Experimental Parasitology ■■ (2015) ■■–■■

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Minireview

The laminated layer: Recent advances and insights into *Echinococcus* biology and evolution

Álvaro Díaz [a,*], Cecilia Fernández [a], Álvaro Pittini [a], Paula I. Seoane [a], Judith E. Allen [b], Cecilia Casaravilla [a]

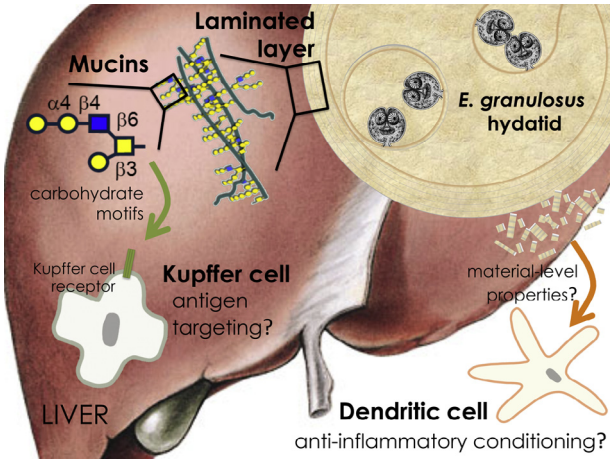
[a] *Cátedra de Inmunología, Departamento de Biociencias (Facultad de Química) e Instituto de Química Biológica (Facultad de Ciencias), Universidad de la República, Montevideo, Uruguay*

[b] *Institute of Immunology and Infection Research, Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, UK*

HIGHLIGHTS

- *Echinococcus* larvae are protected by the acellular, mucin-based laminated layer (LL).
- *E. granulosus* probably uses more mucin protein backbones than *E. multilocularis*.
- In addition, the mucin glycans in *E. granulosus* undergo more elongation.
- The LL glycans may be optimized to interact with liver-specific host receptors.
- The LL also has carbohydrate-independent modulatory effects on innate immune cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form 9 March 2015
Accepted 20 March 2015
Available online

Keywords:
Echinococcus
Laminated layer
Mucin
Carbohydrate
Liver
Lectin

ABSTRACT

The laminated layer is the unique mucin-based extracellular matrix that protects *Echinococcus* larvae, and thus to an important extent, shapes host–parasite relationships in the larval echinococcoses. In 2011, we published twin reviews summarizing what was known about this structure. Since then, important advances have been made. Complete genomes and some RNAseq data are now available for *E. multilocularis* and *E. granulosus*, leading to the inference that the *E. multilocularis* LL is probably formed by a single type of mucin backbone, while a second apomucin subfamily additionally contributes to the *E. granulosus* LL. Previously suspected differences between *E. granulosus* and *E. multilocularis* in mucin glycan size have been confirmed and pinned down to the virtual absence of Galβ1–3 chains in *E. multilocularis*. The LL carbohydrates from both species have been found to interact selectively with the Kupffer cell receptor expressed in rodent liver macrophages, highlighting the ancestral adaptations to rodents as intermediate hosts and to the liver as infection site. Finally, LL particles have been shown to possess carbohydrate-independent mechanisms profoundly conditioning non-liver-specific dendritic cells and macrophages.

* Corresponding author. Fax: +59824873074.
E-mail address: adiaz@fq.edu.uy (Á. Díaz).

http://dx.doi.org/10.1016/j.exppara.2015.03.019

Please cite this article in press as: Álvaro Díaz, et al., The laminated layer: Recent advances and insights into *Echinococcus* biology and evolution, Experimental Parasitology (2015), doi: 10.1016/j.exppara.2015.03.019

These advances are discussed in an integrated way, and in the context of the newly determined phylogeny of *Echinococcus* and its taenid relatives.

Contents

1. Introduction

The taenid cestodes belonging to the genus *Echinococcus* (Thompson and Jenkins, 2014) have two-host life cycles that in all cases include a sexual stage in the intestine of a carnivore and a larval stage in the tissues of non-carnivorous/omnivorous mammals. These life-cycles are based on a predator–prey relationship between the definitive host (harboring the parasite adult stage) and the intermediate hosts (harboring the larval stage). Infection by the larval stages (larval echinococcoses) can affect humans (Brunetti and White, 2012), which are thus accidental intermediate hosts. Intermediate host infection comes about after ingestion of eggs (passed out with the definitive host feces), which hatch releasing oncospheres that penetrate the intestinal wall, and are carried by blood or lymph to internal organs. Oncospheres develop into larvae known as metacestodes, which have a basic bladder-like morphology, and give rise within them to protoscoleces, infective for the definitive hosts.

