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# *Echinococcus granulosus* antigen B: A Hydrophobic Ligand Binding Protein at the host–parasite interface

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A B S T R A C T

Lipids are mainly solubilized by various families of lipid binding proteins which participate in their transport between tissues as well as cell compartments. Among these families, Hydrophobic Ligand Binding Proteins (HLBPs) deserve special consideration since they comprise intracellular and extracellular members, are able to bind a variety of fatty acids, retinoids and some sterols, and are present exclusively in cestodes. Since these parasites have lost catabolic and biosynthetic pathways for fatty acids and cholesterol, HLBPs are likely relevant for lipid uptake and transportation between parasite and host cells. *Echinococcus granulosus* antigen B (EgAgB) is a lipoprotein belonging to the HLBP family, which is very abundant in the larval stage of this parasite. Herein, we review the literature on EgAgB composition, structural organization and biological properties, and propose an integrated scenario in which this parasite HLBP contributes to adaptation to mammalian hosts by meeting both metabolic and immunomodulatory parasite demands.



## 1. Introducing *Echinococcus granulosus* antigen B: an abundant HLBP of a tissue-dwelling larvae

*Echinococcus* (Phylum Platyhelminthes, class Cestoda, family Taeniidae) is a tapeworm parasite, which has a complex, indirect life-cycle comprising different mammalian hosts and parasite stages [1]. The definitive and intermediate hosts harbor the adult worms and the larvae respectively, and have a predator–prey relationship between them, which ensure to uphold the parasite life cycle. The taxonomy of *Echinococcus* spp. has been a controversial issue for decades. Recently, molecular techniques and phylogenetic analysis allowed the identification of new species of *Echinococcus* morphologically indistinguishable. Particularly, some of the traditionally *Echinococcus granulosus* strains or genotypes (named G1–G10) are now recognized as different species within *Echinococcus* genus. Thus, nine valid species of *Echinococcus* are accepted at this time: *Echinococcus multilocularis*, *Echinococcus vogeli*, *Echinococcus oligarthra*, *Echinococcus shiquicus*, *E. granulosus sensu stricto* (traditionally named G1–G3), *Echinococcus equinus* (traditionally named G4), *Echinococcus ortleppi* (traditionally named G5), *Echinococcus canadensis* (traditionally named G6–G10) and *Echinococcus felidis* [2,3]. Therefore, the originally defined *E. granulosus* is a species complex; the term *E. granulosus* sensu lato (*E. granulosus* s. l.) can be used to group the different species and strains comprising *E. granulosus* complex. The larval stage of *E. granulosus s. l.* is the causative agent of cystic echinococcosis (hydatid disease) in a wide range of mammalian species (mainly domestic ungulates) as well as humans. It establishes and gradually grows within host viscera (mainly liver and lungs). Fig. 1 illustrates the cyst structure; it is a unilocular fluid-filled bladder limited by a two-layer wall [1]. The cyst external layer is an acellular, carbohydrate rich material permeable to macromolecules, which is synthesized by the underlying cellular layer called germinal (GL). The latter is a syncitium,

*Abbreviations:* DCs, dendritic cells; EgAgB, *Echinococcus granulosus* antigen B; GL, germinal layer; HF, hydatid fluid; HLBP, Hydrophobic Ligand Binding Protein; LL, laminated layer; SEC-MALLS, size exclusion chromatography coupled to multi-angle laser light scattering

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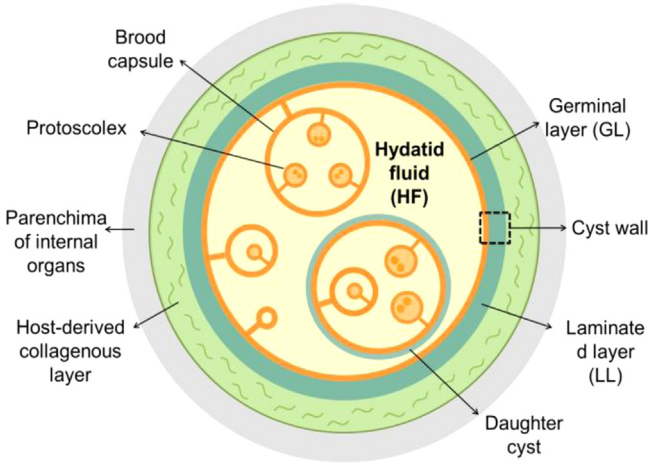


