, 1, 2, 4, 8, 12, 14, 16, 18, 24, 48 and 72 h. GoIL-2 cDNA fragment was amplified by RT-PCR using these mRNAs as templates. An approximately 0.8 kb DNA fragment, in size, was amplified from nine of the 12 mRNAs (Fig. 1). The sequencing results revealed that cDNA fragment of GoIL-2 was

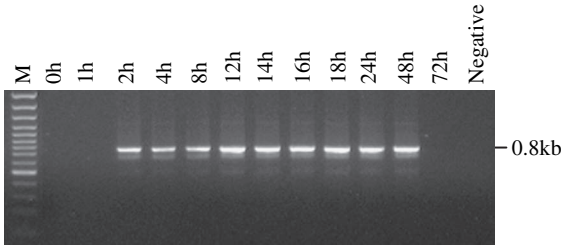

Fig. 1. RT-PCR amplification of GoIL-2 mRNA extracted from goose SMC harvested 0, 1, 2, 4, 8, 12, 14, 16, 18, 24, 48 and 72 h after Con A stimulation.

718 nt in length (GenBank accession no. AY392557). GoIL-2 cDNA fragment encodes a polypeptide of 141 amino acid residues with a predicted molecular mass of 16.29 kDa. Analysis of the signal peptide revealed that there was a potential cleavage site between amino acid residues 21 and 22 (arrow in Fig. 2). The predicted signal peptide of GoIL-2 was similar in size to that of mammalian animals (20 aa) and other birds (22 aa).

The deduced amino acid sequence of GoIL-2 was compared with those of the several avian and mammalian species. The alignment analysis showed that GoIL-2 shared 85% identity to DuIL-2 (GenBank accession no. AY193713), 63% to ChIL-2 (GenBank accession no. AF294321), 64% to TuIL-2 (GenBank accession no. AJ007463), 9% to human IL-2 (GenBank accession no. U25676), and 7–20% to other mammalian IL-2s. As shown in Fig. 2, in IL-2 sequences of the compared avian species, there are 78 conserved amino acid residues including three cysteine residues (position 63, 70 and 119), indicating that there is at least one di-sulfate bond in the molecules. However, Lys^{48} and Asn^{80} are present only in GoIL-2 amino acid sequence. $Thr^{34,45,114}$, $Lys^{73,105}$, $Ile^{75,110}$, $Asp^{86,90}$, Glu^{104} and Arg^{132} are the conserved amino acid residues in goose and duck IL-2s, but are variable in chicken and turkey IL-2s. On the contrary, four amino acid residues (Ala^{14}, Ile^{42}, His^{51} and Leu^{131}) were conserved in chicken and turkey IL-2s, but were variable in goose and duck IL-2s. Phylogenetic analysis (Fig. 3) shows further that the avian IL-2s were subdivided into two monophyletic lineages. The goose and duck IL-2s formed a monophyletic group distinct from ChIL-2 and TuIL-2, confirming the similarity between duck and goose IL-2s.

2.2. Expression and purification of the His-GoIL-2 fusion protein

The gene fragment encoding GoIL-2 protein (without signal peptide) was cloned into the pBAD/His B expression vector, where the araB promoter is controlled by arabinose. After induction with 0.2% L-(+)-arabinose and resolved in a SDS-PAGE, the expressed recombinant GoIL-2 with an approximate molecular weight of 18 kDa

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