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MELANIZATION OF SEPHADEX BEADS BY THE MALARIA VECTOR, *ANOPHELES GAMBIAE*: EFFECT OF BLOOD MEAL, AND MECHANISMS OF REPRODUCTIVE COSTS

Ashraf M. Ahmed

Department of Zoology, Faculty of Sciences, University of El-Minia, El-Minia, Egypt.

Key Words : *Anopheles gambiae*, melanization, follicular apoptosis, follicular resorption, Sephadex beads, reproductive costs.

ABSTRACT

Melanization is the main immune response killing malaria parasite in the gut of the malaria refractory mosquitoes. This study investigates this response against Sephadex beads model in the malaria susceptible *Anopheles gambiae* at 20 h post-beads inoculation. Follicular apoptosis and resorption have been investigated as two suggested mechanisms underlying reproductive costs as a result of this immune response. Thus, one of two different beads was inoculated into mosquito thoracic haemocoel, either a negatively charged CM C-25 or a neutral CM G-25 one. Melanization was relatively strong against neutral G-25 beads in sugar-fed mosquitoes, and become noticeably stronger after a blood meal. In contrast, melanization was relatively weak against negatively charged C-25 beads in both sugar- and blood-fed mosquitoes. This study also shows follicular apoptosis and follicular resorption as two main factors contributing to beads melanization-induced inhibition in mosquito oogenesis. The mean % of follicular resorption was significantly higher in the ovaries of C-25 beads inoculated mosquitoes compared to that in the ovaries of both control and those injected with *Aedes* physiological saline (APS). However, it was significantly lower than that in the ovaries of G-25 beads inoculated mosquitoes-which showed an overall significant more follicular resorption compared to all experimental mosquitoes. Thus, beads melanization and the concomitant follicular resorption seem to be depended on the type of inoculated bead. Such differences may, on one hand, mirror the variation of mosquito immune efficacy against different malaria parasites in natural populations and, on the other hand, limit the malaria immuno-control strategies.

INTRODUCTION

Malaria is regarded as the most serious of the parasitic diseases, causing 300–500 million clinical cases and 1.5–2.7 million deaths annually (www.who.org). It is caused by protozoans of the genus *Plasmodium* and is transmitted by anopheline mosquitoes during blood feeding. Based on the ability to transmit malaria, mosquitoes are categorized into either susceptible or refractory. In susceptible mosquitoes (the malaria vector), malaria parasite succeeds to complete its life cycle. In refractory mosquitoes, the innate immune system is capable of completely blocking of the malaria development inside its body *via* different mechanisms: (i) by physiological incompatibility between the mosquito and the parasite (Nijahout, 1979), (ii) by melanotic encapsulation of ookinetes in the midgut tissues (Collins, *et al*, 1986) or (iii) by preventing sporozoites from penetrating the salivary gland (Rosenberg, 1985).

Therefore, as a result of pesticide resistance of the mosquito vector and the rapid spread of multiple anti-malarial drug-resistance of malaria parasite, the vector immune system became the main recent target of malaria control-aiming researches. This objective is mainly to reduce the competence of the vector to transmit malaria *via* utilizing its immune system to kill the malaria parasite in its midgut. Transmission-blocking immunity is the most rapidly evolving strategy towards this aim (Shahabuddin *et al*.; 1998 Yoshida *et al*.; 2001) including genetic selection of malaria-refractory vector (Collins *et al.;* 1986) and genetically modifying a malaria incompetent vector (James *et al*., 1999 and Kokoza *et al.;* 2000). On the other hand, the success of these immuno-control strategies depends, in my opinion, on tow major parameters; the first is the ability of this modified vector to: (i) inherit the introduced parasite-blocking immune genes to its subsequent offspring in the wild (i.e. to be reproductively fit) and (ii) to be capable of effectively compete with the other wild-mating populations, otherwise they would be at a selective disadvantage. The second parameter is not to impose fitness costs on the immuno-genetically modified vector otherwise will greatly limit these efforts.

Many fitness costs parameters have been shown to be concomitant to refractoriness **(**Ferdig, *et al*., 1993 and Yan *et al.* 1997**)** in the malaria refractory mosquito. In susceptible mosquitoes, stimulating the immune system has also been found to be costly in terms of fecundity reduction (Ahmed *et al*., 2002 and Schwartz & Koella, 2004). Thus, fitness costs imposed upon the

vector as a result of either refractoriness or mounting immune responses are, in my opinion, limiting these strategies. I would, therefore, believe that a better understanding of the fitness costs of mounting immune responses may help in developing a novel and less costly way of blocking malaria transmission by its mosquito vector.

