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Research Paper

Construction of novel cytokine by fusion of chicken IL-2 signal peptide to mature chicken IL-15 and comparison of the adjuvant effects by DNA immunization against *Eimeria* challenge

Dexing Ma[a], Mingyang Gao[a], Jie Li[a], Chunli Ma[b,*], Guangxing Li[a,*]

[a] College of Veterinary Medicine, Northeast Agricultural University, Harbin, Heilongjiang, China
[b] College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang, China

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ABSTRACT

A novel fusion cytokine was constructed by replacing signal peptide (SP) of chicken IL-15 (ChIL-15) with SP of chicken IL-2 (ChIL-2). The fusion cytokine (NChIL-15) was cloned into the expression vector pcDNA3.1(+) to generate pcDNA-NChIL-15. An animal experiment was carried out to evaluate the adjuvant effects of NChIL-15 on DNA vaccine pcDNA-3-1E against *Eimeria acervulina* challenge. The mRNA profiles of ChIL-2 and ChIFN-γ in spleen were characterized by means of real-time PCR. The recombinant positive eukaryotic expression plasmid pcDNA-NChIL-15 were constructed successfully. The protective effects provided by co-immunization with 100 μg pcDNA-3-1E and 50 μg pcDNA-NChIL-15, measured by relative body weight gain (BWG), average lesion score in duodenum and oocyst decrease ratio, showed no significant difference with 50 μg pcDNA-ChIL-15 as an adjuvant on day 6 post infection (PI). However, chickens co-immunized with pcDNA-3-1E and pcDNA-NChIL-15 exhibited significant upregulated level of ChIL-2 and ChIFN-γ transcripts in spleen. Our original data suggests the constructed novel cytokine NChIL-15 could be a potential adjuvant used to enhance the immune protective effects, although the optimized dosage need to be explored further.

1. Introduction

Coccidiosis of poultry, an intestinal disease caused by the apicomplexan protozoan *Eimeria* spp., is responsible for major economic loss to the poultry industry worldwide (Williams, 1999; Shirley et al., 2004). Seven *Eimeria* species including *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella*, *Eimeria brunetti*, *Eimeria necatrix*, *Eimeria mitis* and *Eimeria praecox* have been recognized to infect chickens. Currently, the chemoprophylaxis or vaccines are the common practice to control coccidiosis (Williams, 2002a,b; Li et al., 2004, 2005). Live coccidian vaccines consisting of virulent and attenuated *Eimeria* parasites pose the risk of unintended infection under the immunosuppressive conditions associated with intensive rearing conditions (Lee et al., 2010). In addition, the long-term prophylactic drug usage promotes the development of drug resistance, causes the drug residue in animal food, and adds the great costs of new drug development, which have all stimulated research for alternate approaches to control coccidiosis (Williams, 2002a; Dalloul and Lillehoj, 2006). Recent immunological researches of DNA-based *Eimeria* vaccines are directed toward this goal (Lillehoj et al., 2000; Min et al., 2002; Ding et al., 2004; Du and Wang, 2005; Song et al., 2009; Shah et al., 2011). Apicomplexan protozoan covers complex

* Corresponding authors at: Northeast Agricultural University, No. 59, Mucai Street, Gongbin Road, Xiangfang District, Harbin, Heilongjiang 150030, China. Tel.: +86 451 5519 0723; fax: +86 451 5519 0363.
E-mail addresses: mdxneau@gmail.com, mdxneau2009@yahoo.com.cn (G. Li).

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parasite–host interaction where an array of specialized parasite molecules involved have long been considered as the potential candidates for vaccine-based therapies (Dowse et al., 2008; Sharman et al., 2010). 3-1E antigen is expressed in sporozoite and merozoite developmental stages of *Eimeria*. It has been shown that DNA vaccine carrying 3-1E gene could induce immune protection against coccidiosis (Song et al., 2000; Min et al., 2002; Ma et al., 2011). Administration of conventional chicken cytokines such as ChIL-2 and ChIFN-γ have been reported to enhance the host immune responses to *E. tenella* and *E. acervulina* induced by DNA vaccines (Kim et al., 1999; Göbel et al., 2003; Xu et al., 2006; Shah et al., 2011). ChIL-15 is reported in 2001, and the sequence of ChIL-15 cDNA contained a 187 amino acid open reading frame (ORF) and an unusually long (66 amino acids) signal peptide (SP), and the predicted amino acid sequences of ChIL-15 showed greater homologies with mammalian IL-15s (Lillehoj et al., 2001). ChIL-15 shares many biological functions with ChIL-2 which include stimulating T-cell proliferation and inducing activation of NK cells (Hilton et al., 2002). Several studies reported that coadministration of ChIL-15 as vaccine adjuvants along with *Eimeria* gene vaccines enhances the immunogenicity (Min et al., 2002; Lillehoj et al., 2005a; Ma et al., 2011). Considering its similar biological functions with ChIL-2, we aimed at further exploring the potential adjuvant effects of ChIL-15 in this study. It has been shown that the exchange of human IL-15 (hIL-15) SP coding sequence with that of hIL-2 increased cellular and secreted levels of hIL-15 protein 15- to 20-fold in COS cells, while hIL-2 transcripts with the hIL-15 SP generated 30- to 50-fold less hIL-2 protein than wild-type hIL-2 (Bamford et al., 1998). The previous study had proved that the long SP of hIL-15 was one of the factors that influenced the translation and especially influenced the effective secretion. The expression of hIL-15 protein was regulated not only in the transcription level but also in the translation level (Waldmann and Tagaya, 1999). So the aim of the present study was to construct a novel ChIL-15 cytokine (NChIL-15) by replacing SP of ChIL-15 with SP of ChIL-2, and compare the adjuvant effects by DNA immunization against *Eimeria* challenge.

