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Digenean-gastropod host associations inform on aspects of specific immunity in snails

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ABSTRACT

Gastropod immunology is informed importantly by the study of the frequent encounters snails endure with digeneans (digenetic trematodes). One of the hallmarks of gastropod-digenean associations is their specificity: any particular digenean parasite species is transmitted by a limited subset of snail taxa. We discuss the nature of this specificity, including its immunological basis. We then review studies of the model gastropod *Biomphalaria glabrata* indicating that the baseline responses of snails to digeneans can be elevated in a specific manner. Studies incorporating molecular and functional approaches are then highlighted, and are further suggestive of the capacity for specific gastropod immune responses. These studies have led to the compatibility polymorphism hypothesis: the interactions between diversified fibrinogen-related proteins (FREPs) and diverse carbohydrate-decorated polymorphic parasite antigens determine recognition and trigger specific immunity. Complex glycan structures are also likely to play a role in the host specificity typifying snail–digenean interactions. We conclude by noting the dynamic and consequential interactions between snails and digeneans can be considered as drivers of diversification of digenean parasites and in the development and maintenance of specific immunity in gastropods.

1. Introduction

The emphasis of this review is on the specific nature of the gastropod immune response. After considering various ways to define "specificity" that ended up seeming arbitrary or less relevant to the biology of gastropods themselves, we decided to use as our operational indicator of specificity something provided by one of the most relevant, and certainly one of the better-studied groups of dedicated gastropod parasites, the digeneans (also known as flukes or digenetic trematodes). For our purposes here, specific immunity of snails is considered to be the capability of their internal defenses to recognize a particular taxon or group of closely-related taxa of digeneans and effect immune-mediated elimination, either innately, or in a manner that is improved after a previous exposure to the same or related digeneans. Given that the systematics of digeneans is steadily improving, and the group is remarkably diverse, we can think of them as being relatively well-defined, diverse and relevant tools to probe the nature of gastropod immunity. By no means do we intend to imply that the only manifestations of specificity in gastropod defenses apply to digeneans, nor that snail responses to digeneans fully represent the entire spectrum of snail immune capabilities.

We readily acknowledge that the extent to which invertebrate defense responses are specific, and the extent to which invertebrates are anatomically equipped (e.g. lack of lymphocytic tissues, clonal expansion) to mount specific responses that can be meaningfully enhanced upon re-exposures, are long-standing issues of debate in studies of comparative or evolutionary immunology (Hauton and Smith, 2007; Klein, 1989; Loker et al., 2004). Many recent studies suggest the diversity of molecules available to various groups of invertebrates to counter pathogens and parasites may be much higher than previously considered (e.g. Buckley and Rast, 2012; Ghosh et al., 2011), and that at least some invertebrates can tailor their immune responses to the pathogens encountered. The fruit fly *Drosophila melanogaster* employs various non-self recognition receptors that distinguish Gram (−) from Gram (+) bacteria or other pathogens, and activate different signaling pathways that generate transcriptional profiles to emphasize defense factors with greatest impact on the pathogens encountered (Ferrandon et al., 2007; Hoffmann and Reichhart, 2002; Kraaijeveld et al., 2012). Moreover, previous encounters that result in immune elimination of pathogens lead to immune priming; fruit flies retain increased immune resistance to specific pathogens for about a week (Christofi and Apidianakis, 2013; Pham et al., 2007). Many of the authoritative papers in the field derived from

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studies of deuterostome invertebrates or of ecdysozoan protostomes, with studies of Lophotrochozoa, the other major branch of protostome invertebrates (Erwin et al., 2011), tending to lag behind.

Here we attempt to make the case that some of the hallmark features of more sophisticated invertebrate defenses, including mounting specific responses that can be elevated to heightened levels, are exemplified by gastropod molluscs, particularly in their interactions with members of another prominent lophotrochozoan phylum, the Platyhelminthes, specifically the Digenea. Both gastropods and digeneans are species-rich groups. A fundamental commitment of digeneans to a highly invasive mode of development in gastropods was made long ago, and has been largely retained subsequently. The nature of the digenean development program within gastropods is one that is remarkable for the extent to which it involves proliferative asexual development, with dire fitness consequences for snails. We argue that it is precisely within such a long-standing, biotically complex interaction, one with high fitness stakes and diverse hosts and parasites involved, that one might expect to see some of the most sophisticated aspects of parasite offense and host defense played out.

This review first discusses the nature and range of naturally occurring compatible gastropod-digenean parasite associations – what is often referred to as snail–digenean host specificity – as an indication of specific innate immunity in snails. We then review some of the classic experimental studies (in our view, too often neglected) that demonstrated snails can gain specific immunity, termed acquired resistance, when challenged with particular lineages of digeneans. We then consider more modern studies to interpret the molecular foundations of specific immunity in the snail *Biomphalaria glabrata*. We conclude with some consideration of the potential macroevolutionary impacts of specific immune interactions between digeneans and snails.