Ten or eleven *Echinococcus* species are currently recognized (Lymbery et al., 2014; Thompson and Jenkins, 2014). These include six or seven species split apart from what was historically *E. granulosus*. This (paraphyletic) group of species (Nakao et al., 2013a, 2013b), now called *E. granulosus sensu lato*, uses different ungulate species as intermediate hosts. The metacestode develops as a large, unilocular turgid "cyst" (more correctly termed a "hydatid"), which grows through concentric enlargement only. This group includes *E. granulosus* (*sensu strictu*), i.e. what was formerly known as the "sheep strain", the most studied species of the group. Outside this group, the genus includes the neotropical species *E. vogeli* and *E. oligarthra*, having rodent intermediate hosts and metacestode morphologies with small variations with respect to the hydatid (D'Alessandro and Rausch, 2008). Finally, the genus also includes the sister species *E. shiqicus* and *E. multilocularis*, with lagomorph and rodent intermediate hosts respectively (Vuitton and Gottstein, 2010; Xiao et al., 2006). Larval *E. shiqicus* develops in a hydatid-like morphology. In contrast, the *E. multilocularis* metacestode generates outward protrusions that invade the surrounding host tissue (Mehlhorn et al., 1983). Hence *E. multilocularis* larvae develop as a network of interconnected vesicles and tubules than can take over the whole organ (liver) and even invade other organs. Thus human infection by this species is the most lethal of helminthiasis (Brunetti and White, 2012; Thompson and Jenkins, 2014).

Throughout the genus, metacestodes are bounded by a thin layer of cells (germinal layer, GL), outwardly protected by an acellular structure known as the laminated (or laminar) layer (LL). The LL, unique and distinctive of the genus *Echinococcus*, is undoubtedly a major component of the adaptation of these parasites to dwell in internal organs of immunocompetent mammals over years or decades. In 2011, we published twin reviews summarizing what was known about the LL, and attempting to fill the gaps with informed speculations (Díaz et al., 2011a, 2011b). In short, the LL is a specialized extracellular matrix synthesized by the GL, and more specifically by its outermost syncytial layer, termed the tegument. As the tegument is only one cell-thick, tegumental cells must engage in intense biosynthetic activity in order to generate the building blocks for the massive LL. The LL is based on mucins, i.e. glycoproteins with many points of a particular type of glycosylation (mucin *O*-type glycosylation). Whereas mucins normally form the loose mucus barriers (Corfield, 2015), the LL is much more structured, allowing live hydatids to be turgid. Across the genus, LL thickness varies considerably, reaching 3 mm in *E. granulosus sensu strictu* (and being similarly thick in *E. granulosus sensu lato*), up to 400 μm in *E. vogeli*, 5–38 μm in *E. shiqicus*, and 10–12 μm in *E. multilocularis* (Bortoletti and Ferretti, 1978; Rausch, 1954; Rausch et al., 1981; Xiao et al., 2005). In addition to mucins, the LL of *E. granulosus* contains abundant nanodeposits of a calcium salt of inositol hexakisphosphate ($InsP_6$) (Casaravilla et al., 2006; Irigoín et al., 2002, 2004). This is a curious adaptation, since $InsP_6$ is an intracellular molecule in all other biological systems studied (Irvine and Schell, 2001). The adaptation is absent from *E. multilocularis* on the basis of biochemical evidence (Irigoín et al., 2002). From transmission electron microscopy data, a technique by which the *E. granulosus* $InsP_6$ deposits appear as a conspicuous feature (Irigoín et al., 2004), the adaptation would be present in *E. equinus* (Richards et al., 1983) but absent in *E. vogeli* (Ingold et al., 2001). Thus presence of the calcium $InsP_6$ deposits appears to correlate with adaptation to infect large mammals.

The LL is widely thought to be a crucial element of the host–parasite relationship in the larval echinococcoses. Its roles include shielding the parasite from direct attack by host immune cells, and probably down-regulating local inflammation (Díaz et al., 2011a, 2011b). The evidence for the second aspect is still indirect. It includes the observation that in experimental *E. granulosus* infections, the local inflammation subsides at the same time as the LL is deployed (Breijo et al., 2008). It also includes, for *E. multilocularis,* the observation that the portions of the invasive protrusions that are freshly formed, and thus still devoid of LL, are lined with inflammatory cells, while those nearby areas that are already covered by LL are lined with collagen and fibroblasts (Mehlhorn et al., 1983).

In the following two sections of this review, we focus on recent advances on the peptide (apomucin) and carbohydrate (mucin *O*-glycan) components of the mucins that make up the LL. We then summarize advances on how the LL is decoded by the immune system. We propose that this decoding includes, but is not restricted to, specific interactions between LL mucin carbohydrates and host receptors, and place it in the context of *Echinococcus* evolution.

2. Advances on the LL structure: apomucins

The sequences of apomucins making up the *E. granulosus* LL had been previously inferred from (pre-RNAseq) transcriptomic data, as sequences highly expressed in the GL and essentially absent from the other stages/tissues sampled (Díaz et al., 2011a, 2011b; Parkinson

Please cite this article in press as: Álvaro Díaz, et al., The laminated layer: Recent advances and insights into *Echinococcus* biology and evolution, Experimental Parasitology (2015), doi: 10.1016/j.exppara.2015.03.019