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Eg95: A Vaccine against Cystic Echinococcosis

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Abstract

Hydatidosis or cystic echinococcosis (CE) is caused by the larval stage of the tapeworm *Echinococcus granulosus*. This parasite is cosmopolitan in distribution and causes significant economic losses to the meat industry, mainly due to condemnation of edible offal. Echinococcosis treatment in human is very expensive as it requires extensive surgery or prolonged chemotherapy or use of both. In Asia and Africa, the vulnerable population of developing the disease is around 50 million. Office International des Epizooties (OIE) has recognized CE as a multi species disease. The parasite has acquired the capability to survive long time within the host due to a specific mechanism to evade the host immune system. A specific class of proteins known as secreted and membrane bound (S/M) proteins play key roles in the evasion mechanism. A total of 12 S/M proteins have been reported as immunodiagnostic and immunoprophylactic agents. Of these, Eg95 is a candidate antigen used for immunization of animals. Literature suggests that, Eg95 is a multi-gene family (Eg95-1 to Eg95-7) and exists in seven different isoforms. This chapter will describe minutely efficacy of Eg95 as a vaccine candidate based on animal trial and potentiality of other S/M proteins as immunodiagnostic antigen and immune evasion.

Keywords: *Echinococcus granulosus*, cystic echinococcosis, secreted and membrane-bound (S/M) proteins, vaccine

1. Introduction

Echinococcus granulosus is the etiological agent of cystic hydatid disease (CHD) *alias* cystic echinococcosis (CE). CE is a classic example of cyclozoonosis since for completion of its life cycle, the parasite exploits two vertebrate hosts. The disease is an important cause of human morbidity and mortality specifically among transhumance pastoralists [1] and of worldwide distribution. In case of human, the disease poses a significant burden on health system due to the high cost of treatment including surgery and chemotherapy. Moreover, the disease has negative impact on productive and reproductive performances of farm animals in terms of reduction in production of milk, meat, and wool [2]. The global report suggests that human CE infection ranges from less than 1 per 100,000 to more than 200 per 100,000 in rural population. Prevalence of infection depends on association of man and dog. For

zoonotic importance of the parasite and losses in livestock sector due to this infection, *Echinococcus* infection has been listed in the OIE Terrestrial Animal Health Code and is a notifiable disease for reporting by member countries and territories as per OIE code. In a most cited literature [2], disability-adjusted life years (DALYs) have been estimated as US \$193,529,740 (95% CI, \$171,567,331–\$217,773,513). An annual livestock production loss of US \$141,605,195 (95% CI, \$101,011,553–\$183,422,465) and possibly up to US \$2,190,132,464 has been estimated as well. This has initiated the need to formulate control strategies. Guidelines of control measures include prevention of access of dogs to livestock carcasses, treatment of dogs with suitable anthelmintic, thorough meat inspection and disposal as well destruction of infected viscera and vaccination with Eg95 vaccine (<http://www.oie.int/en/disease/echinococcosis>).

2. Secreted and membrane-bound (S/M) proteins for sustenance within host: a weapon of parasite against host environment

We felt it judicious that, before we discuss about an Eg95 vaccine and secreted and membrane-bound (S/M) proteins of *E. granulosus*, let us brief S/M proteins in general since these biomolecules is the main functionaries for spicing up the immune system. As a general rule, helminth infection is a chronic infection because these harmful creatures survive in the host by its unique feeding strategies and evasion/muting of the host immune system. The weapon they use for winning the battle against the host immune system is S/M proteins (S/M) [3]. These unique biomolecules (S/M proteins) are associated with multitudinous activities such as

S. No.	Name of the parasite	Name of S/M protein	Activity
1.	<i>Schistosoma mansoni</i>	Chemokine binding Protein	Neutralization of chemokine activity (CXCL8, CCL3, CX3CL1, CCL2, CCL5); inhibits neutrophil migration [5] et al.
2.	<i>Fasciola hepatica</i>	Helminth defense molecule-1	Molecular mimicry of antimicrobial peptides, binds to LPS and reduces its activity; prevents acidification of the endolysosomal compartments and antigen processing; prevents NLRP3 inflammasome activation [6, 7].
		Fatty acid binding protein	Suppresses LPS-induced activation via binding and blocking of CD14; induction of alternatively activated macrophages [8]
		TGF-like molecule	Ligates mammalian TGF-b receptor (albeit with a lower affinity) and induces IL-10 and Arginase in macrophages [9]
3.	<i>Brugia malayi</i>	Asparaginyl-tRNA synthetase	Structural homology to IL-8, binds IL-8 receptors CXCR1 and CXCR2; chemotactic for neutrophils and eosinophils; induced regulatory responses and IL-10 in a T cell transfer model of colitis [10]
		TGF-b homolog-2	Ligates mammalian TGF-b receptor and suppresses T cell responses [11]
		Abundant larval transcript	Inhibitor of IFN- γ signaling [11]

