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Abstract	*Fasciola hepatica*, the common liver fluke, causes infection of livestock throughout temperate regions of the globe. This helminth parasite has an indirect lifecycle, relying on the presence of the mud snail to complete its transition from egg to definitive host (Beesley et al., Transbound Emerg Dis 65:199–216, 2017). Within the definitive host, the parasite excysts in the intestine forming a newly excysted juvenile (NEJ) and migrates via the peritoneal cavity to the liver. Disease resulting from infection can be acute or chronic depending on the host and the number of parasites present. Sheep may succumb to a fatal acute infection if the challenge of metacercariae is great enough. However, in cattle chronic disease is the most likely outcome with parasites surviving for long periods of time. Annual losses are estimated to be in the region of US$ 2000 million to the agricultural industry (Beesley et al., Transbound Emerg Dis 65:199–216, 2017). Management of the disease depends heavily on chemotherapy with triclabendazole being the drug of choice, consistent use for over 20 years has resulted in drug-resistant strains emerging worldwide (Beesley et al., Int J Parasitol 47:11–20, 2017). A more sustainable approach to control would be through vaccination and indeed a lead candidate has been identified, cathepsin L1. Despite these promising results the parasite continues to confound our own and host efforts to generate long-lasting and effective immunity. In this brief review we focus our attention on those mechanisms that the parasite

	utilises to circumvent the innate based defense mechanisms within the host.
Keywords (separated by '-')	*Fasciola hepatica* - Immune evasion - Helminth - Immunomodulatory - Cathepsin - Innate immunity

Chapter 8

Evasion of Host Immunity During *Fasciola hepatica* Infection

Robin J. Flynn and Mayowa Musah-Eroje

Abstract

Fasciola hepatica, the common liver fluke, causes infection of livestock throughout temperate regions of the globe. This helminth parasite has an indirect lifecycle, relying on the presence of the mud snail to complete its transition from egg to definitive host (Beesley et al., Transbound Emerg Dis 65:199–216, 2017). Within the definitive host, the parasite excysts in the intestine forming a newly excysted juvenile (NEJ) and migrates via the peritoneal cavity to the liver. Disease resulting from infection can be acute or chronic depending on the host and the number of parasites present. Sheep may succumb to a fatal acute infection if the challenge of metacercariae is great enough. However, in cattle chronic disease is the most likely outcome with parasites surviving for long periods of time. Annual losses are estimated to be in the region of US$ 2000 million to the agricultural industry (Beesley et al., Transbound Emerg Dis 65:199–216, 2017). Management of the disease depends heavily on chemotherapy with triclabendazole being the drug of choice, consistent use for over 20 years has resulted in drug-resistant strains emerging worldwide (Beesley et al., Int J Parasitol 47:11–20, 2017). A more sustainable approach to control would be through vaccination and indeed a lead candidate has been identified, cathepsin L1. Despite these promising results the parasite continues to confound our own and host efforts to generate long-lasting and effective immunity. In this brief review we focus our attention on those mechanisms that the parasite utilises to circumvent the innate based defense mechanisms within the host.

Key words *Fasciola hepatica*, Immune evasion, Helminth, Immunomodulatory, Cathepsin, Innate immunity

1 Immunity to *F. hepatica*

F. hepatica immunity in ruminant hosts mirrors to large extent the response seen to Schistosome species. During experimental infection there is a brief phase of lymphocyte proliferation accompanied by IFN-γ production; thereafter a prolonged phase of IL-4 and initial antibody production follows. Coinciding with onset of patency there is a switch toward an anergic phenotype [3–5].

