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Identification, cloning and characterisation of interleukin-1F5 (IL-36RN) in the chicken

M.S. Gibson [a,*], N. Salmon [a], S. Bird [b,1], P. Kaiser [a,2], M. Fife [a]

[a] Institute for Animal Health, Compton, Berkshire RG20 7NN, UK
[b] Scottish Fish Immunology Research Centre, Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK

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ABSTRACT

The human IL-1 family contains eleven genes encoded at three separate loci. Nine, including IL-36 receptor antagonist (IL-36RN), also known as IL-1F5, are present at a single locus on chromosome 2, whereas IL-18 and IL-33 lie on chromosomes 11 and 9 respectively. There are currently only three known orthologues in the chicken – IL-1β, IL-18 and IL-1RN – which are encoded on chromosomes 22, 24 and unplaced, respectively.

A novel chicken IL-1 family sequence representing IL-36RN (IL-1F5) was initially identified from an expressed sequence tag (EST) library by its similarity to both chicken IL-1RN and chicken IL-1β. Following isolation of the cDNA from the liver of an uninfected bird, a number of unique sequence features were identified. The predicted protein has a longer NH_2-terminus than the human protein; however, as in mammals, this region contains neither a prodomain nor a signal peptide. A putative nuclear export sequence is also apparent, yet a similar motif is absent in mammalian IL-36RN. Although chIL-36RN exhibits low homology with its mammalian orthologues, it encodes a predicted β-trefoil structure whose β-strands are conserved with those of the mouse sequence.

Unlike in mammals, chIL-36RN expression was constitutive in all tissues and cell subsets examined. In response to viral infection, expression was significantly downregulated in a line of birds which are susceptible to the virus.

Chicken IL-36RN, like chIL-1RN, is not encoded at the chIL-1β locus, further emphasising the genomic fragmentation of the large IL-1 gene cluster found in mammals. This suggests differential evolution of this cytokine family since the divergence of birds and mammals from a common ancestor, and underlines the difficulty of determining the full repertoire of chIL-1 family members.

1. Introduction

The interleukin-1 (*IL-1*) gene family in humans comprises eleven members (*IL-1F1-F11*) that act as either pro- or anti-inflammatory cytokines. Although this family of ligands plays a pivotal role in the innate immune response, recent evidence has shown several IL-1 agonists have additional functions, regulating adaptive immune responses in $CD4^+$ T cell subsets and dendritic cells (Sims and Smith, 2010; Vigne et al., 2011).

Three ligand members were originally described – IL-1β, IL-1α, and IL-1RN – along with two receptors (IL-1RI and IL-1RII) and the IL-1 receptor accessory protein (IL-1RAcP). IL-1α and IL-1β are classical pro-inflammatory cytokines that act on the cells of the innate immune system to modulate their function. Both ligands bind a common, shared receptor, IL-1RI, through which signalling is transduced following recruitment of the IL-1RAcP. Due to the extremely potent biological activity of these two ligands, their production at both the mRNA and protein levels is tightly regulated.

Abbreviations: aa, amino acids; BLAST, basic local alignment search tool; Bl-Mo, blood-derived monocytes; BM-DC, bone marrow-derived dendritic cells; BM-MΦ, bone marrow-derived macrophages; BrL, brown Leghorn; CDS, coding sequence; Ct, cycle threshold value; dpi, days post-infection; EST, expressed sequence tag; IBDV, infectious bursal disease virus; IL-36RN, interleukin-36 receptor antagonist; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; Mo-MΦ, blood monocyte-derived macrophages; NES, nuclear export sequence; NLS, nuclear localisation sequence; NMWL, nominal molecular weight limit; PBS, phosphate-buffered saline; PEI, polyethylenimine; PHA, phytohaemagluttinin; PMA, phorbol myristate acetate; RIR, Rhode Island Red; SPF, specified-pathogen-free.