**Fig. 1.** Diagrammatic structure of hydatid cyst. *Echinococcus granulosus* Hydatid or Metacestode (larval stage) is a fluid-filled unilocular cyst which sets in intermediate host viscera (mainly liver and lungs). The cyst wall is formed by laminated (LL) and germinal (GL) layers. The laminated or external layer is an acellular, carbohydrate rich material synthesized by the underlying cellular GL. This internal layer is a syncytium, which generates by asexual reproduction larval worms (protoscoleces) in brood capsules and daughter cysts. The fluid content is called hydatid fluid (HF) and collects larval excretion/secretion products from protoscoleces and GL. The whole structure is surrounded by a collagenous, host-derived layer, which is consequence of the resolution of host inflammatory reaction. Hydatid surrounded by the host-derived layer is known as hydatid cyst.

which generates, by asexual reproduction, parasite larval worms called protoscoleces. A collagenous, host-derived layer surrounds the whole structure, which is a consequence of the resolution of the host inflammation. The fluid inside the cyst (HF) is a complex mixture of parasite secretory–excretory products and host-derived molecules.

One of the major molecules synthesized in large amounts by the cyst is a lipoprotein named antigen B (EgAgB), considered the most specific and relevant *Echinococcus*-genus antigen for human serodiagnosis (reviewed by [4]). EgAgB is ubiquitous within the cyst, being present in HF, the GL and protoscoleces [5–7]. However, the presence of EgAgB in host infected viscera and circulation has not been well documented [8], but the fact that the host develops a strong antibody response against this antigen proves that it reaches the host [9]. The mechanisms used by the larva to transport EgAgB towards host tissues are completely unknown, making uncertain whether EgAgB is present at the host–parasite interface throughout the infection or just at particular time points. The mechanism of EgAgB uptake by host cells is also unknown.

EgAgB was initially purified and characterized on the basis of its antigenic properties. It was described as the thermostable and self-aggregating antigen of HF that accounts for one of the two major precipitation bands obtained by immunoelectrophoresis of HF with sera from hydatid patients [5]. An apparent molecular weight of 160 kDa was initially estimated for EgAgB on the basis of size exclusion chromatography (SEC) [10], but a recent study by SEC coupled to light scattering (SEC-MALLS) indicates that it exists in solution as a heterogeneous population of particles with most species showing an average molecular mass of about 230 kDa [11]. Since immunogenicity properties are associated to protein more than to lipid moieties, the biochemical characterization of EgAgB deepened firstly on its protein composition, and involved the identification of the major immunodominant subunits and epitopes [12–15]. These studies were of foremost importance in terms of EgAgB diagnostic value, but in terms of its chemical and functional characterization, they lost sight of the lipoprotein nature of the molecule. More recently, the identification of the genes encoding the EgAgB protein moiety and their homologous in other cestodes, revealed that these proteins share the ability to bind hydrophobic ligands, constituting a novel cestode-specific family of lipid binding proteins referred to as HLBPs (Hydrophobic Ligand Binding Proteins) [16]. This finding drew our attention to the EgAgB lipid moiety; *in vitro* studies had shown that delipidated EgAgB apolipoproteins were able to bind fatty acids [17], but the identity of the physiological ligands was unknown. We characterized then the native lipid components of EgAgB [11], from which a novel picture for the structural organization of this antigen emerged, having an impact on previous ideas about its biological function. Thus, in the following sections we first introduce the current knowledge from genomic, proteomic and lipidomic studies on EgAgB, and then discuss renewed ideas about the likely role of this antigen in the biology of *E. granulosus s.l.*

## 2. The apolipoprotein component of EgAgB

As stated above, EgAgB was described as a lipoprotein component of HF in 1971 [5]. Lots of effort were made during the following four decades to purify and characterize the EgAgB apolipoprotein component at the gene and protein levels. Proteomic studies were entirely performed using HF as a source of EgAgB, while the cyst GL and protoscoleces have been mostly used as a parasite material for genomic studies. In spite of these efforts, some questions regarding both genomic and proteomic characterization of EgAgB still remain to be answered.