After emerging with the intestine invading NEJs must be sensed by the innate pattern recognition receptor (PRR) network. Evidence from murine models would suggest that the production

of canonical type-2 cytokines IL-25, IL-33, and TSLP are essential 34
at this juncture in initiating the first wave of innate immune 35
responses. Eosinophilia is a core characteristic of the antihelminth 36
response with multiple studies suggesting a sliding scale of impor- 37
tance in helminth clearance. In nematode infection eosinophilia is 38
known to be nonessential in nematode infections for the expulsion 39
of parasites [6]. Swartz et al. have shown that eosinophils play no 40
role in *S. mansoni* infection parameters such as egg deposition, 41
worm burdens, liver enzymes, and granuloma size or number 42
[7]. In *F. hepatica* infection Bossaert et al. showed that eosinophil 43
counts were significantly elevated in infected cattle within 4 weeks 44
of infection and remained so during the course of a 16 week infec- 45
tion period [8]. Zhang et al. demonstrated the presence of biphasic 46
eosinophilia in *F. hepatica* infected sheep, with the peaks occurring 47
at weeks 4 and 9–10 postinfection [9]. The importance of eosino- 48
philia was again demonstrated by Chauvin et al., who demonstrated 49
a positive relationship between the total eosinophil count and the 50
infective dose administered to sheep, signifying a correlation 51
between immune response and intensity of infection [10]. Impor- 52
tantly their role in protective immunity is well supported; Doy et al. 53
suggested a role for eosinophils in resistance developed in immune 54
rats [11]. Immune rats facing a challenge infection showed an 55
increase in eosinophils within the *lamina propria* of the small 56
intestine. Van Milligen et al. described an ex vivo model of the rat 57
gut during infection, in immune rats [12]. Again, eosinophil counts 58
were elevated in the *lamina propria* of immune rats. When NEJs 59
migrated into the mucosa of immune rats they were found to be 60
coated with both IgG1 and IgG2a antibodies and eosinophils. 61
Later work [13] showed that eosinophils were essential for protec- 62
tion in the same model. The presence of parasite-specific antibody 63
would make ADCC the most likely method of killing NEJs. This 64
work is supported by studies of various species placing ADCC at the 65
center of protective immunity against *F. hepatica* NEJs in cattle 66
[14, 15]. 67

Macrophages elicited by helminth infection have been shown 68
to diverge from the normal paradigm of classically activated—nitric 69
oxide producing—antibacterial cells. Gordon summarized and out- 70
lined the mechanisms by which parasitic helminths can interact with 71
MΦ, causing their alternative activation [16]. Alternatively acti- 72
vated MΦ (AAMΦ) are denoted by their production of polyamines, 73
proline, and IL-10. The differential regulation of L-arginine by MΦ 74
has allowed workers to distinguish between these two populations 75
of cells. AAMΦ metabolize L-arginine (Arg-1) using the enzyme 76
arginase. AAMΦ induced by parasite infections have been shown to 77
express a unique panel of markers: the mannose receptor along with 78
a number of unique molecules such as intelectins, resistin-like 79
molecules (RELM), chitinases, or chitinase-like proteins [17]. To 80
date AAMΦ have been found in infections with a wide variety of 81

parasites including *S. mansoni* [18], *Taenia crassiceps* [19], 82
F. hepatica [20], *Litomosoides sigmodontis* and *Nippostrongylus bra-* 83
siliensis [21], *Brugia malayi* [22], and H. polygyrus [23]. Numer- 84
ous studies have shown that AAMΦ regulate the type-2 immune 85
response in various helminth infections and help to limit immuno- 86
pathology. However, the protective role of AAMΦ was shown by 87
Anthony et al. (2005) using *H. polygyrus* [23]. Infection of mice 88   AU4
revealed an accumulation of AAMΦ into the intestine and sur- 89
rounding these worms. Moreover, drug abbreviation of infection 90
giving rise to immunity magnified this sterilizing immune response 91
and macrophage depletion demonstrated that AAMΦ were central 92
to curative response. Importantly, administration of an arginase-1 93
inhibitor demonstrated a direct effect of AAMΦ on worm viability 94
measured via cytochrome oxidase. A direct effect of AAMΦ on 95
F. hepatica viability has yet to be shown but roles in directing or 96
contributing to the Th2 response during infection is well estab- 97
lished in multiple species [24–26]. 98