* Corresponding author. Tel.: +44 1635 578411; fax: +44 1635 577263.
E-mail addresses: mark.gibson@iah.ac.uk (M.S. Gibson), nigel.salmon@iah.ac.uk (N. Salmon), s.bird@waikato.ac.nz (S. Bird), pete.kaiser@roslin.ed.ac.uk (P. Kaiser), mark.fife@iah.ac.uk (M. Fife).
[1] Current address: Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand.
[2] Current address: The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK.

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Further regulation of IL-1α and IL-1β is provided by a naturally occurring antagonist, IL-1 receptor antagonist (IL-1RN), which acts to reduce IL-1 activity by blocking the IL-1RI. Upon binding this receptor, IL-1RN is unable to engage cell surface IL-1RAcP, as it lacks the specific amino acid residues required to perform this function. This prevents signal transduction and subsequent gene transcription, effectively limiting inflammation.

For almost another decade, only a single novel IL-1 ligand, IL-18 (Okamura et al., 1995), was identified. However, during this same period an increasing number of orphan receptors with IL-1R-like features were discovered. The emergence of the human genome sequence revealed a further six IL-1 family ligand genes (IL-1F5-F10) clustered with the original three members on human chromosome 2. Subsequent work has attributed most of these ligands to specific IL-1 family receptors, with some formerly orphan receptors now having defined roles. Whilst this expanded cytokine family remains synonymous with pro-inflammatory responses, it also contains several members that suppress inflammation. These include three IL-1 receptor antagonists, IL-1RN, IL-1F5 (recently renamed IL-36RN (Dinarello et al., 2010)), and IL-1F10 (recently renamed IL-38 (Dinarello et al., 2010)) and an endogenous inhibitor of the innate immune response, IL-1F7 (IL-37) (Nold et al., 2010). There are also a number of receptors that downregulate inflammation. These are the orphan receptor single Ig domain-containing IL-1 receptor-related molecule (SIGIRR; also known as TIR8); the decoy receptor, IL-1RII; a soluble binding protein, IL-18BP (Novick et al., 1999); and a neuronal-specific IL-1RAcP splice variant, IL-1RAcPb (Smith et al., 2009). Additional, soluble forms of the IL-1RI, IL-1RII and ST2 receptors also exist to regulate IL-1 ligand activity.

Whilst the more recently discovered IL-1 family members (IL-1F5-F11) have been less extensively studied, functional roles for most of these ligands are becoming apparent. IL-1F5 (IL-36RN) acts as a receptor antagonist of IL-1RL2 (IL-1Rrp2) (Debets et al., 2001; Towne et al., 2011; Vigne et al., 2011). It inhibits the multiple stimulatory effects induced by the agonists IL-1F6 (IL-36α), IL-1F8 (IL-36β) and IL-1F9 (IL-36γ) by preventing them from binding to this receptor. As with IL-1RN, IL-36RN fails to recruit IL-1RAcP on the cell surface (Towne et al., 2011). IL-36RN also downregulates inflammation through an (as yet unelucidated) interaction with the orphan receptor SIGIRR. In mice and rats, both IL-1β- and lipopolysaccharide (LPS)-induced inflammation is antagonized by IL-36RN in the brain, leading to an anti-inflammatory response. This is characterised by increased IL-4 production. The ability of IL-36RN to mediate this effect is abolished in SIGIRR$^{-/-}$ and IL-4$^{-/-}$ knockout mice (Costelloe et al., 2008). Further evidence of its importance stems from the observation that IL-36RN-deficient individuals display unregulated inflammation and chronic pustular psoriasis, which can be fatal (Marrakchi et al., 2011).