The African human malaria vector, *An. gambiae*, has been chosen for this study to achieve two objectives. The first is to investigate the melanization response against Sephadex® beads, as an alternative model of malaria parasite, in the absence and presence of blood meal. The second is to investigate the mechanism(s) underlying melanization-inhibiting oogenesis in this vector, which may help improving our understanding the immunity-reproduction trade-off race.

MATERIALS AND METHODS

Mosquito colony

The malaria vector, *An. gambiae* (KIL strain), was reared, maintained and used for this study in the Vector Biology and Parasitology Laboratory at Keele University, United Kingdom. Mosquito size gives an indication about blood meal size, which subsequently affects vitellogenesis (Briegel, 1990 and Hurd *et al*., 1995). Thus, mosquitoes were reared in standardised conditions to produce adults of similar sizes as detailed in Ahmed *et al*., (1999). Moreover, a randomly selected group of 20 mosquitoes from each experimental group was checked to verify that they were of similar sizes by measuring their wing lengths (Briegel, 1990). No significant differences in wing sizes of experimental mosquito groups were detected within treatments.

Inoculation of Sephadex® beads into sugar- and blood-fed mosquitoes

To inoculate beads into sugar-fed mosquitoes, 6 days-old mosquitoes were randomly divided into two groups (30 mosquitoes each). One group was inoculated with the negatively charged CM C-25 Sephadex® beads, and the other group was inoculated with the neutral CM G-25 ones (Sigma-Aldrich, UK). Beads were re-hydrated in *Aedes* physiological saline (APS) (13 mM NaCl, 0.5 mM KCl, 0.1 mM $CaCl_2$) prior to inoculation into mosquitoes. Mosquitoes were immobilized by chilling on ice for 5 min prior to beads inoculation. One bead (in 0.25 µl APS) was inoculated into mosquito thoracic haemocoel (one bead/mosquito). Beads inoculation took place using a calibrated heat-pulled micro-capillary needle. Because beads range in size from 40 to 120 µm in diameter, only beads within 40-80 µm in diameter (~ 50 µm) were aspirated into the inoculation needle to reduce the wound caused by injection as possible.

To inoculate beads into blood-fed mosquitoes, 6 days-old

mosquitoes were starved for 12 h prior to feeding on the blood of an anaesthetised CD mouse for 20 min. Immediately after feeding, fully engorged females were randomly selected and assigned to two groups (30 mosquitoes each) and inoculated with the two types of beads as detailed above.

In both of the above experiments, inoculated mosquitoes were maintained in small cages (6 x 6 x 6 cm), kept in their rearing insectary and allowed access to 10% glucose solution. At 20 h post-inoculation, only active mosquitoes (that were capable of moving and flying) were used for dissecting their thoraces for monitoring beads melanization. According to Koella and Sørensen (2002), beads melanization was scored in three degrees; no visible melanization (white bead), patchy (dotted or half melanized bead) and complete melanization (dark black beads) (Fig. 1). Percentages of mosquitoes that showed each melanization degree were then calculated (in 10 mosquitoes from each group).

Detection of follicular resorption

To investigate follicular resorption, 6-days-old mosquitoes were allowed to blood feed as mentioned above. Immediately after feeding, fully engorged females were randomly selected and assigned to 4 groups (30 mosquitoes each). The first and second groups were inoculated with C-25 and G-25 beads respectively as detailed above. The third group was injected with 0.25 µl APS (sham injection). The forth group was left untreated (control). Ovaries from treated mosquitoes were then dissected in APS at 20 h post-blood meal/treatments. Ovaries were then dipped in Neutral Red [0.5% (w/v) solution in citrate-phosphate buffer (0.1 $moll^{-1}$ citric acid/0.1 $moll^{-1}$ sodium citrate) at pH 6 for 1 min to assist the visualization of resorped follicles (Clements and Boocock, 1984). Neutral Red quickly penetrates the epithelium of the resorped follicle and concentrates in its lysosomes. Hence, resorped follicle exhibits an increased uptake of Neutral Red (Bell and Bohm, 1975; Clements and Boocock, 1984) and so yields a deep red colour at a slightly acidic pH (Wilson, 1990) comparing to normal one. Resorped follicles were then counted and % of follicular resorption was calculated/ovary pair (in 10 mosquitoes from each treated group).