2   Mechanisms of Immune Evasion

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Given the depth of information that is known about innate effector 100
mechanisms, there is a corresponding trend for our knowledge 101
regarding specifics of immune evasion to arise from study of the 102
interactions between *F. hepatica* and the innate leukocytes. From 103
herein we will discuss and explore the nature of these interactions 104
and where known their function effects. One of the first in vitro 105
studies of Immunomodulation resulting from *F. hepatica* infection 106
was recorded in 1985 [27]. They reported that the ability of 107
lymphocytes, from infected sheep, to proliferate was reduced even 108
when stimulated with the mitogen, ConA. Similar interactions 109
between leukocytes and excretory–secretory (ES) products were 110
observed by Jefferies et al. [28–30]. They studied the effect of ES 111
products on both human and ovine neutrophils and found that ES 112
products caused neutrophils to polarize, migrate and induced mor- 113
phological changes going from spherical to elongated type cells. 114
They also demonstrated an ability of ES to reduce the oxidative 115
burst of sheep and human neutrophils in response to PMA in a dose 116
dependent manner. This work was one of the first to suggest that 117
the parasite is capable of modulating aspects of the immune system 118
to evade damage or destruction. ES products are a complex of 119
multiple secreted proteins, both actively and passively. Refining 120
the molecules within ES and defining their mode of action has 121
become paramount to understanding parasite evasion and includ- 122
ing key molecules in future vaccination plans. Below we discuss two 123
major classes of parasite modulators, enzymatic and nonenzymatic 124
modulators, giving an overview of the major details we have 125
gleaned from studies to date. 126

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3 Enzymatic Modulators

3.1 Cathepsins

The cathepsin cysteine protease family, containing cathepsin L1 (CL1) are the most clearly defined molecules from *F. hepatica* with immunomodulation capabilities. Early after the initial identification of CL1, Carmona et al. [31] demonstrated that *F. hepatica* CL1 could prevent eosinophil mediated ADCC killing of NEJs. CL1 was capable of cleaving antibody at the Fc-Fab junction, thus preventing cell attachment. Prowse et al. [32] demonstrated again CL1 directly modulates the expression of CD4 on lymphocytes by cleaving the receptor enzymatically. This effect could be reversed in the presence of a specific cathepsin inhibitor. Thus, at a direct level CL1 modulates immune function through its enzyme activities. Brady et al. [33] had earlier described a model of coinfection where *F. hepatica* would suppress mechanisms of defense that were specifically directed at *Bordetella pertussis*. This resulted in a loss of bacterial specific IFN-γ production and a delay in clearance of bacteria from the lungs. In follow up work, O'Neill et al. [34] demonstrated that injection of CL1 would have the same negative effect on *B. pertussis* immune responses as a *F. hepatica* infection. By use of knockout mice, they were able to show that this suppression was partially mediated by IL-4. In IL-4$^{-/-}$ mice IFN-γ levels were elevated in comparison to wild-type mice following injection of CL1, but still were significantly lower than in controls. Administration of a cathepsin enzyme inhibitor revealed that enzyme activity was required for the full suppressive effect. The enzymatic nature of *F. hepatica* CL1 was shown to suppress septic shock in vivo by Donnelly et al. [35]. Moreover, CL1 acted on TRIF and not surface bound TLR4 and use of both chemical inhibition and an active-site mutant CL1 confirmed reliance on protease activity. The requirement for active CL1 was against demonstrated in DCs [36], where CL1 caused partial maturation of DCs in vitro. A downstream functional effect was detectable in terms of attenuated Th17 responses when CL1-exposed DCs were used. Indicating there might be multiple routes to deviation from a Th1 or Th17 response that the parasite can use.