The heterogeneity of EgAgB apolipoprotein component is one of its remarkable features arising from the fact that this component is encoded by a polymorphic multigene family [18,19]. Early studies based on the amino acid sequence of an EgAgB member indicated that this antigen was orthologous of cestode-specific lipid binding proteins with affinity to hydrophobic ligands (referred as HLBP), including immunodominant *Taenia* antigens [20]. Later, a phylogenetic study using additional data from *Taenia solium* antigens confirmed these observations [16] Interestingly, independent gene expansions in these families appear to have occurred in different cestode lineages, giving rise to species and gene-specific monophyletic clades [21,22]. *E. granulosus s.l.* antigen B family comprises five clades named EgAgB1–EgAgB5 [18,23–26]. Until now, the number of copies of EgAgB genes has been a subject of debate. The existence of 10 EgAgB distinct genes (including four and three different genes corresponding to the EgAgB3 and EgAgB4 clades, respectively) was proposed on the basis of the characterization of *E. granulosus s.l.* isolates from different geographic origins [21]. However, a recent analysis of EgAgB loci in the current assembly of *E. granulosus s.s* (G1) genome revealed the presence of seven EgAgB loci clustered on a discrete region of the genome, with three slightly differing copies of EgAgB3 and only one copy of each of the others [27,28]. Outside this cluster only an EgAgB related gene in *E. granulosus s.s* (G1) was identified [28]. The antigen B loci with seven genes arranged in tandem were also identified in *E. multilocularis* genome, while two identical copies of antigen B related gene were identified [27,28]. Additional EgAgB genes in extra-chromosomal DNA arrays cannot be ruled out since they might have slipped the genome assembly process [27]. It is also possible that some variants of EgAgB previously sequenced may reflect genetic polymorphism in this gene family. All EgAgB genes have common structural features. All of them possess two exons, the first one encodes a signal peptide for protein secretion while the second one encodes the mature 8 kDa polypeptide (65–71 amino acids long), traditionally referred to as EgAgB8/1–EgAgB8/5. When comparing the amino acid sequences within EgAgB family, members of the EgAgB1, EgAgB3 and EgAgB5 clades were found to be more similar among each other compared to members of the EgAgB2 and EgAgB4 clades and *vice versa* (Fig. 2A).

