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Fasciola hepatica: Humoral and cytokine responses to a member of the saposin-like protein family following delivery as a DNA vaccine in mice ☆

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Abstract

The humoral and cellular responses to DNA vaccination of BALB/c mice with a novel antigen from the *Fasciola hepatica* saposin-like protein family (FhSAP-2) have been investigated. Two constructs were produced containing the FhSAP-2 DNA sequence, one intended for extracellular secretion of FhSAP-2 protein, and one expressing FhSAP-2 in the cytoplasm of a transfected cell. The constructs were tested in HEK 293T cells, with the secretory construct producing less detectable FhSAP-2 relative to cytoplasmic construct when observed by fluorescence. The size of expressed protein was confirmed by Western blot of cell lysate, but FhSAP-2 was undetectable in cell supernatants. Both, secretory and cytoplasmic constructs as well as FhSAP-2 recombinant protein were tested in mice. The antibody response elicited in mice vaccinated with the rFhSAP-2 induced high levels of IgG_1, IgG_2, and IgE as well as high levels of IL-10 and IFNγ indicating a mixed Th1/Th2 response. Vaccination of mice intramuscularly with the cytoplasmic FhSAP-2 construct resulted in a dominant IgG_{2a} isotype antibody as well as a dominant IFNγ cytokine, with significant IgE, IgG_1, and IL-10 responses also present, suggesting a mixed Th1/Th2 profile. Isotype and cytokine profiles elicited by the FhSAP-2 secretory construct were similar to those obtained with the cytoplasmic construct but at levels that were significantly lower. The results demonstrate that FhSAP-2 can be delivered as a DNA vaccine construct and induces a stronger Th1 response than the recombinant protein alone. This could result in an improvement in the immunoprophylactic potential of this candidate vaccine against *F. hepatica*.

Index Descriptors and Abbreviations: Fasciola hepatica saposin-like protein family; Trematode; Humoral and cellular response; DNA vaccination; FhSAP-2, *Fasciola hepatica* saposin-like protein; IL-10, interleukin 10; IFNγ, γ interferon; Th1, T-helper cell response type-1; Th2, T-helper cell response type-2; NK, natural killer; CMV, cytomegalovirus; DBB, denaturing binding buffer; PCR, polymerase chain reaction; LB, Luria broth; *E. coli*, *Escherichia coli*; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminotetracetic acid; DNA, deoxyribonucleic acid; PBS, phosphate-buffered saline; Con A, Concanavalin A; ELISA, enzyme linked immunosorbent assay; IFA, immunofluorescence assay; IM, intramuscular; TPA, tissue plasminogen activator; GST, glutathione *S*-transferase; CatL, cathepsin L; MHC, major histocompatibility complex; PMSF, paramethylsulfonilfluoride; HP, high performance

1. Introduction

Fascioliasis, caused by *Fasciola hepatica*, represents a recognized but unsolved agricultural problem responsible for economic losses estimated at around $3 billion US per year to rural agricultural communities and commercial producers worldwide, including the United

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States (Chen and Mott, 1990). Recent reports indicate that *F. hepatica* is also an important human pathogen and is increasingly recognized as causing significant human diseases; with 2–4 million people infected with this parasite from which, a conservative estimated indicate that 360,000 infected people are in the Department of La Paz of the Bolivian Altiplano (Bjorland et al., 1995; Chen and Mott, 1990). Animals become infected with the liver fluke following ingestion of vegetation contaminated with the infective metacercariae. The parasite excysts, and the juvenile form penetrates the intestinal wall and moves to the liver, where it causes extensive hemorrhage and perforation. After approximately 8 weeks the flukes begin to move into the bile duct, their final place of residence as adult flukes. Liver fluke infections can be highly pathogenic and can lead to severe morbidity and even death of the host. The flukicide triclabendazole is the most effective drug for controlling *Fasciola* (Overend and Bowen, 1995; Suhardono et al., 1991); however, the prohibitive cost of treatment prevents its wide use by rural producers in developing countries. Moreover, resistance to triclabendazole has been reported in sheep infected with *F. hepatica* (Overend and Bowen, 1995), suggesting that selection of resistant parasites may eventually compromise the effectiveness of this drug. Vaccines represent the most attractive long-term alternative to change this scenario.