Detection of follicular apoptosis

Sample of five ovary pairs from each of blood-fed C-25 and G-25 beads-inoculated mosquitoes were dissected at 18 h post-treatments/blood feeding as detailed above. Ovary pairs were then collected individually in Phosphate Buffered Saline (PBS) (one ovary pair/100 µl) and kept on ice.

Ovaries were then used for detecting caspase-like activity using CaspaTag™ Fluorescein Caspase (VAD) Activity kit (Intergen Company) according to the manufacture's instructions. An amount of 2.5 µl of 30x Working Dilution FAM-Peptide-FMK was added directly to each ovary pair and mixed gently. Ovaries were then incubated for 1 h at 37°C under 5.0% CO_2 while protected from light. Ovaries were then washed twice in 1x Working Dilution Wash Buffer. Each ovary pair was then moved onto a clean slide and mounted in Vectashield® mounting medium, and immediately examined under a fluorescence microscope using a band pass filter (excitation 490 nm, emission 520 nm). Fluorescing follicles, containing caspase-like activity (undergoing apoptosis), were clearly detectable.

Statistical analysis

Statistical analysis was undertaken using MINITAB software (MINITAB, State College, PA, Version 13.1). Data of follicular resorption were first tested for normality prior to any further analysis. Since data were not normally distributed, Kruskal-Wallis test was used to determine the overall effects of beads-inoculation on follicular resorption. Individual comparisons between test and control groups were made using the non-parametric test, Mann-Whitney *U*. Wing measurements of experimental mosquitoes were compared using Mann-Whitney *U* for the same statistical reason.

RESULTS AND DISCUSSION

In this study, it is important to clarify three points: (i) because of the limitations of working with human malaria parasite, melanization of Sephadex bead-model was alternatively used to study this most effective response, which mimic what would occur against *Plasmodium* in the vector, (ii) since the number of ookinetes invading mosquito's midgut peaks at 20 h post-infective blood meal (Ahmed, 2004), it is therefore the suitable time for the mosquito immune system to work efficiently against this weak malaria stage. Thus, I used this time point to investigate melanization response against this beads-model and (iii) the size of the blood meal taken by mosquito may participate as a direct/indirect factor in most of the differences detected in this study (i.e. differences in melanization, follicular apoptosis and follicular resorption). Therefore, blood meal size was accounted for to be certain that these differences were not because of different sized blood meals. Thus, wing size (the distance from the wing tip to the distal end of the alula) was used as an estimate of body size, and hence, blood meal size for all experimental mosquitoes (Briegel, 1990). Hence, wing sizes of experimental mosquitoes are mentioned in the

proper places within each relevant experiment in the discussion.

Melanization of beads by sugar-fed mosquitoes

Beads were inoculated into mosquito thoraces, and the three degrees of melanization were then monitored at 20 h post-inoculation (Fig. 1). Percentage of mosquitoes, which showed each melanization degree, was calculated out of 10 active beads inoculated mosquitoes. Fifty percent of mosquitoes showed un-melanized C-25 beads (negatively charged), whereas, only 20% showed un-melanized G-25 beads (neutral) (Fig. 2 A). Half melanization or patching degree was monitored in 40 or 30% of mosquitoes against C-25 or G-25 beads respectively (Fig. 2 A). Complete melanization was monitored in 10 or 50% of mosquitoes against C-25 or G-25 beads respectively (Fig. 2 A). These results suggest that the strongest response, the complete melanization, was exhibited by higher % of mosquitoes against the neutral G-25 beads. This indicates that the strength of melanization response could be dependent on the type of inoculated beads as demonstrated in other studies (e.g. Schwartz & Koella, 2002). This difference in melanization response may be as a result of a specific surface microenvironment (Gorman, *et al*., 1998) and/or the difference in surface charges (Zahedi *et al*., 1992). These differences in surface characters affect the non-self recognition pattern, which, in turn, affect the initiation of melanization response. This may reflect what happened in reality when the immune system works effectively against one pathogen and not the other, or when two strains of mosquitoes respond differently towards the same pathogen. For example, *Plasmodium* susceptible *An. gambiae* poorly melanized the negatively charged C-25 beads whereas, the refractory ones strongly melanized the same beads very quickly (Paskewitz and Riehle, 1994). This, in fact, is mirroring the response of both of mosquito strains towards the ookinetes while developing in their guts.