S. No.	Name of the parasite	Name of S/M protein	Activity
4.	<i>Necator americanus</i>	Metalloproteinases	Causes proteolysis of eotaxin, but not of IL-8 or eotaxin-2 [12]
		<i>Ancylostoma</i> secreted protein-2	Binding to CD79A on B cells, downregulation of lyn,PI3K, and BCR signaling [13]
5.	<i>Necator brasiliensis</i>	Acetylcholinesterase	Degrades acetylcholine, reduces neural signaling; induces proinflammatory cytokines with diminished type 2 cytokines in transgenic AChE-expressing trypanosome infection [14]
6.	<i>Echinococcus multilocularis</i>	T cell immunomodulatory protein	Induces release of IFN- γ from CD4+ T cells in vitro [15]

Table 1.
Secreted and membrane-bound (S/M) protein of some important helminths of man.

penetration and establishment in the host; modulation of the host immune system and uptake of metabolites from the host [4]. Due to continuous exposure of S/M proteins with the host immune system, some of the candidate biomolecules exhibit immunodiagnostic or immunoprophylactic activities. Here we provide some of the examples of S/M proteins of helminths involved in survival strategies within the challenging host environment (**Table 1**).

3. Introducing S/M proteins of *E. granulosus*

This is better to understand about S/M proteins of *E. granulosus* because S/M proteins are used for control of the disease. We will not make the list long for the convenience of the readers and will zoom down to only four of them. Out of four, two are diagnostic antigens (Antigen B and Antigen 5) and two (Eg95 and 14-3-3 protein) are of immunoprophylactic value. Under this subheading, “Introducing S/M proteins of *E. granulosus*,” we will brief on three S/M proteins except for Eg95, which we will elaborate later in this chapter.

3.1 Antigen B

Antigen B (AgB) is an oligomeric thermostable lipoprotein. The antigen was first described by Oriolet al. [16]. The protein was separated from the hydatid fluid by size-exclusion chromatography as a 160 kDa protein. This protein is abundantly present in *E. granulosus* hydatid fluid. AgB has already been characterized as an immunomodulatory protein, capable of inducing a permissible immune response to the parasite development. This protein is an oligomeric lipoprotein composed of 8 kDa related subunits [17]. Molecular studies revealed that AgB is encoded by a gene family with five major gene clusters, namely AgB1 [18], AgB2 [19], AgB3 [20], AgB4 [21], and AgB5 [22]. Bhattacharya et al. [23] carried out an exhaustive study on AgB families of Indian isolates of *E. granulosus*. AgB1 revealed homology to *Echinococcus canadensis* (G8) and *E. granulosus sensu stricto* (G1/G2). AgB3 was homologous to *Echinococcus ortleppi* (G5) alias cattle strain. Predicted amino acid sequence of AgB4 was homologous to bovine isolates identified earlier.

3.2 Antigen 5

Antigen 5 (Ag5) is a major antigen of *E. granulosus*. This has been identified to have immunodiagnostic value. This is also known as Capron's arc 5 antigen because this antigen formed an arc by immunoprecipitation reaction with the serum samples of patients suffering from the disease [24]. Progress in the molecular characterization of Ag5 has been limited. Ag5 is a thermo labile protein. By size-exclusion chromatography, this has been eluted as 60–70 kDa protein [25]. By discontinuous gel electrophoresis, this has been found that, in native form Ag5 has a major component (67 kDa) and a minor component (57 kDa). Under reducing condition, Ag5 dissociates in two major peptides of 38 kDa and 22 kDa [26, 27]. Ag5 is a major component of hydatid cyst fluid, which is suggestive of its role as a key molecule in the biology of *E. granulosus*. This antigen plays an important role for development and sustenance of parasite within intermediate host till the transmission of the parasite to the definitive host [25].

3.3 14-3-3 Protein

14-3-3 proteins are a group of molecules that are of different isoforms. These molecules are distributed in a broad range of cells in all eukaryotic organisms. These groups of molecules are highly conserved in nature and were first reported from brain tissue. In recent time, they were found to play crucial roles in eukaryotic cell cycling. 14-3-3 Proteins bind with specific ligands containing phosphorylated serine/threonine residues to form homo- and heterodimer complexes, and this process is regulated by phosphorylation. Several mechanisms of action of 14-3-3 proteins have been reported; such as induction of conformational change of target molecules, the physical occluding of specific features, the scaffolding, and the change of cellular localization. The 14-3-3 proteins are acidic protein with a relative molecular weight of 30 kDa. This group of proteins show 50% identity within and across species, small (30 kDa), acidic proteins that show about 50% amino acid identity both within and across species. In mammals, seven isoforms have been identified (b-beta, c-gamma, f-zeta, r-sigma, e-epsilon, g-eta, and s-tau). The 14-3-3f isoforms also termed as *E14t* have been identified in *E. multilocularis* (Gen Bank accession no. U63643) and *E. granulosus* (Gen Bank accession no. AF20790). 14-3-3 Proteins have been found in metacestode, oncospheres, and protoscoleces of *E. multilocularis*. In *E. granulosus* these proteins have been found in protoscoleces and have potential role in the biology of this parasite. In one of the studies from India by Pan et al. [28], this was found that, there was over expression of 14-3-3 protein (zeta isoform) in drug induced protoscoleces of *E. granulosus* comprised to control group. This particular finding indicated that this protein may be used as biomarker in drug-induced protoscoleces.