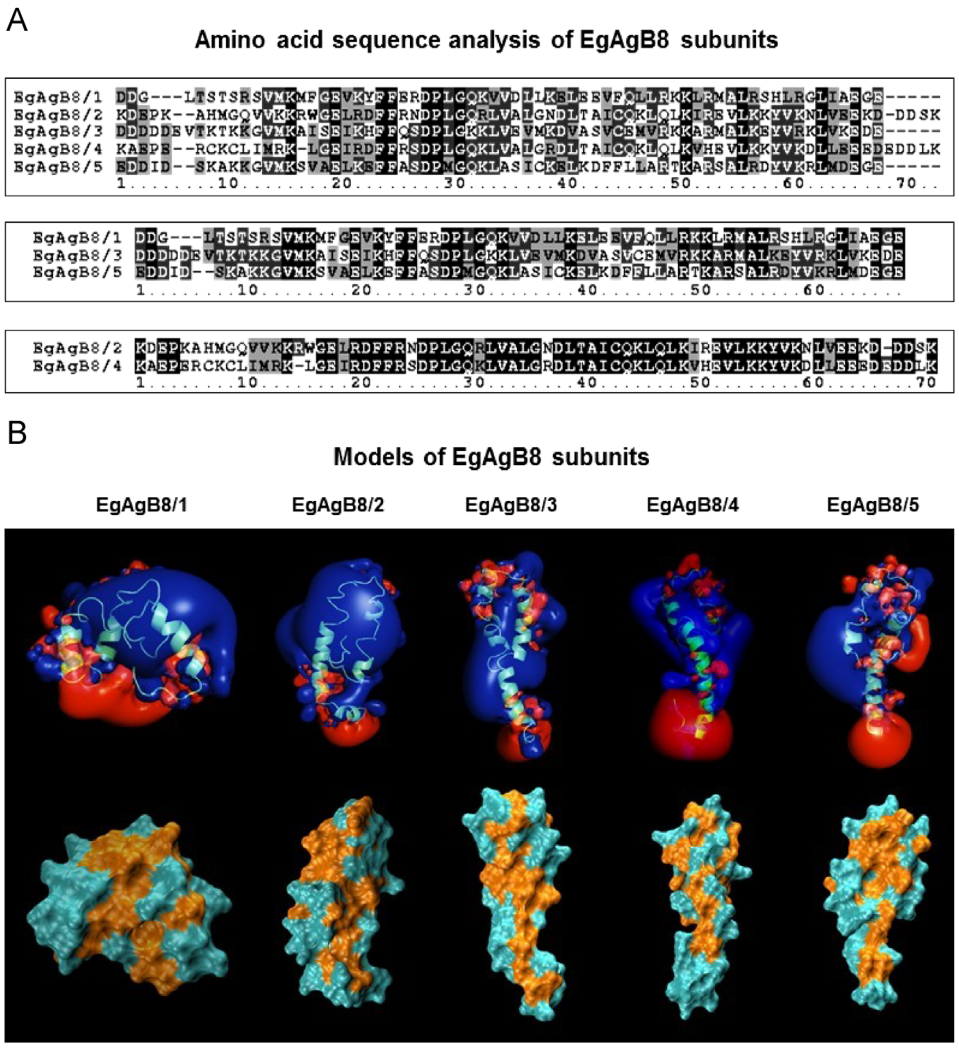


**Fig. 2.** Amino acid sequence analysis and models of EgAgB8 subunits. A) Alignment and analysis of EgAgB8 subunits were undertaken using Biology Workbench 3.2 (University of Illinois, National Center for Supercomputing Applications, USA). Alignment of amino acid sequences of the mature peptides of EgAgB8/1, EgAgB8/2, EgAgB8/3, EgAgB8/4 and EgAgB8/5, using ClustalW from Biology Workbench 3.2. Fully conserved, identical and similar (conservative substitutions) amino acids are highlighted on black, dark gray and gray, respectively. Comparison between EgAgB8 mature peptides shows that EgAgB8/1, EgAgB8/3 and EgAgB8/5 are more closely related between them than with EgAgB8/2 and EgAgB8/4 and *vice versa*. Accession numbers: EgAgB8/1: AAD38373, EgAgB8/2: AAC47169, EgAgB8/3: AAK64236, EgAgB8/4: AAQ74958 and EgAgB8/5: BAE94835. B) Models of the plausible structures of the subunits of EgAgB8. In the first row the electrostatic profile of the apolipoproteins is shown with positive surfaces in blue (dark gray) and negative in red (light gray). The hydrophobic characteristics of the proteins are depicted in the second row with the non-polar amino acids in cyan (light gray) and the hydrophilic ones in orange (dark gray). According to the models, the possibility of interaction with ligands and the aggregation between monomers are evidenced, perfectly consistent with experimental data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Regarding the secondary structure of EgAgB apolipoproteins, studies based on circular dichroism and optical rotatory dispersion spectra of the native lipoprotein suggest a helical content of about 50% [10]. Later on, analysis by circular dichroism of immunopurified EgAgB suggested that over 65% of its secondary structure corresponds to α-helix [14]. On the other hand, a 40% of α-helical content was estimated in similar studies using recombinant EgAgB subunits [29]. Differences in the percentage of α-helical content between native and recombinant subunits may be due to dissimilar protein folding and/or post-translational modifications or non-covalent interactions with their hydrophobic ligands. Interestingly, under thermal denaturalization most of the secondary structure of EgAgB subunits is retained, which could explain the thermostability of the immunogenic properties of the native antigen. In agreement with these results, *T. solium* HLBPs have also been predicted as α-helix rich proteins using conventional tools for secondary structure modeling [22].

Previously, in an attempt to characterize the structural properties of the subunits EgAgB1, EgAgB2 and EgAgB3 from EgAgB apolipoproteins, elongated models of three dimensional structures were proposed [29]. Now we have refined the structural model providing plausible tertiary structures of EgAgB apolipoproteins using homology modeling. Multiple sequence alignment were obtained and used as reference for the structural prediction of the proteins by homology modeling. Based on structural templates, conformations of each apolipoprotein were then constructed using Modeller9v3 and electrostatic profiles were predicted using Adaptive Poisson–Boltzmann Solver (APBS) program. According to the models, all apolipoproteins showed predominantly alpha helical structures in agreement with the previous data mentioned above (Fig. 2B). Moreover, the position of hydrophilic and hydrophobic amino acids defines pocket-like regions where hydrophobic ligands could interact with the proteins, and a partial charge distribution showing a plausible electrostatic profile for molecular aggregation. Interestingly, in EgAgB8/1, the configuration recalls an acyl-CoA binding protein-like structure with the hydrophobic pocket surrounded by a couple of helices [30]. In addition, this model preserves the geometry of the immunogenic region ($^{23}$EVKYFFER$^{30}$) where key amino acids are exposed to the solvent, in agreement

with those previously reported [14]. Since the amino acid sequences of the different subunits have a high grade of homology, mainly between EgAgB8/1, EgAgB8/3 and EgAgB8/5 (Fig. 2A), further analysis is necessary to determine if all subunits can adopt a similar fold such as that proposed for EgAgB8/1.

Another interesting feature of EgAgB apolipoproteins is their ability to form oligomers, which agrees with the electrostatic profile predicted by tertiary structure modeling. Early studies analyzing EgAgB composition by SDS-PAGE showed a regularly spaced group of bands with apparent molecular sizes of 8, 16, 24 and 32 kDa, which became less abundant as their molecular size increased (Fig. 3A) [31].

Demonstration that these high molecular weight components are built from the 8 kDa subunits came from the analysis of the subunit composition of native EgAgB, in which the amino acid sequences of tryptic peptides isolated from the 8, 16 and 24 kDa bands were compared with that of EgAgB8/1 and EgAgB8/2 monomers [32]. The mechanisms involved in oligomerization have not been elucidated yet, but the involvement of covalent disulfide bonds can be ruled out because the bigger oligomers also occur in SDS-gel run under reducing conditions [31]. Interestingly, recombinant EgAgB8/1, EgAgB8/2 and EgAgB8/3 subunits were also found to be capable of self-assembling generating homo-oligomers of 16 and 24 kDa, but also high-order oligomers [29,33]. Differences in the oligomerization properties of recombinant subunits were observed: rEgAgB8/2 and rEgAgB8/3 oligomers showed greater structural stability than rEgAgB8/1 oligomers, and oligomerization of rEgAgB8/3 lead to a more heterogeneous population of compact oligomers according to their size distribution and resistance to proteolysis [33]. Finally, it is worth to mention that oligomerization may represent a footprint-like feature of native *Echinococcus* antigen B, since it does not seem to be shared by other HLBPs [34].

