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Molecular cloning and characterization of chicken NK-lysin

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Abstract

NK-lysin is an anti-microbial and anti-tumor protein expressed by NK cells and T lymphocytes. In a previous report, we identified a set of overlapping expressed sequence tags constituting a contiguous sequence (contig 171) homologous to mammalian NK-lysins. In the current report, a cDNA encoding NK-lysin was isolated from a library prepared from chicken intestinal intraepithelial lymphocytes (IELs). It consisted of an 850 bp DNA sequence with an open reading frame of 140 amino acids and a predicted molecular mass of 15.2 kDa. Comparison of its deduced amino acid sequence showed less than 20% identity to mammalian NK-lysins. The tissue distribution of NK-lysin mRNA revealed highest levels in intestinal IELs, intermediate levels in splenic and peripheral blood lymphocytes, and lowest levels in thymic and bursa lymphocytes. Following intestinal infection of chickens with *Eimeria maxima*, one of seven *Eimeria* species causing avian coccidiosis, NK-lysin transcript levels increased 3–4-fold in $CD4^+$ and $CD8^+$ intestinal IELs. However, cell depletion experiments suggested other T lymphocyte subpopulations also expressed NK-lysin. The kinetics of NK-lysin mRNA expression indicated that, whereas infection with *E. acervulina* induced maximum expression only at 7–8 days post-infection, *E. maxima* and *E. tenella* elicited biphasic responses at 3–4 and 7–8 days post-infection. Finally, recombinant chicken NK-lysin expressed in COS7 cells exhibited anti-tumor cell activity against LSCC-RP9, a retrovirus-transformed B-cell line. We conclude that chicken NK-lysin plays important roles during anti-microbial and anti-tumor defenses.

Keywords: NK-lysin; Chicken; Cloning; *Eimeria*; Intraepithelial lymphocytes

1. Introduction

NK-lysin and granulysin are homologous cationic peptides produced by natural killer (NK) cells and

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cytotoxic T lymphocytes (CTLs). Both are members of a larger group of proteins, referred to as saposin-like proteins, that are found along with perforin in cytolytic lymphocyte granules (Munford et al., 1995; Liepinsh et al., 1997). These proteins share a common predicted structure and perform a variety of biological functions (Pena and Krensky, 1997). Anti-microbial and anti-tumor cell properties of NK-lysin and granulysin have been reported with targets including Gram-positive and Gram-negative bacteria, and protozoan parasites (Andersson et al., 1995; Tschopp and Hofmann, 1996; Stenger et al., 1998; Andreu et al., 1999; Hata et al., 2001; Jacobs et al., 2003). In particular, NK-lysin showed high anti-bacterial activity against *Escherichia coli* and *Bacillus megaterium*, and lytic activity against YAC-1, a NK cell sensitive tumor cell line (Andersson et al., 1996). The microbiocidal and tumor cytolytic activities of NK-lysin, like those of other saposin-like proteins, are believed to be due to its ability to form pores in the cell membrane due to its α-helical structure (Ruysschaert et al., 1998; Zhang et al., 2000).

Avian coccidiosis is caused by several species of the genus *Eimeria* and is considered to be one of the most economically important diseases of domestic poultry. For many years, prophylactic use of anti-coccidial drugs as feed additives has been the primary means of controlling the disease. However, the use of coccidiostats has drawbacks because of the increasing emergence of drug resistant field strains of *Eimeria* (Allen and Fetterer, 2002). Vaccines may offer alternatives to drugs as a means of controlling coccidiosis since avian coccidia are highly immunogenic, and primary infections stimulate immunity to homologous parasite challenge. Although several promising vaccine candidates have been described (Lillehoj et al., 2000a, 2000b; Dalloul and Lillehoj, 2005), efforts to develop an effective, commercially feasible coccidiosis vaccine have been slow.

The putative chicken homologue of NK-lysin was previously reported in chicken splenocytes, thymus and intestinal intraepithelial lymphocytes (IELs) (Tirunagaru et al., 2000; Cui et al., 2004; Min et al., 2005). In prior work, we constructed a chicken cDNA library from *Eimeria*-infected intestinal IELs with the goal of identifying endogenous anti-microbial factors expressed by host cells during coccidiosis (Min et al., 2005). Contig 171, composed of 87 overlapping expressed sequence tags (ESTs), was provisionally identified as a NK-lysin-like sequence based on its homology to mammalian NK-lysins and granulysin. Interestingly, ESTs derived from this gene occurred with the highest prevalence in the library, an indication that its encoded gene product was an important component of the intestinal immune response to coccidiosis. The current study was conducted to clone, express, and characterize contig 171 and confirm its identity as NK-lysin.

2. Materials and methods

2.1. Cloning of chicken NK-lysin

Construction of the IELs cDNA library in the pCMV-SPORT6 vector was described previously (Min and Lillehoj, 2004). Contig 171 (GenBank accession no. CD728315) was identified based on sequence homology to mammalian NK-lysin sequences available in GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). The coding sequence was amplified from total RNA of IELs by RT-PCR using the following restriction enzyme-anchored primers: *Nco* I anchored forward primer, 5′-CCCCATGGGGGAAGGGA-CAGCATGGCCGCTGCTC TC-3; *Not* I anchrored reverse primer, 5′-CGCGGCCGCGGGCTCTGGC CGTGCTCAG CCC-3′ (restriction sites are underlined). PCR products were digested with *Nco* I and *Not* I (Roche, Indianapolis, IN), ligated into the corresponding restriction endonuclease sites of pET32a(+) (Novagen, Madison, WI), and transformed into BL21(DE3) competent cells (Invitrogen, Carlsbad, CA). For expression in COS7 cells, pET32-NK-lysin was digested with *Kpn* I and *Not* I, gel-purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA), subcloned into pTriEx-4 under control of the CMV immediate early promoter (Novagen), and transformed into TOP 10 competent cells (Invitrogen).

2.2. Real-time RT-PCR

Oligonucleotide primers for NK-lysin and GAPDH quantitative RT-PCR are listed in Table 1. The levels of NK-lysin transcripts were normalized to those of GAPDH using the Q-gene program (Muller et al., 2002). Amplification and detection were carried out