Significant progress has been made over the past decade to determine the repertoire of immune function genes present in the chicken (Kaiser, 2010). Whilst our knowledge of the different cytokine families has grown rapidly, several cytokines present in mammals lack an obvious avian orthologue. The availability of the chicken genome sequence (ICGSC, 2004) has led to the identification of a number of novel cytokines. However, even after three separate genome builds; the quality of the chicken genome sequence, both its assembly and its annotation, is unsurprisingly poor by comparison to that of the human genome. There are a considerable number of regions on many of the chicken chromosomes which contain large sequence gaps. For some of the micro-chromosomes, there is no sequence information for the entire chromosome. As such, it is difficult to know how many of the genes found in humans, which are presumed missing in the chicken, are genuinely absent, with the probable exception of those that are missing from loci with conserved synteny.

To date, only three members of the chicken IL-1 family have been cloned and functionally characterised: IL-1β (Weining et al., 1998), IL-18 (Schneider et al., 2000) and IL-1RN (Gibson et al., 2012). All three of these cytokines exhibit biological activity resembling that of their mammalian orthologues. Despite the apparent absence of further IL-1 ligands, the entire human IL-1 receptor family is conserved in the chicken (Gibson, unpublished results). This indicates that the chicken genome may encode additional, as yet undiscovered, ligand genes. In the current chicken genome build, the locus containing chIL-1β exhibits limited conserved synteny with the nine gene human IL-1 cluster, but lacks any further IL-1 genes. This region of the chicken genome includes orthologues of two genes (SLC20A1 and CKAPL2) that flank the human IL-1 cluster; however, no other direct orthologous genes are present.

Here we report the discovery and characterisation of IL-36RN for the first time in a non-mammalian species.

2. Materials and methods

2.1. Identification of chicken IL-1 family members

A search of the NCBI Chicken Genome Resources EST database (http://www.ncbi.nlm.nih.gov/genome/guide/chicken/) identified expressed sequence tag (EST) sequences that corresponded to putative chicken IL-1 orthologues. For all members of the human IL-1 family yet to be identified in the chicken, the full gene sequence, full amino acid (aa) sequence and the aa signature motif were analysed with the basic local alignment search tool (BLAST) against the chicken genome sequence using the ENSEMBL genome browser. A single EST sequence (GenBank ID: BU247129) corresponding to a novel chicken IL-1 family gene was translated and analysed by TBLASTN against all eukaryotic animal genomes in ENSEMBL to confirm its putative identity. Sequences (positive hits) from species containing orthologous genes were aligned using ClustalX v1.83 (Thompson et al., 1997).

Following amplification and cloning, a novel chIL-36RN aa sequence was analysed for the presence of a signal peptide using the SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/) (Bendtsen et al., 2004; Emanuelsson et al., 2007)) and for potential protease cleavage sites using peptide cutter (http://www.expasy.org/tools/peptidecutter/) (Gasteiger et al., 2005)). The novel chIL-36RN protein sequence was analysed for structural similarity to known protein domains present in the ProDom database (http://www.prodom.prabi.fr/prodom/current/html/form.php). The sequence was BLASTP queried against all families of protein domains. Using the NetNES prediction server (http://www.cbs.dtu.dk/services/NetNES/), which uses hidden Markov models and neural networks, the chIL-36RN aa sequence was analysed for the presence of a nuclear export sequence. Phylogenetic analysis was carried out using MEGA v5.0 (Tamura et al., 2011) with bootstrap analysis with 500 bootstrap datasets. The secondary structure of the protein was predicted using PSIPRED v3.0 (http://www.bioinf.cs.ucl.ac.uk/psipred/).

2.2. Cloning and sequencing of the IL-36RN cDNA

In order to obtain a full length clone of IL-36RN, the original EST clone (GenBank ID: ChEST734c4/BU247129.1) was acquired from MRC Geneservice (http://www.geneservice.co.uk/products/cdna/chickenEST.jsp) and sequenced. Sequence-verified PCR products of the full length chicken IL-36RN CDS were directionally cloned into the His-tagged expression vector pHLSec (kindly provided by James Birch, IAH; vector details in (Aricescu et al., 2006)) between *Age*I and *Kpn*I restriction sites. The complete amplified cDNA sequence was submitted to the EMBL Nucleotide Sequence Database (EMBL-Bank ID: HE605042).