CHAPTER IV

DISCUSSION

Exsheathment is an important and necessary process for the development of microfilariae into L3 infective larvae (Ewert 1965). In the present study, exsheathment of *B. malayi* microfilariae was found to occur in the midgut of the refractory *Ae. aegypti* (Thailand strain) from 5 min to 48 h PIBM and before penetration into the hemocoel. Although we cannot completely exclude the possibility of exsheathment occurring during penetration of the midgut wall in some microfilariae, the finding of large numbers of exsheathed microfilariae in the midgut and SEM observations of only exsheathed microfilariae penetrating the gut wall, both indicate that most if not all exsheathment occurred in the midgut. These results using a refractory strain of *Ae. aegypti* contrast with those for *B. malayi* microfilariae in the susceptible Black eye Liverpool strain, which do not exsheath until they penetrate the midgut wall (Yamamoto et al 1983; Agudelo-Silva and Spielman 1985; Perrone and Spielman 1986), and similarly also contrast with those in the susceptible vector *Oc. togoi* that only sheathed *B. malayi* microfilariae penetrate the midgut epithelium (Jariyapan et al. 2013). Although Chen and Shin (1988) have reported that *B. pahangi* microfilariae tend to carry their sheaths into the hemocoel of both susceptible (Liverpool) and refractory (Bora-Bora) strains of *Ae. aegypti* within 2 h after feeding on a filarial-infected rat, some of the microfilariae were exsheathed in the midgut of both strains. Perrone and Spielman (1986) have demonstrated that exsheathment of *B.*

malayi microfilariae in the *Ae. aegypti* (Black eye Liverpool strain) is enhanced by movement against the constricting midgut wall. The sheath of *B. pahangi* is suggested to be weakened or broken at the anterior end of the microfilariae during penetration of the midgut of *Ae. aegypti*, Black eye Liverpool strain (Christensen and Sutherland 1984). In the current study sheathed *B. malayi* microfilariae in the midgut were surrounded by small particles and maceration of the sheath was also observed in the *Ae. aegypti* midguts, suggesting that the midguts of the refractory mosquitoes might have protein(s) and/or enzyme(s) and/or other factor(s) that induce and/or accelerate degradation of the sheath and consequent exsheathment. To our knowledge, this is the first report of such small particles and maceration of the microfilarial sheath in the midgut of a refractory strain of *Ae. aegypti*. It will be important to investigate other refractory strains of *Ae. aegypti* and/or mosquito species to determine whether similar observations are made as in the Thailand strain and determine whether this is a general mechanism.

During blood meal digestion, several proteolytic enzymes are released in the *Ae. aegypti* midgut to degrade blood meal proteins into peptides and amino acids, for example, trypsins (Felix et al. 1991; Barillas-Mury and Wells 1993; Kalhok et al. 1993), chymotrypsins (Jiang et al. 1997; Bian et al. 2008), aminopeptidases (Billingsley and Rudin 1992; Noriega et al. 2002), carboxypeptidases (Edwards et al. 2000; Isoe et al. 2009a), and serine proteases (Isoe et al. 2009b). Tchankouo-Nguetcheu et al (2010) have analyzed the response of *Ae. aegypti* midguts to infection with Chikungunya and Dengue 2 viruses. This study reported that differentially regulated proteins in response to viral infection include structural, redox, regulatory proteins, and enzymes for several metabolic pathways. Some of these proteins, such

as those with antioxidant properties, are probably involved in cell protection. Tchankouo-Nguetcheu et al (2010) have also proposed that the modulation of other proteins such as transferrin, hsp60 and alpha glucosidase may favor virus survival, replication and transmission. Recently, the first proteomic analysis of the *Ae. albopictus* midgut has been reported (Saboia-Vahia et al. 2012). Fifty-six proteins were identified with sequence similarity to entries from the *Ae. aegypti* genome. Five functional networks among the identified proteins include a network for carbohydrate and amino acid metabolism, a group associated with ATP production, and a network of proteins that interact during detoxification of toxic free radicals, among others. Several enzymes, for example, endopeptidase and papaya extract protease, as well as calcium ions, pH conditions, temperature and ivermectin have been proved to be effective in stimulating the exsheathment of microfilariae of *B. pahangi*, *B. malayi*, *Litomosoides carinii* and *W. bancrofti* (Weinstein 1963; Devaney and Howells 1979; Rao et al. 1992). However, to date no specific protein(s) and/or enzyme(s) and/or factor(s) involved in exsheathment of microfilariae in the midgut of *Ae. aegypti* have been identified. Therefore, further work to identify and characterize the small particles and maceration of the *B. malayi* microfilarial sheath in the midgut of the refractory *Ae. aegypti* (Thailand strain) is required. Further, as exsheathment and midgut penetration are important processes for development of microfilariae, the potential interruption of transmission by interfering with the parasite molecules involved in exsheathment and penetration could be important in the development of transmission control strategies.

Exsheathed *B. malayi* microfilariae were observed penetrating the PM and midgut epithelium into hemocoel of the *Ae. aegypti* (Thailand strain) suggesting that

the PM of the refactory strain was not a barrier against migration of the microfilariae. These observations are in accordance with several previous studies that have reported the PM is not a barrier against migration of *Brugia* microfilariae to the hemocoel in susceptible *Ae. aegypti* (Black eye Liverpool strain) and *Oc. togoi* (Ewert 1965; Christensen and Sutherland 1984; Jariyapan et al. 2013).

Melanization is an innate immune response mounted against foreign organisms in insects (Christensen et al. 2005, Zou et al. 2010; Thomas et al. 2011; Sakamoto et al. 2011; Tokura et al. 2014). In this study, melanized *B. malayi* microfilariae were discovered in the hemocoel at 96 h PIBM suggesting that the *Ae. aegypti* (Thailand strain) used melanization reactions against this parasite. An explanation might be that as the microfilariae were too large to be phagocytosed by hemocytes, therefore, the mosquitoes synthesized melanin as a defensive reaction against molecules on the surface of the body of the exsheathed microfilariae. Consequently, the microfilariae were unable to develop further to a larval stage. Melanization of sheaths and microfilariae of *B. malayi* have also been observed in *An. quadrimaculatus* and *Ae. aegypti* (Nayar and Knight 1995). Recently, Saeung et al (2013) have reported melanization of *B. malayi* L1 larvae in refractory vectors, *An. paraliae, An. sinensis* and *An. nitidus*. Two refractory mechanisms, direct toxicity and/or melanotic encapsulation, have been suggested to be involved in the refractoriness of development in the thoracic muscles of the mosquitoes (Saeung et al. 2013). In *Cx. p. pipiens*, Michalski et al (2010) have demonstrated that *B. pahangi* and *B. malayi* microfilariae in the mosquito midgut have compromised motility, sharp bends in the body and internal damage, revealed as the presence of many fluid or

carbohydrate-filled vacuoles in the hypodermis, body wall, and nuclear column, indicating that the *Cx. p. pipiens* midgut has factor(s) that damage the microfilariae.

The results obtained in the present study suggested that the *Ae. aegypti* (Thailand) mosquitoes have refractory factor(s) for the NSP *B. malayi* development in both the midgut and hemocoel.