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Molecular Cloning and Sequencing of Chicken Il-6 Cds and Study its Comparative Genomic Analysis with Some Avian Species

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ABSTRACT

The Indian poultry industry contributes approximately Rs.22000 crores to the GNP and supports the livelihood of 2.0 million people. But, there is a big gap between the production and per capita availability of the poultry products. It is because of broiler stocks are either plateaued or on the verge of selection limit due to continued long term selection or even losses due to disease. cDNA encoding Chicken IL-6 was cloned from Con A stimulated Chicken Peripheral blood mononuclear cells using oligonucleotide primer..The ChIL-6 cDNA is 710nt long encoding protein of 240 aa. ChIL-6 shows respectively, 95.2%, 86.7%, 75.3% and 55.2% identities with Quail IL-6, DuIL-6, guinea fowl IL-6 and TuIL-6 in cDNA and also shows respectively 93.6% , 92.5% , 57.7% and 2.7%, identities with Turkey IL-6, Quail IL-6, duck IL-6 and Guinea fowl IL-6 in amino acid sequence. This study provides a useful data for further immunological studies of IL-6 in avian immune systems.

KEYWORDS : cDNA,Chicken IL-6, Avian Species"

Introduction

The Indian poultry industry has emerged as the most dynamic and fastest expanding segment in animal husbandry sector during the last 3-4 decades with an average annual growth rate of 10-15 % in broiler chickens and 5-6 % in egg production. The Indian poultry industry contributes approximately Rs.22000 crores to the GNP and supports the livelihood of 2.0 million people**.** In spite of spectacular growth, however, there is a big gap between the production and per capita availability of the poultry products. The modern existing layer and broiler stocks are either plateaued or on the verge of selection limit due to continued long term selection. Therefore, preventing the losses due to diseases may attain further increase in production. The modern manage mental practices, however, endeavor to control the diseases; still have lapses at many levels and mutations in pathogenic organisms may result into disease outbreaks.

Avian immune system is a highly evolved and complex system, involving different cell types and soluble factors that act in concert to elicit immune response against invading pathogens and thus play a pivotal role in survivability Immune response and disease resistance in chicken have found to be affected by multiple genetic and environmental factors. Many factors regulating the immune response in bird have been identified and characterized in last decade. To maintain the local and systemic homeostasis in response to challenge from invading microorganisms and tissue injury, patho-physiological adjustments are made by extensive variety of communication molecules including cytokines, chemokines, cytokine inhibitors, hormones, neurotransmitters, eicosanoids and reactive oxygen intermediates.

Cytokines are a unique family of growth factors. Cytokines also have enormous potential in the control of infectious disease in poultry. Their use as novel therapeutic agents in disease has begun to be explored. Cytokines may also have the potential to act as vaccine adjuvant that may specifically activate the immune system to produce effective protection. The main function of cytokines is in the activation and regulation of the cells of immune system. One particular approach of interest is to incorporate the gene encoding the cytokine of interest into a DNA vaccine or into a viral or bacterial vaccine vector

Interleukin-6 (IL-6) is a multifunctional cytokine that plays major role in regulating immune responses, acute phase reactions, and haematopoies [1]. IL-6 is produced by many different cell types and act on B cells, T cells, hepatocytes, haematopoiesis progenitor cells and cell of central nervous system [2]. Its multifunctional nature is reflected in the variety of names that were originally assigned to IL-6 such as B cell differentiation factor (BCDF), interferon β2 and hepatocyte stimulating factor. IL-6 can switch the differentiation of monocytes from dendritic cells to macrophages, and that IL-6 can modulate the Th1/Th2 response apparently by inhibiting Th1 differentiation by up regulation of suppressor of cytokin signaling 1. IL-6 deficient develop normally but they are unable to efficiently control vaccinia virus and *Listeria monocytogenes* infections.

IL-6 activity has been found in several infectious diseases of chickens. IL-6 is produced during Chicken Eimeria infections[3]. IL-6 modulates the transcription of several liver-specific genes during acute inflammatory states. Altered IL-6 serum levels have been described in several hematological diseases such as plasmacytoma or Castle -man's disease, mesangial osteoporosis, and rheumatoid arthritis.

For this study blood sample from junglar vein of white leghorn were collected and the variation in ChIL-6 with some avian species is analyzed. But this is done by direct sequencing of the amplified fragment bypassing the cloning strategy.

Materials and Methods

Blood sample was collected from junglar vein of white leghorn. PBMC was isolated using the histopaque-1077. PBMC were cultured in RPMI medium supplemented with 10%FCS were plated in 6 well plate and induced with final conc. Of 15µg/ml of Con A (mitogen). Total RNA from stimulated PBMCs were isolated using RNAgents total RNA isolation system. First strand of cDNA from RNA sample was prepared by Revert AidTM first strand cDNA kit. PCR was carried out for amplification of IL-6 gene with first strand cDNA using forward (5'GAGAAGCCGCACCATGAACT 3')and reverse primer (5'GGATTGTGCCCGAAC TA AAAC3'). The thermo cycler was set as 94°C for 3min, then for 35 cycles 94°C for 45s, 56°C for 45s, and 72°C for 1min, 72°C for 10min. RT-PCR products were detected in 1.6% agarose gel.

