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Scribe Summary

Lymphatic filariasis (LF) is a mosquito-borne disease caused by parasitic nematodes that results in the abnormal swelling and enlargement of body parts in humans (Zamanian et al, 2015). LF is a neglected tropical disease that has infected more than 60 million people and poses a risk to more than a billion people living in areas that have a high risk of transmission (Dietrich et al, 2019). There are three species of parasitic filarial nematodes that cause this disease: *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (Dietrich et al, 2019). Characterizing the mechanisms and pathways by which these parasites interact with their hosts is crucial for fully understanding the pathology of LF and eventually developing a cure. It has been discovered that one of the methods by which *B. malayi* interacts with its human host’s cells is by releasing exosome-like vesicles that contain microRNAs (miRNAs) (Zamanian et al, 2015). Characterization of miRNAs found in *B. malayi* is an important step in understanding the miRNA regulatory pathways and networks in filarial parasites. It is of particular interest to identify miRNAs that could be involved in immune evasion of *B malayi*, as these would probably be the ones contained in the released exosome-like vesicles (ELVs). 99 miRNA families were found to be conserved among helminths, arthropods, and vertebrates (Poole et al, 2014). It is unlikely that the highly conserved miRNA families play a role in parasite specific pathways, which leaves questions to be answered. Are any of these families unique to filarial parasites, or even *B. malayi* specifically? If so, which of these parasite specific miRNAs could play a role in host immune response evasion?

Following this inquiry into the interactions between B. malayi and the host immune system, there is a focus on the immune response of humans to invasion, specifically macrophage activity, as humans are the definitive host for the parasite. However, there is no mention of the immune response of the intermediate host, the mosquito. Do mosquitos respond to infection by the nematode and what exactly is the immune response?

The Zamanian paper focuses on macrophages during the definitive host immune response to infection. It was shown that about 40-50% of macrophages internalized exosome-like vesicles released by the parasite to some degree. Only about 10% of macrophages internalized ELVs at higher levels (Zamanian et al, 2015). Why is this immune response to the ELVs not efficient in destroying the vesicles? Could it be that the researchers were investigating the incorrect immune cells and that there were other cells more involved in protecting against the contents of ELVs?

There is a lack of understanding of host-parasite interactions in lymphatic filariasis as the manipulation of host biology and the mechanisms by which it is conducted are not well defined. This leads to the question of why *Brugia malayi* was chosen as the organism studied in this paper if there are two other parasitic nematodes that cause LF (*Wuchereria bancrofti* and *Brugia timori*)? Why not study all three parasites for a holistic view of the mechanisms behind LF infection, particularly the release of miRNA containing exosome-like vesicles?

1. **Are any of these families unique to filarial parasites, or even *B. malayi* specifically?**

Of the 99 *B. malayi* miRNA families identified to be conserved among helminths, arthropods, and vertebrates, 7 were found to have homologues in the helminths *Caenorhabditis elegans* and *Ascaris suum*. Interestingly, another 6 of the families were found to have homologs identified in arthropods, but not vertebrates. This implies that these miRNA families play ecdysozoa specific roles. Within these 6 families, half were found to have homologs in mosquitos specifically, which is of particular interest since they are the intermediate host of the parasite (Poole et al, 2014).

For 12 of the initial 99 families, homologs could not be found in neither arthropods, nor vertebrates. This suggests that these are helminth or even filarial specific miRNA families. 9 of these 12 families seemed to be specific to filarial parasites – orthologues were identified for 8 of them in at least one other filarial species but not in arthropods, vertebrates, or other helminths (Poole et al, 2014).

1. **If so, which of these parasite specific miRNAs could play a role in host immune response evasion?**

Of these putative filarial specific miRNAs, two miRNAs were of particular interest, miR-9535 and miR-9536. No orthologues were identified for miR-9535, raising the possibility that this microRNA could be specific to *Brugia malayi* and play roles in functions specific to the worm, such as host immune evasion. miR-9536 is intriguing due to its location in an intron of Bm1\_03065, a *cut-1* cuticlin gene fragment. Due to its location in this gene, expression of the miRNA likely has to do with the expression of the cuticlin gene and therefore involved in molting and cuticle synthesis (Poole et al, 2014). Since an important aspect of the LF disease pathology is the molting of *B. malayi* to the L3 larval stage in mosquitos, this could indicate a role in immune evasion as well.

1. **Do mosquitos exhibit an immune response to infection by *Brugia malayi* microfilariae?**

Melanization is a process that plays a role in multiple physiological processes in insects such as wound healing and immune system response (Nakhleh et al, 2017). It is a complex biochemical cascade that eventually results in melanin pigment being deposited around the pathogen, in this case the *B. malayi* microfilariae. This is determinantal to the development of the worm, as it can no longer access nutrients to grow (Dedkhad et al, 2018). Melanization is toxic to parasitic worms, bacteria, fungi, and even some viruses (Nakhleh et al, 2017). The site of *B. malayi* exsheathment is the midgut of mosquitos, where melanization has been observed after infection as well as in other muscles of the insect (Dedkhad et al, 2018). This indicates that melanization is an innate immune response to infection by *Brugia malayi*.

1. **Why is the host immune response to the ELVs not efficient in destroying the vesicles? Could it be that the researchers were investigating the incorrect immune cells and that there were other cells more involved in protecting against the contents of ELVs?**

Individuals infected with LF have been shown to have an impaired lymphocyte proliferation. There is a reduction in filarial-specific B and T lymphocyte precursors, as well a lower amount of CD3+ T cells and B cells that respond to the parasite (King, 2001). This could indicate that the immune cells more involved in the immune response to B. malayi are the lymphocytes and not the macrophages, which could account for the inefficient response from the cells.

1. **Why was *Brugia malayi* was chosen as the organism studied in this paper if there are two other parasitic nematodes that cause LF (*Wuchereria bancrofti* and *Brugia timori*)? Why not study all three parasites?**

The Zamanian paper was actually the first study to identify the exosome-like vesicles of filarial nematodes, and the researchers discovered them in *Brugia malayi* (Wu et al, 2019 and Zamanian et al, 2015). In addition to this being the first instance of these ELVs being identified, *B. malayi* is the easiest of the three parasites to work with in a laboratory setting. The parasites are easy to raise, and the livestock needed to maintain them is standardized for *B. malayi*. *W. bancrofti* is currently being studied, but not at the same levels as *B. malayi*. *B. timori* is unfortunately hard to find to study.

While the discovery of these exosome-like vesicles is an exciting advance in understanding lymphatic filariasis, there are still many challenges faced in fully characterizing the roles of ELVs. There is still no definitive classification of the ELVs or the miRNAs contained within them. Identifying the functions of the microRNAs contained in these exosome-like vesicles is crucial to determining the host targets being manipulated by *B. malayi* during infection. This could also elucidate any conserved effectors released by the parasite. It is also wondered whether there is specificity in the host tissues or cells targeted during infection. The answer to this could explain why the abnormal swelling and enlargement symptom of lymphatic filariasis occurs only in a few specific body parts. And if it is the case that specificity occurs, what are the underlying molecular mechanisms behind this selectivity (Zamanian et al, 2015)? Answering these questions will bring to light certain interactions between the parasite and its host, which will address the overarching limitation of studies in this field – the lack of understanding of host-parasite interactions. As mentioned earlier, the mechanisms by which host biology is manipulated are not well defined. Future research could further investigate these interactions and biological pathways, answering the questions posed by the current gaps in knowledge.

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