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Identification and functional characterization of the house finch interleukin-1β

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ABSTRACT

Interleukin-1β (IL-1β), an inflammatory cytokine of the IL-1 family, is primarily produced as a precursor protein by monocytes and macrophages, then matures and becomes activated through proteolytic catalysis. Although the biological characteristics of avian IL-1β are well known, little information is available about its biological role in songbird species such as house finches that are vulnerable to naturally-occurring inflammatory diseases. In this study, house finch IL-1β (HfIL-1β) was cloned, expressed, and its biological function examined. Both precursor and mature forms of HfIL-1β consisting of 269 and 162 amino acids, respectively, were amplified from total RNA of spleen and cloned into expression vectors. HfIL-1β showed high sequential and tertiary structural similarity to chicken homologue that allowed detection of the expressed mature recombinant HfIL-1β (rHfIL-1β) with anti-ChIL-1β antibody by immunoblot analysis. For further characterization, we used primary splenocytes and hepatocytes that are predominant sources of IL-1β upon stimulation, as well as suitable targets to stimulation by IL-1β. Isolated house finch splenocytes were stimulated with rHfIL-1β in the presence and absence of concanavalin A (Con A), RNA was extracted and transcript levels of Th1/Th2 cytokines and a chemokine were measured by qRT-PCR. The addition of rHfIL-1β induced significant enhancement of IL-2 transcript, a Th1 cytokine, while transcription of IL-1β and the Th2 cytokine IL-10 was slightly enhanced by rHfIL-1β treatment. rHfIL-1β also led to elevated levels of the chemokine CXCL1 and nitric oxide production regardless of co-stimulation with Con A. In addition, the production of the acute phase protein serum amyloid A and the antimicrobial peptide LEAP2 was observed in HfIL-1β-stimulated hepatocytes. Taken together, these observations revealed the basic functions of HfIL-1β including the stimulatory effect on cell proliferation, production of Th1/Th2 cytokines and acute phase proteins by immune cells, thus providing valuable insight into how HfIL-1β is involved in regulating inflammatory response.

1. Introduction

Interleukin-1beta (IL-1β) is the most studied prototypical pro-inflammatory cytokine because of its crucial role in the initiation of inflammation and regulation of innate and adaptive immune responses (Netea et al., 2015). IL-1β lacks a signal peptide and is primarily expressed by activated macrophages, monocytes, and dendritic cells as an inactive precursor form and remains in the cytosol, requiring proteolytic processing at its *N*-terminal region for optimal bioactivity (Black et al., 1988; Thornberry et al., 1992; Arend et al., 2008). Subsequently, it is cleaved by either an intracellular cysteine protease caspase-1 activated by inflammasome (Thornberry et al., 1992; Martinon et al., 2002) or by inflammasome-independent enzymatic processes such as neutrophil-derived serine proteases and pathogen-released enzymes (Netea et al., 2010). This cleaved IL-1β is secreted into the extracellular milieu, where it can induce its own transcription as mature and bioactive IL-1β. By binding to IL-1 type I receptor (IL-

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1R1), secreted IL-1β exerts its biological activities including T cell activation, B cell proliferation, and antigen recognition along with the induction of inflammatory genes, chemokines, and cell adhesion molecules (Burns et al., 2003; Dinarello, 2009). In mammals, IL-1β induces the development of Th17 cells in combination with IL-6 or TGF-β, while the production of IL-23 is IL-1β dependent in monocytes which contributes to maintenance of Th17 cells (Weaver et al., 2007; Dong, 2008; van de Veerdonk et al., 2009). IL-1β also induces synthesis of cyclooxygenase type 2 (COX-2), type 2 phospholipase A, and inducible nitric oxide synthase (iNOS), leading to the production of prostaglandin-E2 (PGE2), platelet activating factor (PAF), and nitric oxide (NO) that causes fever, lower pain threshold, vasodilatation, and hypotension (Dinarello, 2009). Additionally, IL-1β is responsible for triggering the synthesis of the acute phase protein serum amyloid A (SAA), IL-6, neutrophil-selective CXC chemokines, and macrophage inflammatory protein-2 (McColl et al., 2007). An abnormal increase of IL-1β secretion is associated with the pathogenesis of auto-inflammatory diseases such as cryopyrin-associated periodic syndromes, which is related to an over-activation of caspase-1 (Campbell et al., 2016).