Melanization of beads by blood-fed mosquitoes

This experiment investigates the effect of blood meal on beads melanization response at 20 h post-beads inoculation/blood meal. Mosquitoes were allowed to a complete blood meal and immediately inoculated with beads. Blood meal has slightly improved melanization response against C-25 beads, which were completely melanized by 20% of blood-fed mosquitoes compared to only 10% of sugar-fed ones (Fig. 2 A & B). However, un-melanized beads were recorded in 50 % of blood-fed mosquitoes, which is similar to that

in sugar-fed ones (Fig. 2 A & B). On the other hand, melanization of G-25 beads was greatly improved in blood-fed mosquitoes relatively to sugar-fed ones (Fig. 2 A & B). Hundred percent of mosquitoes successfully melanized G-25 beads, since 40% of mosquitoes half-melanized and 60% completely melanized these beads (Fig. 2 B). Blood meal size was accounted for to be certain that differences in melanization response are not because of lack of nutrition or starvation. This was justified by allowing mosquitoes to become fully engorged and by measuring wings, which showed no significant difference in wing sizes between C-25 and G-25 inoculated mosquitoes, as they were 2.89 ± 0.1 and 2.87 ± 0.1mm respectively [Mann-Whitney *U*, ($P > 0.05$), n = 20].

These results suggest that blood meal has an enhancing role of melanization response as early as 20 h post-blood meal, which was dependent on the type of inoculated beads. These findings are in consistence with Koella and Sørensen, (2002), who demonstrated the same effect of blood meal in slightly different experimental design. They inoculated mosquitoes with beads 24 h post-blood meal and monitored beads melanization 24 h post-inoculation (i.e. 48h post-blood meal). Thus the current results suggest that blood meal activates melanization response as early as 20 h post-blood meal, which coincides with the peak of ookinete invasion of mosquito gut (Ahmed, 2004).

In the *Plasmodium*-vector model, in despite of being taken with the blood meal by mosquitoes, the parasite succeeds to complete its life cycle without being melanized. This may indicate that the parasite suppresses the immune system of its vector. An evidence for this is the gametes-free blood meal from malaria infected chickens has considerably reduced the efficacy of mosquitoes to melanize Sephadex beads (Boëte *et al*., 2002 and 2004). So, it seems that the parasite has its own active strategy to suppress the mosquito's immune response even in the presence of blood meal. I would therefore expect that the parasite starts this protective strategy while still in its vertebrate host. This could be *via* affecting the quality of the blood, as the immuno-competence of mosquito depends on a sufficient nutritive blood meal (Schwartz and Koella, 2002). Although however, I still suggest that the blood meal activates the immune system of mosquito in such effective level that, in one way or another, is promising and helpful towards utilizing the immune system of mosquito to block malaria development in its gut.

Mechanism of reproductive costs

Anopheles gambiae mosquitoes that melanized Sephadex® beads showed a resultant reproductive costs in terms of fecundity reduction as they laid

fewer eggs comparing to control ones (Hilary Hurd, Keele University, UK, personal communication, data are not shown). In the present study, two mechanisms underlying this fecundity reduction have been demonstrated.

A)- Follicular apoptosis

Naturally, apoptosis (programmed cell death) is essential for the removal of unwanted cells and is critical for both restricting cell numbers and for tissue patterning during development of living organism. Central to the apoptosis process is the family of cysteine proteases, known as caspases, which are activated by death-inducing signals, and constitute the biochemical core of the mechanism of apoptosis (Alnemri *et al*., 1996). Caspases are site-specific proteases acting in a cascade to bring about the sub-cellular changes characteristic of apoptosis (e.g. DNA fragmentation, chromosomal condensation and stimulation of phagocytosing the apoptotic cellular fragments (Hengartner, 2000). In insects, one of the important roles of apoptosis is regulating normal oogenesis process (Dorstyn *et al*., 2000). We detected increase in caspases activity in the developing ovarian follicles of malaria infected *An. stephensi* (Hopwood, *et al.*, 2001) and *An. gambiae* mosquitoes (unpublished data) which significantly inhibited oogenesis process.