## 3. The hydrophobic ligands of EgAgB apolipoproteins

The lipoprotein nature of EgAgB was first described by Oriol and collaborators [5] on the basis of the high content of lipids non-covalently bound to the protein component (lipids were mostly removed by organic solvent extraction); this was a logical conclusion even though in this work these properties were measured in an enriched EgAgB preparation obtained from HF (containing another antigen relevant for hydatid disease diagnosis, later to be known as antigen 5). Thereafter, as a result of the identification of EgAgB as a HLBP member, the lipid binding properties of EgAgB were studied *in vitro* using fluorescent fatty acid analogs [17]. Similarly to other HLBP members [16,20,22,35–38], fatty acids were found to be candidates for EgAgB hydrophobic ligands since the native antigen as well as the recombinant EgAgB8/1 and EgAgB8/2 apolipoproteins bound a fluorescent palmitic acid analog (16-AP) with high affinity. However, in comparison with other HLBPs, *in vitro* binding studies suggested that EgAgB ligands may include a more limited set of lipids since EgAgB apolipoproteins did not bind the undecanoic fatty acid analog DAUDA, ANS or DACA [17]. In any case, this experimental approach could not reveal the identity of the lipids physiologically bound to EgAgB apolipoproteins. The composition of the native lipid moiety of EgAgB was studied by Obal and collaborators [11] using EgAgB purified by immunoaffinity from HF. This study confirmed that EgAgB is a complex macromolecule with a high content of lipids, which reaches between 40% and 50% of the total mass. Analysis of EgAgB lipid component by high performance thin layer chromatography revealed that this component is highly heterogeneous, containing a wide mixture of lipid classes ranging from highly hydrophobic lipids (mainly triacylglyceroles and sterol esters) to a variety of phospholipids (mainly phosphatidylcholine) (Fig. 3B). In addition, gas liquid chromatography analysis showed that 16:0, 18:0

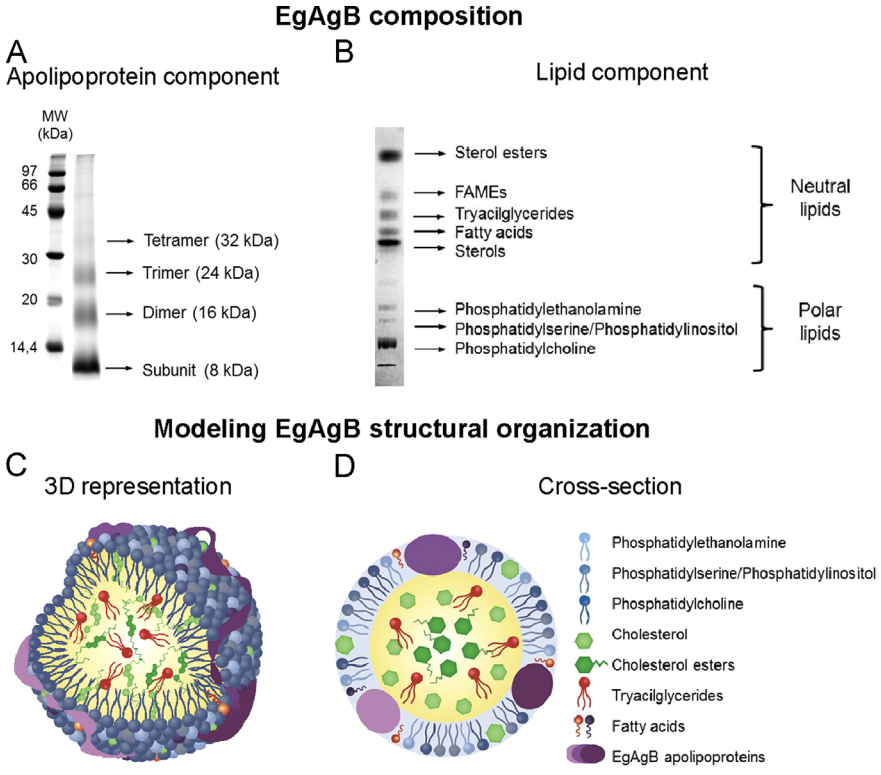


**Fig. 3.** EgAgB composition and structural organization. A) Protein composition by SDS-PAGE: EgAgB apolipoprotein subunits (8 kDa) form higher molecular weight oligomers (16, 24 and 32 kDa). B) Lipid class composition by high performance thin layer chromatography. A wide variety of neutral and polar lipids is present in EgAgB. C) 3D representation of the structural organization proposed for EgAgB. D) Schematic representation of an EgAgB lipoprotein cross-section according to the proposed model. Notice that these representations illustrate putative similarities between EgAgB and vertebrates plasma lipoproteins; nucleus contains most hydrophobic lipids surrounded by a layer of phospholipids where apolipoproteins are embedded.