The PCR products were eluted from agarose gel using kit (Qiagen, USA). The purified PCR products were cloned into pTZ57 R/T vector. The ligation reaction was prepared in 10µl reaction using T4 DNA ligase. Transform DH5α cells (*E. Coli* cells) with the ligated DNA, fresh competent cells were prepared. To minimize the number of clones to be handled, clones were initially checked by performing colony PCR and later confirmed by plasmid-PCR. DNA sequencing was performed for cloned PCR products from chicken IL-6**.** Sequencing was done 5'-3' as well as 3'-5' using the M13 Forward and M13 reverse primers, respectively**.** The sequences obtained were first checked manually and blasted to ascertain that sequences were of desired candidate genes. The related sequences identified from blast results were retrieved from Genebank. The different sequences were edited and the se-

quences corresponding to the concerned region were cut and saved.

Subsequently, the sequences were aligned using CLUSTALW**.** MEGA(Version 4.0) software was used to estimate nucleotide as well as amino acid **v**ariability. The genetic distances were estimated as Kimura 2-parameter distances, while the genetic distances between the amino acid sequences from different poultry species were estimated as poisson correction distances using MEGA software. Phylogenetic trees were constructed with NJ procedure using MEGA Version 4.0

Results and Discussion

The mRNA was isolated from chicken PBMC stimulated with 15µg/ml Con A. The chicken IL-6 cDNA fragment was amplified by RT-PCR using mRNA as template. An approximately 762bp DNA fragment, in size was amplified from mRNA. The sequencing result revealed that cDNA fragment of ChIL-6 was 710nt in length.

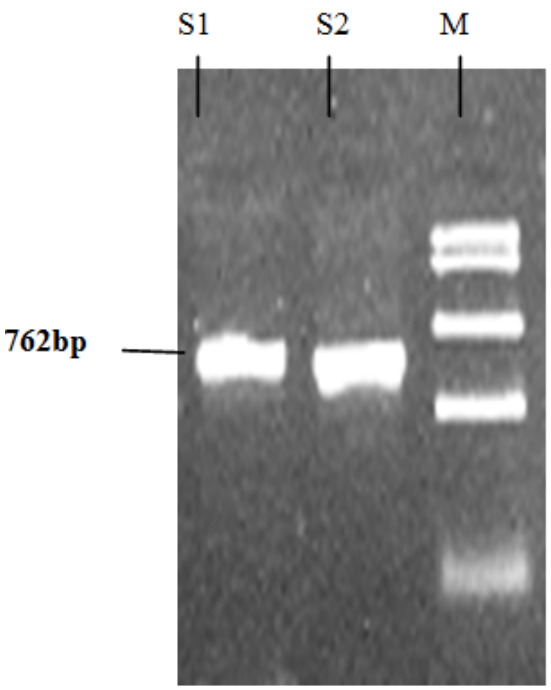

Fig .1. RT-PCR amplification of ChIL-6 extracted from Chicken PBMC harvested after Con A stimulation.

ChIL-6 cDNA fragment encodes a polypeptide of 241 amino acid residues. The amino acid sequence of ChIL-6 was compared with some Avian species. The alignment analysis showed that ChIL-6 shared 93.6% identity to Turkey IL-6, 92.5% to Quail IL-6, 57.7% to duck IL-6 and 2.7% to Guinea fowl IL-6 (Table 1). In IL-6 sequences of compared avian species there are conserved amino acid residues including three cysteine residues (99,132 and 165) indicating that there is atleast one di sulfate bond in the molecules. However, Asp, Met, Ser are present only in ChIL-6 amino acid sequence.

Table 1 : Pair wise genetic distance based on amino acid diversity in IL-6 peptide.

Percent Identity

Divergence	1	2	3	4	5		
1		2.7	57.7	92.5	93.6	1	Chicken_target_gene
2	410.0		1.8	2.7	3.2	2	Guinea_fowl
3	8.0	524.0		100.0	96.8	3	Peking_duck
4	8.0	524.0	0.0		96.8	4	Quail
5	6.7	471.0	2.5	2.5		5	Turkey
	1	2	3	4	5		

Phylogenetic analysis further shows that Avian IL-6 was subdivided into three monophyletic lineages. The quail, duck and turkey IL-6 formed a monophyletic group distinct from ChIL-6 as well as distinct from Guinea fowl IL-6 (Fig.2.).

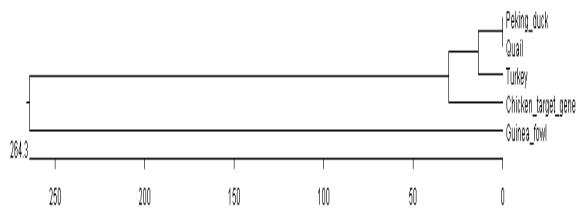

Fig 2. Phylogenetic tree based on amino acid variation in IL-6 between chicken and poultry species. The numbers of staff gauge indicate the evolution distance of different species

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REFERENCES

1. Kishimoto, T., Akira, S., Narazaki, M. and Taga, T. (1995) Interleukin-6 family of cytokines and gp130. Blood 86, 1243-1254. | 2. Satoh, T., Nakamura, S., Taga, T., Hirano, T., Kishimoto, T. and Kaziro, Y.(2001) Induction of neuronal differentiation in PC12 cells by Bcell stimulatory factor 2/interleukin 6. Molecular Cellular Biology 8, 3546-3549. | 3. Lynagh, R., Bailey, M. and Kaiser, P. (2005) Interleukin-6 is produced during avian eimeria infections. Veterinary Immunology and Immunopathology 76, 89-102