Although many attempts have been made, no commercially available vaccine against fascioliasis exists. However, a number of *Fasciola* proteins have been evaluated as protein vaccines against fascioliasis, including glutathione *S*-transferase (GST, Morrison et al., 1996; Sexton et al., 1990), cathepsin L protease (Dalton et al., 1996; Wijffels et al., 1994), and fatty acid binding proteins (Estuningsih et al., 1997; Hillyer et al., 1987; Muro et al., 1997; Tendler et al., 1996). Results from these trials have demonstrated that each of these antigens, delivered as proteins, can induce a protective effect measured as either a reduction in worm burdens and/or a reduction in parasite egg output (Spithill and Dalton, 1998).

Recently, we described the molecular cloning and expression of a *F. hepatica* antigen termed FhSAP-2 that by its structural homology with a *F. hepatica* NK-lysin (Reed et al., 2000), three amoebapores of *Entamoeba histolytica* (Leippe et al., 1994), a porcine NK-lysin (Andersson et al., 1995), and several other related proteins (Bruno et al., 1995; Sano et al., 1992) falls in the saposin-like/NK-lysin protein family of *F. hepatica*. The rFhSAP-2 is also an 11.5 kDa *F. hepatica*/*Schistosoma mansoni* cross-reactive antigen that contains three-intrachain disulfide bonds and is expressed by the fluke at an early stage of infection of its mammalian definitive host (Espino and Hillyer, 2003). Recent vaccination studies of ours demonstrated that rabbits vaccinated with rFhSAP-2 and challenged with *F. hepatica* metacercariae had lower parasite burdens, fewer parasite eggs, and less liver damage than non-vaccinated controls (Espino and Hillyer, 2004). Because the protection induced by rFhSAP-2 is partial, the immunogenicity of this vaccine needs be improved. An attractive alternative could be the vaccination with nucleic acids in the form of a DNA construct.

In DNA immunization, a plasmid encoding an antigen is delivered into tissues such that the host expresses the protein in vivo (Wolff et al., 1990) and mounts an immune response (Tang et al., 1992). Analysis of the immune response generated by DNA immunization has shown that both the humoral and cellular arms of the immune system are activated by DNA vaccination. In particular, cytotoxic-T-lymphocytes are induced; such cells are poorly activated by immunization with protein vaccines in absence of toxic adjuvants (Donnelly et al., 1997). Thus, DNA vaccination induces a broader immune response, which may then enhance the efficacy of the vaccine. As a result, antigens, which may not induce a protective response as protein, may do so when delivered as a DNA construct; examples include DNA encoding the protein hsp65 from *Mycobacterium tuberculosis*, which protect immunized mice against subsequent challenge against *M. tuberculosis*. Immunization of mice with the protein encoded by the vaccine afforded no protection (Tascon et al., 1996). Results of this kind demonstrate that there are qualitative and/or quantitative differences in the immune responses generated by DNA vaccination compared to protein vaccination. The majority of reported antigens that have been examined as DNA vaccines have been derived from viral pathogens (Loirat et al., 2003; Mester et al., 1999; Smith et al., 2004; Tonegawa et al., 2003). However, DNA vaccines have also been tested for parasites, in particular malaria (Hoffman et al., 1997), *Leishmania* (Tapia et al., 2003), and flukes of the genus *Schistosoma* (Da'dara et al., 2002). As part of a program for optimization of our FhSAP-2 vaccine, and a prelude to DNA vaccine protection studies we describe herein the construction of DNA vaccines encoding FhSAP-2, we have evaluated the ability of these constructs to direct expression of FhSAP-2 in mammalian cells and we have assessed the utility of DNA vaccination as a means of inducing specific humoral and cytokine response to the vaccine candidate.

2. Materials and methods

2.1. Recombinant FhSAP-2 purification

Expression of rFhSAP-2 into the pBAD-HisB expression vector is induced using 0.02% L-arabinose for 4 h at 37 °C as previously described (Espino and Hillyer, 2003). After induction, the bacteria are harvested by