4. Eg95: a brief introduction

Concept to develop Eg95 (16.6 kDa protein) was initiated on the basis of identification of individual oncosphere components that stimulate host-protective immune responses in sheep. Marathon effort was made on this aspect by Heath and Lawrence [29] on identification and characterization of host protective antigen hitherto its testing in vaccine trial. For raising the hyperimmune sera, the group of workers used whole *E. granulosus* oncospheres; non-denatured oncosphere extract treated by freezing, thawing and sonication; extract of immature oncospheres; denatured extract of oncospheres. By using an Geenzyme linked immunoblot assay

(EITB), a doublet immunodominant peptide of 23 kDa and 25 kDa was identified. The fraction that contained the 23 and 25 kDa molecules was able to stimulate protection in sheep. These studies suggested that one or both of the 23 and 25 kDa somatic oncospherical antigens of *E. granulosus* were host-protective components even after denaturation. This was the first indenture of native Eg95 vaccine preparation.

4.1 Isoforms of Eg95 antigen

A protein isoform is known as protein variant. They rise from a single gene or a gene family. Protein isoforms are formed due to alternative splicings or variable use of promoter or sometimes may be due to post-transcriptional modification of a single gene [30, 31]. Agene family of Eg95 for common sheep strain (G1) of *E. granulosus* has been described by Chow et al. [32]. They is Eg95-1 (Gen Bank ID: AF134378), Eg95-2 (AF 199351-52), Eg 95-3 (AF199353), Eg 95-4 (AF199349), Eg 95-5 (AF 199350), Eg 95-6 (AF199347), and Eg 95-7 (AF199348). Out of seven members of Eg95 family, Eg95-7 is pseudo gene.

Based on phylogenetic analysis (**Figure 1**), this was evidenced that Eg95 gene family is having two clusters (Eg95 1-4 and Eg 95 5-6). From India, an elaborative study was done to know the genetic diversity of Eg95 [33]. A total of 24 isolates collected from cattle, buffalo, sheep, goat, human, and dog were analyzed. Genotypic characterization of these isolates revealed that all isolates belonged to G1 genotype except one buffalo isolate, which was characterized as cattle strain (G5). Phylogenetically, the Eg95 coding gene characterized from Indian isolates of *E. granulosus* belongs to the Eg95-1/Eg95-2/Eg95-3/Eg95-4 cluster.

Figure 1.
Phylogenetic analysis of Eg95 family based on predicted amino acid sequence (Eg 95-7 could not be included since it is a pseudo gene).

4.2 Eg95 as vaccine

As a vaccine Eg95 is very effective to control *E. granulosus*. This is known that, after vaccination, there is antibody mediated and complement-mediated lysis of invading oncospheres. Initially let us mention a trial on vaccine, which was conducted in Rio Negro, Argentina. In the trial, lambs were vaccinated with Eg95 prepared by University of Melbourne, Australia. Primary immunization was done at 30 days of age and booster dose was applied at the age of 60 days. Final and penultimate dose was provided to the sheep at the age of 1–1.5 years. Immunological evaluation of vaccinated animals confirmed the presence of IgG antibody response, which persisted for a period of 5 years [34, 35]. Like sheep, Eg95 vaccine has been tested in cattle and goat. In cattle, after successful immunization, immunity persisted up to 12 months. This vaccine has been found safe and effective in pregnant sheep and cattle as well as in young small ruminants. A further elaborative study

indicated that, after immunization with Eg95 vaccine in pregnant animals, there is passive transfer of maternal antibody response, which persisted for 3 months in lambs and 5 months in calves [36]. In a very recent note, immunoinformatics analysis and molecular docking tool have been employed to screen the antigen epitopes of *E. granulosus* with a novel purpose to design multi-epitope vaccine comprising of T and B cell epitopes. The multi-epitope vaccine was able to activate B lymphocytes to produce specific antibodies, which were predicted to confer protection in human being against the metacestode infection. This was further predicted that this multi-epitope vaccine was able to activate T lymphocytes and capable of immunological clearance. Further, four CD8⁺ T cell epitopes and four B cell epitopes of *E. granulosus* tegument antigen were also predicted. Ultimately multi-epitope vaccine was predicted with the addition of linker protein [37].

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