and 18:1($n-9$) were the most abundant fatty acids present in the EgAgB lipid moiety. Altogether these observations demonstrated that EgAgB requires adopting a very well organized structure to accommodate high amounts of lipid components in a single particle, leading to propose similarities with animal lipoproteins found in both invertebrate hemolymph and vertebrate plasma [39]. A representation of this structural organization is shown in Fig. 3C: the most hydrophobic lipids would be sequestered in a central core, surrounded by an external hydrophilic shell that contains the EgAgB apolipoproteins and amphipathic lipids (mostly phospholipids and unesterified cholesterol). A structure like this could explain the heterogeneity observed when analyzing the molecular mass of EgAgB by SEC-MALLS, because these lipoprotein particles could accommodate variable amounts of lipids. Moreover, taking into account the lipid: protein mass ratio and the average molecular mass, the native EgAgB particle would be similar to the smallest HDL (referred as $HDL_3$, [40]) and, it would expose around a dozen of apolipoproteins on its surface. The exposure of several apolipoproteins would allow the establishment of multiple interactions with their molecular targets that increases the avidity of the particle interactions and the strength of the signals derived from them. Until now, there is no information on the lipid composition of other HLBPs; this is an important piece of information to determine whether the hydrophobic ligands bound to EgAgB apolipoproteins are a feature of this family.

## 4. Novel hypothesis for the role of EgAgB in parasite biology

Cestodes, including *Echinococcus* spp., have evolved shaped by the environment of the host organs where they lodge, which offers an abundance of diverse nutrients, but a low oxygen tension. Among other characteristics, cestode metabolism has been strongly conditioned by this microaerobic environment. Lipids cannot be used as fuel for energy production at low oxygen tension, and the availability of a variety of nutrients would allow the uptake of host-derived lipids as building blocks to fulfill anabolic demands (reviewed by [40]). This scenario correlates with the fact that cestodes have lost both degrading and biosynthetic pathways for common fatty acids and sterols. In particular, no enzymes for either fatty acid anabolism or squalene synthesis (the precursor of the whole family of animal sterols) have been found in the *E. granulosus s.s.* transcriptome (data base http:/www.compsybio.org/partigene/) or *Echinococcus s.s* genome [28,41]. In agreement with these observations, early metabolic studies demonstrated that sterol synthesis in *E. granulosus s.l.* is interrupted at the farnesyl or nerolidol pyrophosphate level and that the hydatid incorporates radioactively labeled, host-derived cholesterol during experimental infection in mice [42,43]. Thus, the significant amount of fatty acids, triacylglycerols and sterols that EgAgB carries are, in all likelihood, host derived, supporting the idea that this HLBP could have an important role in the uptake and transport of host-derived lipids to parasite tissues. The underlying molecular mechanisms are so far unknown, but some hypothesis may be considered (Fig. 4). Firstly, uptake of host lipids by EgAgB probably requires a direct interaction with host soluble lipoproteins or cell membranes, since lipids exist in association with carrier and transfer proteins in a physiological milieu. EgAgB interactions with host lipoproteins have not been explored, but it may occur similarly as between vertebrate plasma lipoproteins. This lipid exchange may proceed at host tissues or even inside the hydatid cyst, since apolipoprotein A-I was found to be present in HF [44], suggesting that HDL goes across the GL by some unknown pathway (Fig. 4, solid black arrow). Alternatively, uptake of lipids from host cells cannot be discarded because EgAgB has been shown to interact *in vitro* with some resident and inflammation-recruited immune cells such as dendritic cells (DCs) and neutrophils [45–47]. The receptors involved and the fate of EgAgB following cell interactions have not been examined. Secondly, to deliver host-derived lipids to parasite tissues EgAgB should interact with parasite receptors. In this sense it is important to note that in *E. multilocularis* and *E. granulosus s.s.* genomes, antigen B gene cluster is flanked by *EmLDLR* or *EgLDLR* genes, which encode proteins displaying significant sequence similarities to low density lipoprotein (LDL) receptors, and containing one single class A LDL receptor domain common between lipoprotein receptors [27]. Indeed, this domain is shared by various lipoproteins receptors including the VLDL receptor, the LDL receptor-related protein/alpha 2- macroglobulin receptor and the LDL receptor, which contains seven successive class A domains in its N-terminal end [48–50]. Furthermore, another LDL receptor-like molecule, bearing around 20 copies of the class A domain, is present in *E. granulosus s.s* genome [28,41].