2. Materials and methods

2.1. Animals, parasites and DNA vaccine

One-day-old specific pathogen-free (SPF) White Leghorn chickens purchased from Harbin Veterinary Research Institute (Harbin, China) were reared in a coccidian-free environment. Oocysts of *E. acervulina* Shanghai (SH) strain were kindly provided by Dr. Huang Bing (Shanghai Veterinary Research Institute, Shanghai, China). The oocysts were propagated in 3-week old SPF chickens, and the sporulated oocysts were stored in 2.5% potassium dichromate solution at 4 °C. The plasmid pMD18T-mChIL-15 harboring mature ChIL-15 (mChIL-15) gene fragment, pcDNA-3-1E and pcDNA-ChIL-15 were previously prepared in the Laboratory of Veterinary Immunopathology, Northeast Agriculture University, China.

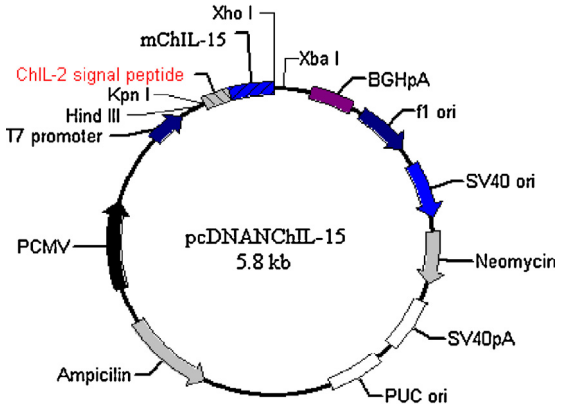

Fig. 1. Schematic illustration of the eukaryotic expression plasmid pcDNA-NChIL-15. The novel cytokine by fusion of ChIL-2 signal peptide to mature ChIL-15 (mChIL-15) was inserted between the *Hin*d III and *Bam*H I sites of pcDNA3.1(+) vector.

2.2. Construction of eukaryotic expression plasmids harboring NChIL-15

The SP coding sequence of ChIL-2 and the initial 23 bp of mChIL-15 protein-coding sequence were contained in two forward primers NChIL-15-F1 and NChIL-15-F2 (Table 1). The Splicing by Overlap Extension (SOE) technique was used to construct the novel cytokine NChIL-15. Briefly, the first round PCR amplification was done with the template pMD18T-mChIL-15 using primers NChIL-15-F2 and NChIL-15-R (Table 1). The amplified fragment was then used as the template to perform the second-round PCR amplification using primers NChIL-15-F1 and NChIL-15-R. The PCR product was subcloned into the *Hin*d III/*Bam*H I site of pcDNA3.1(+) vector (Invitrogen, USA) to generate plasmid pcDNA-NChIL-15 (Fig. 1). The positive plasmids were confirmed by nucleotide sequence analysis.

Table 1
Primer sequences with their corresponding PCR product size.

Primer name	Primer sequence (5′–3′)	PCR product (base pairs)	
NChIL-15-F1	5′-CCCAAGCTT*ATGATGTGCAAAGTACTGATCTTTGGCT-GTATTTCGGTAGCAATGCTAATGACTA*-3′ *Hind* III	420	432
NChIL-15-F2	5′-*GTATTTCGGTAGCAATGCTAATGACTA*CAGCTTATGGA AATCACTGTAAGTGGTCAGACGT -3′		
NChIL-15-R	5′-GACCTCGAGTCCCAGCTTGCGTATTTT-3′ *Xho* I		

Note: The SP coding sequence of ChIL-2 (italicized) and the initial 23 bp of mature ChIL-15 protein coding sequence (box display) were contained in two forward primers NChIL-15-F1 and NChIL-15-F2. The overlap sequence in the two forward primers was shown italicized and underlined. The primers NChIL-15-F2 and NChIL-15-R, and NChIL-15-F1 and NChIL-15-R were used for the two rounds of PCR amplification, respectively.