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3.2 Peroxiredoxin

A second class of enzymes derived from ES products has also been well documented for their roles in host immunomodulation. Peroxiredoxin (formerly Thioredoxin Peroxidase) is a 2-cys redox enzyme which can traditionally protect DNA from redox damage [37]. It is weakly recognised by the host with antibodies against Prx declining into chronic infection [37]. This in itself may parallel the period of infection when Prx is most potent, at the point during which macrophage recruitment during NEJ invasion is highest. The effect of Prx on macrophages, resulting in AAMΦ, has been demonstrated in multiple species. In mice, Prx causes strong

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induction of arginase-1, FIZZ1 and Ym1 [20] while in ruminants it 173
was shown that arginase-1 and IL-10 were upregulated by Prx 174
[38]. In ruminants acidic mammalian chitinase (AMCase) was 175
also identified as being upregulated following Prx exposure. While 176
chitinases are ancient enzymes known to degrade chitin, commonly 177
found in arthropods, there are no chitin-substrates in *F. hepatica*— 178
which raises the question of its function. Importantly, Prx was 179
shown to cause AAMΦ independent of IL-4/IL-13 which indi- 180
cated a mechanism for the parasite to by-pass canonical type-2- 181
signalling. Furthermore, when neutralized by immunization 182
prior to infection it was revealed that Ym1, indicating AAMΦ in 183
the peritoneal cavity, was reduced as was the subsequent IL-4 184
response [39], ultimately indicating a role for Prx-induced 185
AAMΦs propagating a type-2 immune environment. While the 186
enzymatic function of Prx is essential for its function, the precise 187
mechanism by which it establishes the AAMΦ phenotype remains 188
unknown and may yet present a viable route to *F. hepatica* control. 189
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4 Nonenzymatic Modulators

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Recently a number of parasite modulators have emerged that do 192
not rely on enzymatic activity to polarize or subvert host immune 193
effector mechanisms. However a common feature among these 194
immunomodulators is their homology to host proteins with 195
immune functions. 196

4.1 HDM

F. hepatica helminth defense molecule (FhHDM) was initially 197
identified through a proteomic screen and phylogenetic analysis 198
confirmed that it shares structural similarities with human LL-37, 199
an antimicrobial peptide [40]. Initial characterization suggested 200
that FhHDM could bind to LPS and block septic shock in vivo. 201
Further details on the mechanism of action of FhHDM revealed 202
FhHDM bound to lipids in the membrane was internalized and 203
subsequently blocked antigen presentation on the MHC-II com- 204
plex [41]. During the internalization phase it was shown that 205
lysosomal acidification was blocked and this resulted in decreased 206
inflammasome activation and subsequent IL-1β secretion [42]. The 207
consequence of blocking antigen presentation within infection 208
might allow for evasion of adaptive responses during infection; 209
however IL-1β has more recently been shown to suppress the 210
protective responses against intestinal *Trichuris muris* [43]. Thus, 211
it is possible that while inhibiting antigen presentation benefits 212
F. hepatica survival a benefit of blocking IL-1β remains to be 213
uncovered. 214
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4.2 TLM

TLM, TGF-like molecule, was first described from a screen of the *F. hepatica* genome. It presented with restricted expression, being highly expressed within the NEJ stage and low levels of expression within the adults. Initial experiments demonstrated that TLM retained similar qualities to TGF signalling in other worms and promoted viability and motility in vitro. Sulaiman et al. [15] later demonstrated that effects of TLM were not parasite restricted. Solid-phase binding assays demonstrated that TLM could indeed bind host TGF receptor complexes and resulted in activation of host STAT signalling. Phenotyping of macrophages exposed to TLM demonstrated a deviation from the AAMΦ spectrum with a significant increase in markers associated with a regulatory response including PD-1 and CTLA4. Ultimately, preexposure to TLM resulted in a reduction in macrophage-mediated ADCC killing of the NEJ parasite. This presents a clear pathway from stage-specific secretion of a modulator through to a host tissue specific.

5 Summary

We present here a brief overview of some of the best characterized modulators, enzymatic and nonenzymatic, their modes of actions and phenotypic effects. Recent evidence would suggest that our attention should shift to components of the tegumental coat. In recent studies the crude tegumental coat has been shown to inhibit mast cells [44] and DCs [45] in driving Th1 responses. Interestingly some of the effects of tegumental antigens have shown to be both mannose receptor dependent and independent [46, 47], indicating that the composition of the tegumental antigen is complex and will require much further study. Elucidating the mechanisms of action of *F. hepatica* evasion molecules will benefit vaccine development and future biotherapeutics.

Acknowledgments

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