According to these suggestions, the simplest pathway by which EgAgB may facilitate the uptake of host-derived lipids and deliver them to parasite tissues would involve interactions with host lipoproteins and parasite receptors within the Hydatid (Fig. 4, solid gray arrows). Alternative or complementary mechanisms would involve EgAgB uptake of lipids from host cells or lipoproteins outside the hydatid cyst; this represents a more complex scenario because it would imply a further EgAgB interaction with parasite receptors at the external surface of GL, or a back and forth traffic mechanism across the hydatid cyst wall (Fig. 4, dotted arrows). It is worth to mention that experimental evidence supporting a role of HLBP in fatty acid binding and transportation across the larval wall has been obtained for *T. solium* HLBP [16]. Finally, the expression of several isoforms of EgAgB apolipoproteins may be related with these parasite metabolic needs; a variety of host-derived lipid molecules have to be transport through the GL to reach protoscoleces growing in brood capsules. Since EgAgB genes are differentially expressed by distinct hydatid structures (*i.e.* protoscolex and GL) [21], it may be possible that the apolipoprotein composition of EgAgB particles varies within hydatid structures, adapting different EgAgB apolipoproteins to interact with particular host and/or parasite molecular targets. Moreover, the existence of parasite redundant mechanisms to ensure lipid supply is reasonable. *Echinococcus* spp. genome encoded apolipoprotein A-I binding protein, known to be secreted into the *E. multilocularis* HF as well as the surrounding host medium, may also contribute to the uptake and transfer of host cholesterol to larval structures [51].

In addition to its role as a lipid carrier, evidence exists supporting the idea that EgAgB may act as modulator of the host immune response by influencing the activation and/or differentiation of innate immune cells. This hypothesis emerged from studies showing that EgAgB was capable of interfering with some inflammatory properties of myeloid immune cells. Previous studies have shown that EgAgB inhibited the chemotactic response of neutrophils to bacterial products [45,47]. These studies were carried out using denatured EgAgB8 apolipoproteins (eluted from SDS-PAGE); we observed similar results on MCP-1/CCL-2 induced chemotaxis of monocytes using immunopurified EgAgB lipoprotein, indicating that these *in vitro* modulatory effects are present in the entire lipoprotein particle (see Supplementary information). Furthermore, EgAgB was found to modulate the differentiation and activation of DCs [46]; this would be of foremost importance since DCs are key sentinels of the innate immunity because of their ability to trigger local inflammation in response to danger signals as well as to specifically activate T lymphocytes at the lymph node. The presence of EgAgB during human monocyte differentiation to DCs interfered with cell differentiation (measured as a decrease in the percentage of $CD1a^+$ immature DCs) as well as with DC ability to respond to lipopolysaccharide (evaluated in terms of cytokine secretion and costimulatory expression markers). Furthermore, EgAgB seems to be capable of inducing a non-conventional DC phenotype (low expression of costimulatory CD80 and CD86 receptors, but high