In the present study, follicular apoptosis has been detected as early as 18 h post-bead inoculation in blood-fed mosquitoes. Apoptotic follicles were monitored in the ovaries of mosquitoes inoculated with C-25 and G-25 beads (Fig. 3 A1 & A2 and B1 & B2). Moreover, apoptotic follicles were seen in relatively larger numbers in the ovaries of mosquitoes inoculated with G-25 beads compared to those inoculated with C-25 ones (Fig. 3 A2 and B2). This suggests that melanization response could have triggered more follicular caspases activity, which may depend on the strength of melanization response. Higher magnification (Fig. 3 E) shows a whole fluorescing follicle, which may suggest caspases activities taking place in all follicular cells, and subsequently, all follicular cells are dying at the same time. When caspases activity was inhibited in malaria infected mosquito, follicular resorption was significantly decreased (Hopwood, *et al.*, 2001). This supports my premise that apoptosis in the follicular epithelium initiates follicular resorption and thus, considered as one of the possible pathways towards follicular resorption afterwards.

B)- Follicular resorption

In general, mosquito egg production is cyclical and synchronous. The blood meal initiates the maturation of the ovarian follicles, resulting in oviposition of an egg batch at the end of a gonotrophic cycle. Many insects are able to adjust their egg production according to physiological conditions such as nutrient supply and mating success (Clements and Boocock, 1984). One way, in which this is achieved, is by resorption of some, or all, of the ovarian follicles at some stage during oogenesis. The present study shows this process in the ovaries of blood-fed non-injected (control), APS-injected (sham injected) and beads inoculated mosquitoes at 20h post-blood meal/treatments. Resorped follicles were detected as dark reddish in colour, as a result of uptaking neutral red (Fig. 3 C & D), which stained their internal contents (Fig. 3 F). Resorped follicles were counted, and % of follicular resorption was calculated per ovary pair in 10 mosquitoes from each experimental group. Individual comparisons using Mann-Whitney *U* test showed no significant difference in the mean % of follicular resorption between control and sham injected mosquitoes ($P > 0.05$, n = 10) (fig. 4). As a comparison, this % significantly increased in the ovaries of mosquitoes inoculate with both C-25 and G-25 beads ($P < 0.05$, n = 10) (Fig. 3 C & D and Fig. 4). Ovaries from mosquitoes inoculated with G-25 beads showed significant higher mean % of follicular resorption compared to those from mosquitoes inoculated with C-25 beads ($P < 0.05$, n = 10) (Fig. 3 C & D and Fig. 4). These results suggest increase in follicular resorption as a result of melanization response, which significantly increased with the increase of melanization degree. Nutrition pressure is unlikely to participate as a direct reason for this follicular resorption in this study for two reasons: (i) the control and treated blood-fed mosquitoes used in this study were fully engorged with nutritive blood from the same mouse and (ii) the blood meal size was accounted for to be certain that differences in follicular resorption were because of bead melanization and not because of different sized blood meals. This is justified as no significant differences were detected in mosquito sizes (wing sizes of control, shame injected, C-25 and G-25 inoculated mosquitoes were 2.91 ± 0.11, 2.89 ± 0.1, 2.90 ± 0.15 and 2.88 ± 0.12 mm respectively [Mann-Whitney *U*, ($P > 0.05$), n = 20] indicating similar blood meals in all experimental mosquitoes.

Our previous study (Hopwood *et al*., 2001) detected follicular apoptosis in the ovaries of malaria infected mosquitoes as early as 16 h post-infection which precedes follicular resorption. In the present study, follicular apoptosis was clearly shown at 18 h post-beads inoculation. This may

suggest that follicular cells undergo apoptosis prior to resorption process, which I propose as a suggested mechanism underlying fecundity reduction as a result of melanization response. Evidences for this are: (i) when we injected malaria-infected mosquitoes with 0.25 µl of the apoptosis inhibitor, z-VAD.fmk, follicular resorption was significantly reduced (Hopwood, *et al*., 2001) and (ii) fecundity of mosquitoes that did not melanize beads did not significantly differ from control ones in a similar study by Schwartz and Koella (2004).