2. Host specificity of digenean–snail interactions: an indicator of the underlying innate capacity for specificity of the gastropod immune system

One of the most prominent features of gastropod biology is their involvement as first intermediate hosts in the life cycles of most of the estimated 25,000 species of digenetic trematodes (Esch et al., 2002). Miracidia of digeneans colonize snails and initiate a program of asexual reproduction that yields sporocyst and/or redia stages, and culminates in the production of cercariae, usually after 4–6 weeks. Infection heavily impacts snail fitness because digeneans generally castrate their hosts, yet infected snails may persist in releasing cercariae for months and sometimes years. All the while, the digenean larvae lie in intimate contact with the snail host's tissues.

The associations between digeneans and their snail hosts are specific, and digeneans are said to be host specific with respect to their snail hosts (see Adema and Loker, 1997 for general discussion and examples, and Section 2.1 below). Digenea are highly specialized parasites; almost the entire lineage has committed to molluscs (mostly gastropods) as first intermediate hosts. The capacity of any given digenean to infect snails is not limitless across all snail species; distinctive patterns of host use have emerged. Such characteristics of digenean–snail specificity can help probe and understand the nature, including specificity, of the gastropod immune system.

2.1. Characteristics and variations of digenean–snail specificity

Although the literature has been characterized as implying that a particular digenean species can develop in only one species of snail (Donald et al., 2004), digenean–snail host specificity is not so simply summarized. Extensive studies of specificities of the medically important schistosomes (see Basch, 1976; Brown, 2005 for good historical reviews) indicate that *Schistosoma mansoni* only infects snails of a single genus, *Biomphalaria* (family Planorbidae). At least 18 of the circa 34 species of *Biomphalaria* are susceptible to this trematode (DeJong et al., 2001). Specificity can in some cases be even more pronounced, such that some strains of species like *B. glabrata* are susceptible to *S. mansoni*, whereas others are resistant (Richards et al., 1992), yet other *B. glabrata* strains are susceptible to one strain of *S. mansoni* and resistant to another *S. mansoni* strain (Lie, 1982). Species of *Helisoma*, the sister genus of *Biomphalaria*, are uniformly refractory to *S. mansoni* infection. Many other planorbid snails from different genera have been exposed to *S. mansoni* but consistently fail to take the infection (see Sullivan and Yeung, 2011). Similarly, *Schistosoma haematobium* normally develops only in certain species of *Bulinus* snails (Rollinson et al., 2001). This "rule" regarding host specificity for schistosomes is rarely violated. *Planorbarius metidjensis*, not a particularly close relative of *Biomphalaria* or *Bulinus*, can be infected by both *S. haematobium* (Kechemir and Combes, 1982) and *S. mansoni* (Barbosa et al., 1959) but this snail is probably of little or no significance in natural transmission. *S. haematobium* is unable to infect *Biomphalaria* just as *S. mansoni* is unable to infect *Bulinus*.

The relationship between *S. mansoni* and *Biomphalaria* provides further insight regarding host specificity. After the genus *Biomphalaria* first diversified in the Americas, a snail similar to modern-day *B. glabrata* is believed to have colonized Africa and to have given rise to the African *Biomphalaria* species about 1.4–4.3 million years ago (DeJong et al., 2001). Strong incompatibility or resistance among the 12 African species of *Biomphalaria* to *S. mansoni* are rarely reported, although *Biomphalaria alexandrina* seems to be relatively less compatible to *S. mansoni* from various sources (Frandsen, 1979). The general compatibility between African *Biomphalaria* and *S. mansoni* is not surprising given this parasite most probably evolved on the African continent. By contrast, among the 17 South American *Biomphalaria* species, 8 species are generally refractory. Although South America is where *Biomphalaria* first diversified, *S. mansoni* did not encounter *Biomphalaria* there until 400–600 years ago, upon the arrival of infected Africans. In the absence of *S. mansoni*, some – but not all – lineages of *Biomphalaria* in the Americas have evolved and have retained (and shared with African *Biomphalaria*), distinctive properties that allow exploitation by *S. mansoni*. Once these biological features were established, even without continuous selective pressure from *S. mansoni*, they have been retained by those snails such that they can still support *S. mansoni* infections. Although there is a tendency for sympatric combinations of *S. mansoni* and *Biomphalaria* to be more compatible than allopatric combinations, often *S. mansoni* isolates from one geographic area readily infect *Biomphalaria* snails from distant areas (Kagan and Geiger, 1965; Frandsen, 1979).

Not surprisingly, the degree of host specificity exhibited by different digenean species is variable, even including among closely related species. The liver fluke *Fasciola hepatica* is able to develop in some, but not all, representatives of the following snail genera: *Galba*, *Lymnaea*, *Omphiscola*, *Stagnicola*, *Pseudosuccinea*, and *Radix* (Caron et al., 2014). This hardly seems very specific, but all of these genera are found within a single molluscan family, the Lymnaeidae. *Echinostoma paraensei* can develop in members of three different pulmonate families (Maldonado et al., 2001). Whereas *Clonorchis sinensis* is able to infect snails of five caenogastropod families, the closely-related *Opisthorchis viverrini* infects only one of these families, the Bithyniidae (Petney et al., 2013).

Although fewer experimental studies have been done with marine snails, specificity with respect to digeneans seems to apply here too. For example, for genetically-defined (characterization of 16S