In avian species, chicken IL-1β (ChIL-1β) was first identified and cloned from the chicken macrophage cell line HD11 stimulated with LPS (Weining et al., 1998). ChIL-1β has a similar gene structure to mammalian homologues (Giansanti et al., 2006) with 34% and 33% amino acid identity with the respective human and mouse orthologs; however, it lacks a conserved aspartic acid residue thus preventing the caspase-1 cleavage. Nonetheless, *N*-terminally truncated ChIL-1β lacking the predicted pro-domain exhibits significantly enhanced biological activity suggesting that precursor cleavage is critical for its maximal activity (Gyorfy et al., 2003). Another phylogenetically conserved aspartic acid residue was later discovered by cleavage of avian proIL-1β with either sea bass or human caspase-1, which is distinct from the cleavage site of mammalian homologues (Reis et al., 2012). Consistent with mammalian homologues, ChIL-1β expression is significantly enhanced following viral, bacterial, and protozoal infections. ChIL-1β mRNA expression was induced in the gut following *Eimeria* infection (Laurent et al., 2001; Hong et al., 2006a,b), enhanced mRNA level was also observed in macrophages from turkeys suffering from poult enteritis and mortality syndrome (PEMS), as well as in bursal cells from IBDV-infected chickens (Heggen et al., 2000; Eldaghayes et al., 2006). *Salmonella* spp. led to up-regulation of IL-1β mRNA in chicken cell lines and heterophils (Iqbal et al., 2005; Kogut et al., 2005). Macrophages exposed to either *Escherichia coli* or *Mycoplasma synoviae* increased IL-1β transcription (Lavric et al., 2008). These reports further highlight the important role of IL-1β in controlling the pathogenesis of many diseases.

The properties of IL-1β have been well studied in domestic poultry but not in wild birds, which are in close contact with domesticated animals and may act as natural reservoirs for many zoonotic pathogens. The house finch, *Haemorhous mexicanus*, is a small passerine songbird that originally inhabited western North America and later expanded to the eastern U.S. (Hill, 1993). House finches are relatively easy to capture and examine in captivity making them ideal organisms for studying the ecology of wildlife diseases, and they favored over domesticated birds to study the co-evolutionary relationship between host and pathogen during emergence of other diseases (Hurtado, 2012). Most recently, differential mRNA expression of IL-1β across populations following experimental *Mycoplasma gallisepticum* (MG) infection was documented (Adelman et al., 2013). However, the biological role of IL-1β in wild house finches still needs to be elucidated. To clarify this matter, we first cloned the precursor and mature forms of house finch IL-1β (HfIL-1β), then investigated its basic function by measuring immune cell proliferation and differential mRNA expression of Th1/Th2 response elements, acute phase protein and antimicrobial peptide by activated immune cells.

2. Materials and methods

2.1. Birds and tissue collection

House finches were captured in either July of 2012 or June–July of 2015 using cage traps and mist nets in Montgomery County, VA under permits from VDGIF (044569/2012 and 050352/2015) and USFWS (MB158404-1). All finches were housed at constant day length and temperature, and were fed an *ad libitum* pelleted diet prior to and throughout experiments (Daily Maintenance Diet, Roudybush Inc. Woodland, CA). Following capture, adult individuals from both sexes were identified based on their plumage characteristics and tested for the exposure to the pathogen as described in Park et al. (Data in Brief, submitted). After testing, only healthy birds that showed no clinical signs of disease and had no pathogen load (Grodio et al., 2008) were randomly selected for the subsequent experiments. All tissue samples, including brain, heart, liver, small intestines (duodenum, jejunum, ileum), spleen, thymus, bursa, lung, proventriculus and gizzard were collected from two individuals to assess HfIL-1β tissue distribution. Additionally, the primary cells were isolated from spleens and livers of 10 randomly selected birds for further biological experiments.

2.2. Sequence and structural analyses

Nucleotide and amino acid sequences of HfIL-1β were aligned with other orthologous sequences obtained by BLAST search using Clustal Omega (Sievers and Higgins, 2014). The phylogenetic tree was constructed from the alignment using the neighbor joining (NJ) method within the MEGA4 program, with Poisson correction and complete deletion of gaps (Tamura et al., 2007). The stability of the branching order was confirmed by performing 1000 bootstrap replicates. The theoretical molecular weight (MW) and isoelectric point (pI) were estimated using a Compute pI/MW tool from ExPASy (http://www.expasy.org). The three-dimensional structure of HfIL-1β was built by comparative modeling at the Robetta server (http://robetta.bakerlab.org) (Kim et al., 2004). The model was superimposed with the X-ray structure of ChIL-1β using Discovery Studio 2.0 (Accelrys Inc., CA) and PyMOL (DeLano Scientific, CA).

2.3. Construction of recombinant HfIL-1β (rHfIL-1β) expression plasmid

Both precursor and mature forms of HfIL-1β genes were amplified from total RNA extracted from house finch spleen using the primers designed based on partial genomic sequences of house finch (provided by D. Hawley) (Table 1). Using 1 μg of total RNA, the first-strand cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad, CA). The full-length HfIL-1β was amplified using the following conditions: initial denaturation at 92 °C for 2 min, 35 cycles of denaturation at 92 °C for 15 s, annealing at 54 °C for 15 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. Synthesized precursor and mature forms of HfIL-1β were directly inserted into pCR2.1-TOPO vector (Invitrogen, CA) and transformed into *E. coli* TOP10 (Invitrogen). Transformants containing recombinant plasmid were selected by a combination of PCR screening and endonuclease digestion with *EcoR* I (New England Biolabs, MA), and confirmed by sequencing (Biocomplexity Institute at Virginia Tech, VA). For sub-cloning into a prokaryotic or eukaryotic expression vector, mature and precursor forms of HfIL-1β were digested with endonucleases *Bgl* II and *Xma* I (New