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Structural and functional homology among chicken, duck, goose, turkey and pigeon interleukin-8 proteins

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Abstract

Interleukin (IL)-8-encoding regions of five avian species were cloned, sequenced and characterized. Each IL-8-encoding region is 312 nucleotides long and encodes IL-8 which is 103 amino acids. Pairwise sequence analysis showed that sequence identities of IL-8-encoding regions ranged from 87% to 100%. The IL-8 protein identities varied from 84% to 100%. Phylogenetic analysis indicated that IL-8-encoding regions and encoded proteins of chicken, duck, goose and turkey clustered together and evolved into a distinct phylogenetic lineage from that of pigeon which evolved into a second lineage. The results from binding reactivities of antiserum against each recombinant IL-8 (rIL-8) protein to homologous or heterologous rIL-8 proteins, chemotactic activities of each rIL-8 protein or reduction levels of the chemotactic activity of rIL-8 protein which was pretreated with homologous or heterlogous antiserum have suggested that all five IL-8 proteins were functionally active, and shared structural and functional identity with each other.

Keywords: Interleukin (IL)-8; Chicken IL-8; Avian species; Chemotaxis assay; Homology; Phylogenetic analysis; Molecular cloning

1. Introduction

Interleukin-8 (IL-8) is a member of the chemokines which is structurally related to cytokines with chemotactic activity (Baggiolini et al., 1997). Most chemokines consist of four conserved cysteines which are important for their tertiary structures. Based on the spacing between the first two cysteines, chemokines can be subgrouped into CXC, CC, or CX_3C subfamilies. A fourth subgroup, the C chemokine subfamily, lacks two of the cysteines. A cDNA of the chicken CXC chemokine 9E3/CEF4 has been cloned (Bedard et al., 1987; Sugano et al., 1987). Based on the analysis of biological activity in wound healing and chemotactic activity, 9E3/CEF4 has been strongly suggested as the chicken homologue of IL-8 (Barker et al., 1993) or growth-regulated oncogene (GROα) (Martins-Green et al., 1991). Subsequently, the chicken 9E3/CEF4 CXC chemokine has been identified to be the chicken IL-8 (ChIL-8) (Kaiser et al., 1999). ChIL-8 possesses a Glu-Leu-Arg (ELR) motif immediately preceding the first cysteine residue, which was shown to involve in receptor binding in human or chicken IL-8 (Hebert et al., 1991; Kaiser et al., 1999). In addition, cDNA sequences of IL-8-encoding regions of duck and goose

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have been reported in GenBank (accession nos.: AB236334 and AB213393, respectively).

In addition to chicken, duck, and goose, IL-8 of turkey and pigeon has not been cloned and characterized. To characterize the evolution of IL-8 among avian species, IL-8-encoding regions of chicken (Ch), duck (Du), goose (Go), turkey (Tu), and pigeon (Pi) IL-8 were cloned, sequenced, and characterized. The results showed that chicken, duck, goose, and turkey IL-8 at their nucleotide and amino acid levels clustered together, but separated from those of pigeon. Cross-species reactivities of IL-8 between these five avian species were examined by the binding reactivities of antiserum against each recombinant IL-8 (rIL-8) protein to homologous or heterologous rIL-8 proteins, the chemotactic activities of each rIL-8 protein or the reduction levels of the chemotactic activity of rIL-8 protein which was pretreated with homologous or heterologous antiserum. The results showed that all five rIL-8 proteins were functionally active and shared structural and functional identity with each other.

2. Materials and methods

2.1. Experimental birds

Adult Muscovy ducks, White Roman geese, and Bronze turkeys were obtained from the poultry farm, experimental station of the University. Adult Rock pigeons were obtained from the poultry farm, National Chayi University, Chayi, Taiwan. Adult specific-pathogen free (SPF) White Leghorn chickens were obtained from the SPF flock managed by the Animal Health Research Institute, Council of Agriculture, Taiwan. All animal experimental protocols were approved by the Animal Care and Use Committee, National Chung Hsing University. The experiments were carried out based on the Ethical Rules and Laws of the University.

2.2. Cell culture

Peripheral blood mononuclear cells (PBMCs) of chicken, duck, goose, turkey, or pigeon were prepared from blood that was drawn in a heparinized (10 U/ml) syringe through the wing vein puncture. PBMC were separated by centrifugation through Ficoll/Hypaque (Ficoll type 400, Sigma) and removed, washed twice with 0.5 mM phosphate-buffered saline (PBS), and resuspended in RPMI 1640 culture medium. Cells were placed in six-well tissue culture plate (Nunc) and incubated at 37 °C and 5% CO_2 for 2 h. The non-adherent PBMC were removed by thoroughly washing with PBS three times, recultured for 12 h, and non-adherent PBMC were used as the lymphocyte fraction. The monocyte fraction was adherent cells remaining after removal of the non-adherent cells. Monocytes were scraped with a cell scraper. Both lymphocyte and monocyte were resuspended at 5×10^6 cells/ml with the same medium and then used for further assays.

2.3. Cloning and nucleotide sequencing of IL-8-encoding regions

Total RNA prepared from monocytes of each avian species was extracted and used for the synthesis of cDNA fragments of IL-8-encoding genes, respectively. Cells were washed twice with PBS and treated with 2.5 μg/ml of lipopolysaccharide (LPS) for 10 h. Medium was removed and cells were lysed in TRIzol solution (Life Technologies) at 20 °C for 5 min. Total RNA was then extracted and isolated based on the manufacturer's instruction manual.

cDNA of IL-8-encoding region for each avian species was generated by reverse transcription-polymerase chain reaction (RT-PCR) using each the total RNA samples as the templates (Wu et al., 2007). Primer sequences and locations were chosen according to the cDNA sequence of ChIL-8 (Sugano et al., 1987). The forward primer (5′-AACAAGCCAAACACTCC-3′) corresponds to the 5′ region of the gene encoding ChIL-8 (nucleotides 54–70). The reverse primer (5′-GGATTTTTCCTGTCTGGAA-3′) is complementary to the 3′ end of the gene encoding ChIL-8 (nucleotides 411–393). Both primers were used to amplify each IL-8-encoding region of five avian species with Taq polymerase. Amplified cDNA products were then cloned into TOPO II T/A cloning vector (Invitrogen). Recombinant plasmids were used to transform *Escherichia coli* (*E. coli*) TOP10F (Invitrogen). Transformants containing the recombinant plasmid were screened from the white bacterial colonies. At least two independent cDNA clones were sequenced in both directions. The sequences were analyzed by ABI Prism 337 sequence analyzer (PerkinElmer). Sequencing was extended across the cDNA using synthetic oligonucleotide primers at intervals of 500–600 bases.

2.4. Phylogenetic analysis

Nucleotide sequences from IL-8-encoding regions of five avian species were aligned to construct a phylogenetic tree for their relationship analysis using a Clustal method of DNASTAR software (DNASTAR Inc., Madison, WI, USA).