



Evolution of Th1 and Th2 biased cytokines in divergent species of animals

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The innate immune response initiation requires recognition of conserved, molecular structures broadly shared by microbes, and pathogens that are known as pathogen-associated molecular patterns (PAMPs) and is mediated by germline-encoded pattern recognition receptors (PRRs) (Janeway and Medzhitov 2002). On detection of PAMP within the host's body, PRRs trigger intracellular signaling cascades that execute the first line of host defense through the expression of a variety of pro-inflammatory molecules leading to maturation of dendritic cells, responsible for the subsequent activation and shaping of adaptive immunity, thereby bridging the two arms of immunity (Heath *et al.* 2015). The receptors like TLRs and NLRs have direct involvement in the fitness of the individuals by conferring resistance against various microbes (Zhang *et al.* 2010). Several reports are available on the evolution of domain architecture as well as functional roles of many PRR domains (Johnston *et al.* 2011), but, the evolutionary relationships between TLR and an NLR domain is largely unknown (Jones *et al.* 2001). Thus, our present research work targets the determination of evolution of the PRR domains (TIR and NACHT domains of TLR and NLR genes, respectively) in divergent animal species about the impact of natural selection, their chronological divergence and structural attributes implying the functional specification (Takeda *et al.* 2003). In comparison to other species, avian cytokines have been poorly defined, both in terms of structure and function (Sharma *et al.* 2007).

The multiple sequence alignment was done using online tool clustal omega on coding sequences (retrieved from National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov)) of cytokines namely T helper 1 cells (Th1) (Th1 biased-TNF, IFN- γ , IL1 β , IL2, IL8, and IL18) and T helper 2 cells (Th2) (Th2 biased-IL6, IL10, and IL13) of divergent species (mammals, avians and fish). The best evolutionary model was determined based

on the least Bayesian information criterion (BIC) scores using MEGA6 (Tamura *et al.* 2013). The phylogenetic tree (maximum likelihood method with bootstrap re-sampling) (Felsenstein 1981) was constructed using 12 cytokines and 22 divergent species, each one representing a set of divergent class of animals for comparable interpretation. Protein structure prediction analysis was performed for 22 specific sequences of different species (avians, mammals, and fish).

The phylogenetic tree (Fig. 1) exposed that Th1 biased cytokine, IFN- γ showed separate clusters of mammals, avians, and fish. Th2 biased cytokine IL12 showed that all the species were dispersed except *Canis lupus* and *Sus scrofa*. Th2 biased cytokine i.e. IL13 showed that chicken IL13 was completely dispersed from mammals, and closely related to IL8 and TNF. Further, the phylogenetic tree was constructed for Th1 biased and Th2 biased cytokines which showed that the Th1 cytokines were clustered and Th2 cytokines were dispersed in which IL13 *Gallus gallus* was completely dispersed from IL13 rat.

In this pairwise distance matrix, the coloured boxes showed divergent species among similar cytokines and red values showed similar species among different cytokines.

Protein structure prediction analysis was performed for 22 specific sequences of different species (avians, mammals and fish). Secondary structure prediction of the sequences revealed number of sheets and helices among different types of cytokines domains in different species (avians, mammals and fish). Almost same number of sheets were present in all the species. The absence of an extended strand in parallel and/or anti-parallel beta sheet conformation structure was seen in almost all the sequences. The 3D protein structure analysis showed beta pleated sheets, α -helix and loop structures. Ramachandran plot validation revealed more than 90% of the structure in favoured regions and 95–100% of the structure was lying in allowed regions. Most of the Th1 (IL8, TNF, IFN γ , IL2, IL18) and Th2 (IL6, IL10, IL13) biased cytokines were clustered separately, however, IFN γ , IL-12 and IL13 clustered separately for the mammalian, avian and fish. These interleukins could have evolved independently from the respective ancestral sequences. The structural differences of the predicted peptide sequences of