In the immunity point of view, and at earlier stage, the foreign body (e.g. bead in this study) must be first recognized, resulting in activating the prophenoloxidase cascade, which in turn activates the melanization cascade (Barillas-Mury *et al*., 2000; Dimopoulos *et al*., 2001). The processes involved in both egg production and bead melanotic encapsulation are utilizing a similar melanization pathway, beginning with the enzymatic activation of tyrosine, the precursor of melanin (Paskewitz & Christensen, 1996). I would, therefore, assume that the reproductive cost of mounting immune responses may have happened as a result of a re-allocation of the limited reserves during the formation of melanin from tyrosine. Whichever way *via* which resource re-allocation operates, physiological link(s) between immune stimulation and oogenesis exists, although it remains undefined. Thus, I would expect a direct competition between the defence and reproductive systems for nutrients required for oogenesis and for the synthesis of molecules involved in the immune cascade, as proposed by Ferdig *et al*. (1993). Moreover, it is perhaps worth noting that the follicular apoptosis, and subsequently, follicular resorption reported in the present study are indeed due to the melanization of beads and not due to the melanization response induced for wound healing after bead inoculation, since injecting APS, using the same needle, showed no significant difference in the mean % of follicular resorption compared to that of control mosquitoes (Fig. 4) [Mann-Whitney *U*, ($P > 0.05$), n = 10].

In conclusion, involving of the vector immune system in the battle against malaria is not only costly in terms of reproductive fitness (*via* follicular apoptosis an resorption) but also of some other fitness components for example, shorter egg-to-adult developmental time and shorter mean longevity of adults of refractory mosquitoes compared to the susceptible ones (Yan *et al.*, 1997). Thus, the present study puts forward one more evidence in support of the hypothesis that there is a trade-off between immune system and reproduction operates in mosquitoes

such that reproductive success may be curtailed when the insect mounts an immune response. This, in my opinion, greatly limits the success of the most recent immuno-control strategies aiming at utilizing the mosquito immune system to block malaria transmission. Thus, I would strongly recommend that care should be taken while thinking about these strategies.

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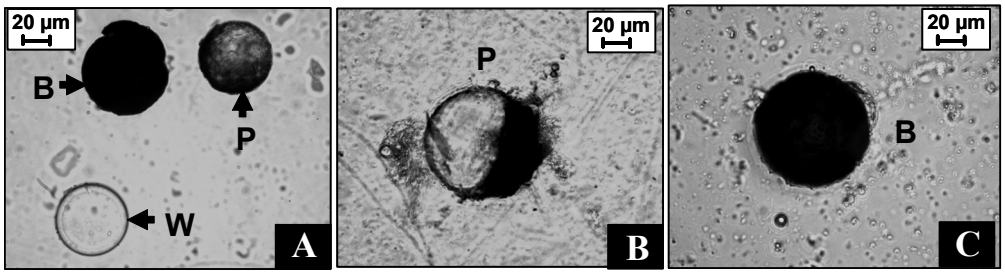

Fig. 1. Melanization degrees of Sephadex® beads by *An. gambiae* mosquito. One bead, in 0.25 µl APS, was inoculated into mosquito thorax (one bead/mosquito). Thoraces were dissected for monitoring melanized beads at 20h post-inoculation. Based on the intensity of melanization, beads were categorised into three degrees; un-melanized or white (**W**; fig. A), half melanized or patchy (**P**; fig. A or B) and completely melanized or black (**B**; fig. A & C).