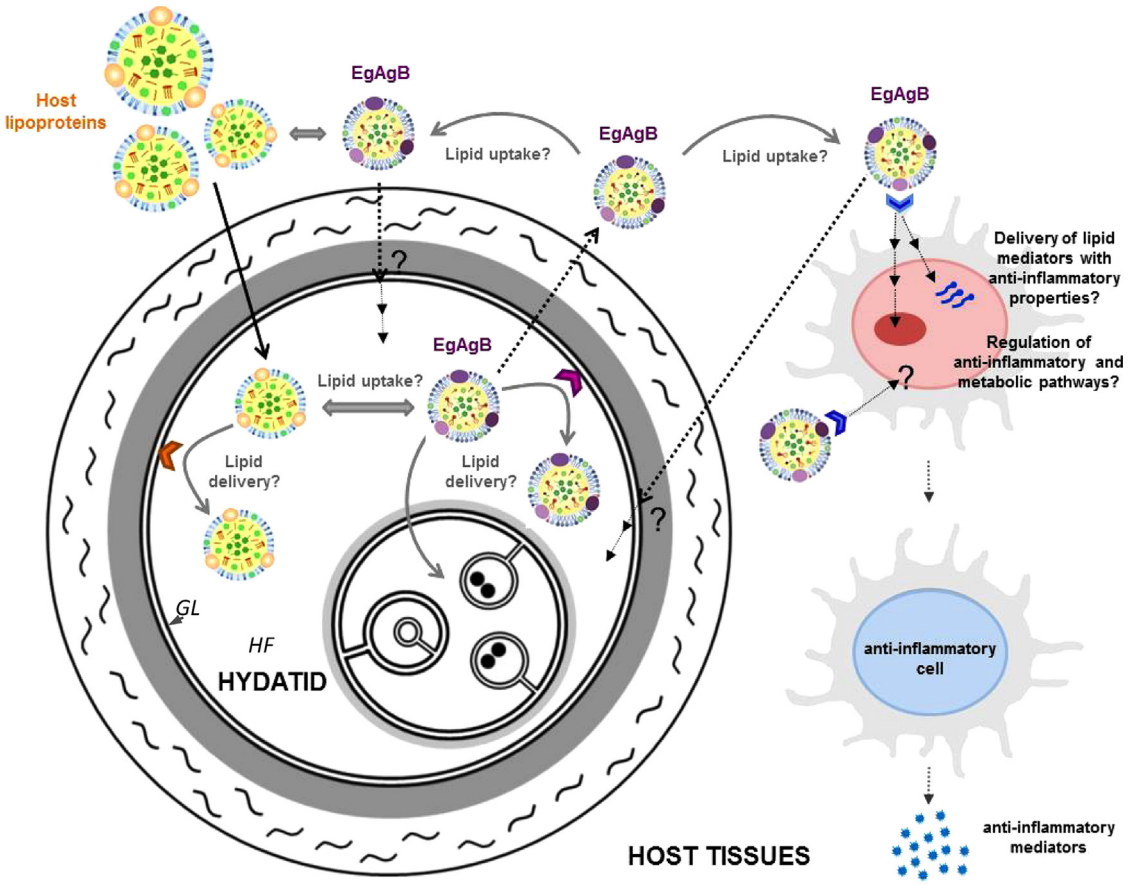


**Fig. 4.** Novel hypothesis for the role of EgAgB in parasite biology. *E. granulosus s. l.* must take fatty acids and cholesterol up from its host since it is unable to synthesize these lipids *de novo*. EgAgB has similarities with plasma lipoproteins of vertebrates, carrying a variety of neutral and polar lipids including triacylglycerides and sterols. EgAgB and host lipoproteins are present in the HF. Thus, inside the cyst, EgAgB may interact with host lipoproteins and parasite receptors to uptake lipids and deliver them to parasite tissues, respectively (gray arrows). Alternatively, host lipoproteins may directly provide lipids to parasite tissues via interaction with GL receptors (gray arrow). EgAgB reaches also host tissues using unknown mechanisms. Outside the cyst, EgAgB may also uptake lipids from host lipoproteins or cells and then, transfer these lipids to the parasite. This transfer may occur at the external face of the GL or may involve a back and forth traffic mechanism across the hydatid cyst wall (dotted arrows). In parallel, EgAgB interactions with immune cells (resident macrophages and DCs, recruited neutrophils and inflammatory monocytes) may influence cell functions by triggering regulatory signaling pathways. This signaling may occur through EgAgB binding to cell receptors at the cell surface, or via delivery of parasite-modified lipids with regulatory properties. EgAgB apolipoproteins differentially expressed in parasite structures could mediate particular interactions with distinct host and parasite molecular targets.

expression of the costimulatory CD40 receptor), which would be responsible for driving T helper cell differentiation towards a Th2-type profile [46]. Nevertheless, studies using native EgAgB are needed to analyze whether the entire lipoprotein caused the same effects on DCs, since studies were performed using a denatured EgAgB. Overall, these reports support that EgAgB is capable of exerting modulatory effects on myeloid cells, but the underlying molecular mechanisms have not been elucidated yet. EgAgB may bind cell membrane receptors to trigger regulatory signaling pathways, or alternatively, may use cell receptors to delivery parasite-modified lipids with regulatory properties (Fig. 4). A couple of putative steroid modifying enzymes have been found in the *E. granulosus s.s.* transcriptome (our unpublished observations).

According to the hypothesis discussed above EgAgB may be involved in essential parasite functions for adaptation to host environment: mechanisms to provide host-derived lipids for anabolic demands, and signaling pathways to skew host immunity towards a more permissive or tolerant response. Interestingly, metabolism and immunity seem to have co-evolved to coordinate their activities through common regulatory axes in order to maintain homeostasis. Examples of these connecting links include the modulation of insulin signaling by diffusible or intracellular inflammatory mediators, and the influence on the immune response of a variety of nutrients, which are ligands of immune sensors pathways (reviewed by [52]). Thus, exploring EgAgB interactions with host immune cells may be an interesting model to look for a putative linkage between lipid metabolism and inflammatory pathways.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.plefa.2014.09.008.

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