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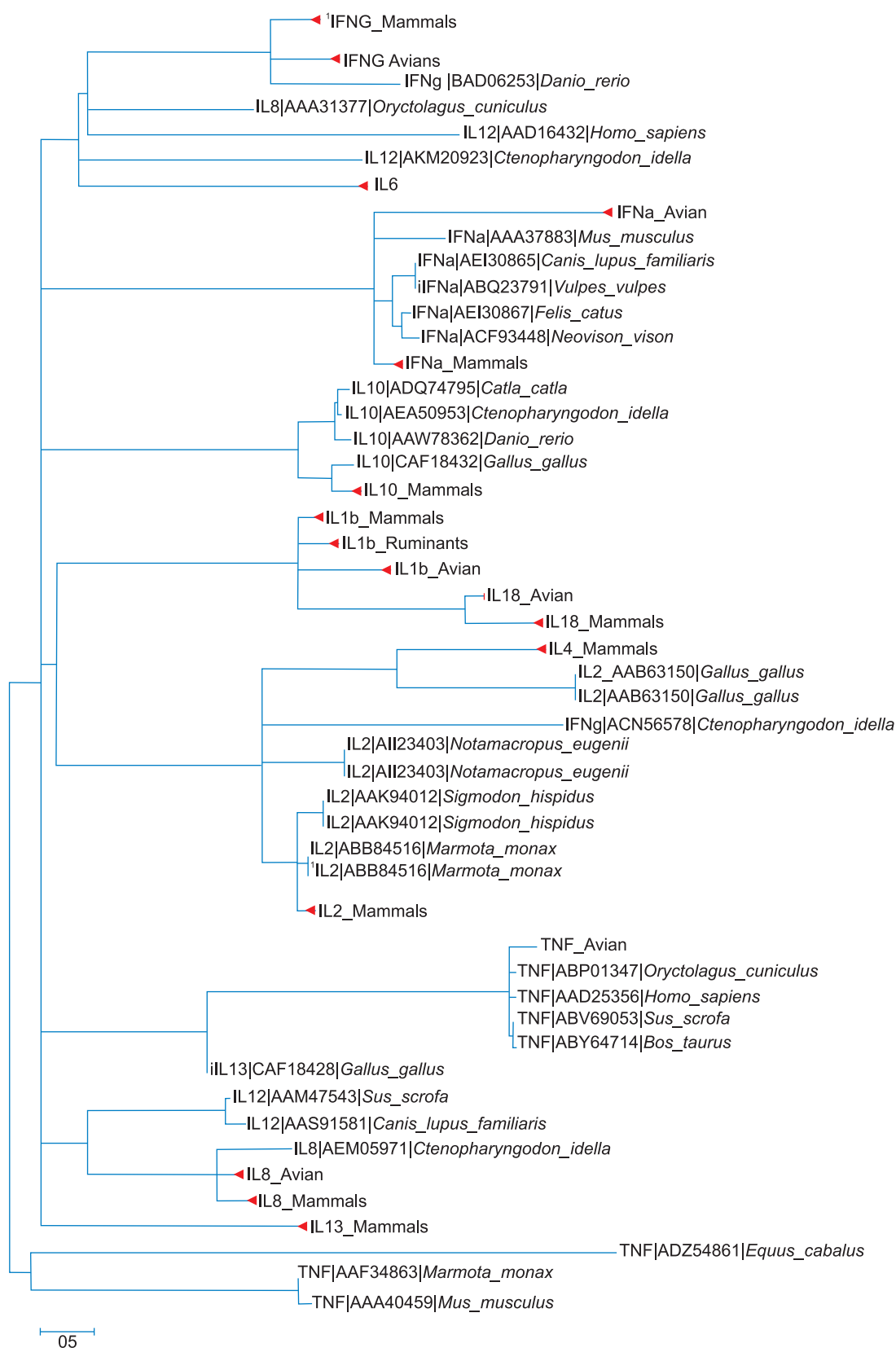


Fig. 1. Phylogenetic tree constructed from CDS encoding the active domains of 22 divergent cytokines, using maximum likelihood method (bootstrap resampling).

TNF and IFN γ among avian, mammals and fish were caused by InDel of amino acids.

It can be concluded from the study that most of the Th1 (IL8, TNF, IFN γ , IL2, IL18) and Th2 (IL6, IL10, IL13) biased cytokines were clustered separately. The structural differences of the predicted peptide sequences of TNF and IFN γ among avian, mammals and fish were caused by InDel of amino acids. Th1 and Th2 biased cytokines from a divergent animal species were studied *in silico* to shed light on the evolutionary perspective of the cytokines in host defense mechanism. Results indicate that these cytokines could have co-evolved independently in divergent species from ancestral sequence.

SUMMARY

Proteins or peptides that play a major role in immune as well as inflammatory responses via activation and regulation of other cells and tissues are known as cytokines. Their role in mammals is well defined, with a vast number of publications describing the structure of cytokines and their role in health and disease. Total 22 full-length CDS (and corresponding peptide) of the domains were selected as representatives of each type of cytokine, belonging to divergent animal species, for the biocomputational analysis. The secondary and tertiary structure of the different cytokine (peptide sequence) domains was predicted to compare the relatedness among the domains under study. Multiple sequence alignment and phylogenetic tree results indicated that IFN-gamma formed separate clusters for mammals, avian, and fish. In IL12, all were dispersed except *Canis lupus* and *Sus scrofa*. In IL13, chicken was completely dispersed from mammals and closely related to IL8 and TNF. These interleukins could have evolved independently from the respective ancestral sequences.

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REFERENCES

- Felsenstein J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* **17**(6): 368–76.
- Heath A W, Devey M E, Brown I N, Richards C E and Playfair J H. 2015. Interferon-gamma as an adjuvant in immune-compromised mice. *Immunology* **67**: 520–24.
- Janeway C A and Medzhitov R. 2002. Innate immune recognition. *Annual Review of Immunology* **20**: 197–216.
- Johnston P A, Liu H and O'Connell T. 2011. Applications in ovo technology. *Poultry Science* **76**: 165–78.
- Jones M A, Wigley P, Page K L, Hulme S D and Barrow P A. 2001. *Salmonella enterica* serovar Gallinarum requires the *Salmonella* pathogenicity island 2 type III secretion system but not the *Salmonella* pathogenicity island 1 type III secretion system for virulence in chickens. *Infection and Immunity* **69**: 5471–76.
- Sharma J M and Burmester B R. 2007. Resistance to Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpesvirus. *Avian Diseases* **26**: 134–49.
- Takeda K, Kaisho T and Akira S. 2003. Toll-like receptors. *Annual Review of Immunology* **21**: 335–76.
- Tamura K, Stecher G, Peterson D, Filipinski A and Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis Version 6.0. *Molecular Biology and Evolution* **30**: 2725–29.
- Zhang Q, Zmasek CM and Godzik A. 2010. Domain architecture evolution of pattern-recognition receptors. *Immunogenetics* **62**: 263–72.