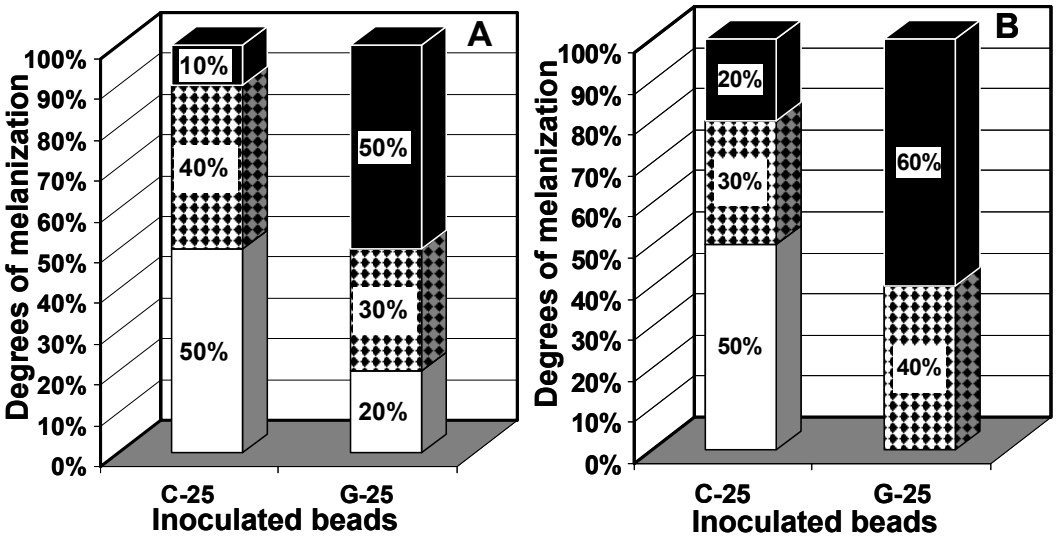

Fig. 2. Degrees of melanization of Sefadex® beads by the immune system of *An.gambiae*. Bars represent the mean % of 10 replicates of mosquitoes (n = 10) that showed different melanization degrees. Beads were inoculated into the thoraces of sugar-fed mosquitoes (**A**) or blood-fed mosquitoes (**B**) immediately after taking a blood meal. Thoraces from each mosquito groups were dissected at 20h post-inoculation for scoring beads melanization degrees. White bars represent the % of mosquitoes that showed un-melanized beads. Dotted bars represent the % of mosquitoes that showed patchy or half melanized beads. Black bars represent the % of mosquitoes that showed completely melanized or black beads.

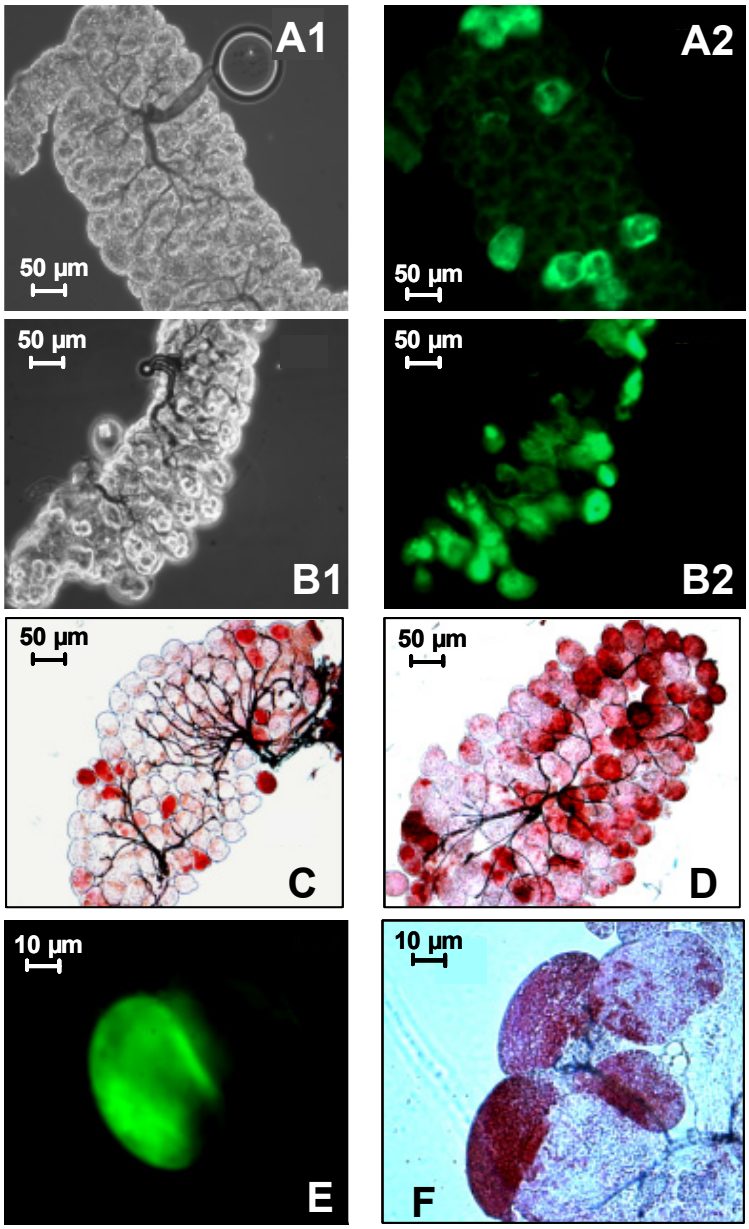

Fig. 3. An illustration plate showing apoptosis and resorption of the developing follicles in the ovaries of blood-fed *An. gambiae* as a result of melanizing Sephadex® beads. Ovaries from beads inoculated mosquitoes were dissected and used for monitoring follicular apoptosis or follicular resorption at 18h or 20h post-blood meal/inoculation respectively. Apoptotic follicles (green in colour) were examined under a fluorescence microscope to view green fluorescence. **A1** and **A2** represent ovaries from mosquitoes inoculated with C-25 beads seen under normal light microscope and fluorescence microscope respectively, which show relatively fewer apoptotic follicles compared to mosquitoes inoculated with G-25 beads (**B1** & **B2**). Resorped follicles (reddish in colour) were counted and percentages of follicular resorption were calculated/ovary pair. **C & D** represent resorped follicles in ovaries from mosquitoes inoculated with C-25 and G-25 beads respectively, showing significantly higher % of follicular resorption in an ovary from G-25 inoculated mosquito (**D**). **E** represents higher magnification for an apoptotic follicle showing detection of caspases activity within the whole follicular body. **F** represents higher magnification for a group of resorped follicles showing them in dark red as a result of uptaking Neutral Red.

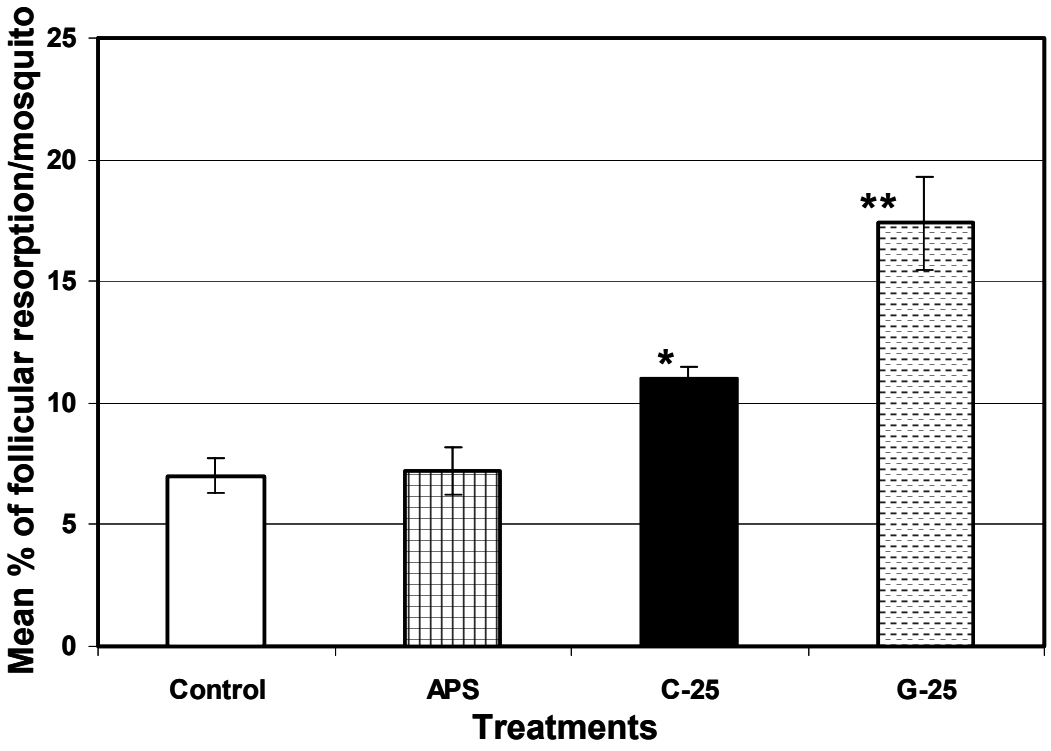

Fig. 4: Follicular resorption in *An. gambiae* at 20h post-treatments. Percentages of resorped follicles/ovary pairs were calculated from ovaries of control (blood-fed), APS-injected, C-25 inoculated and G-25 inoculated mosquitoes at 20h post-treatments. Data were first tested for normality prior to further individual analysis using Mann-Whitney *U* test, as they were not normally distributed. Error bars represent the standard error of means of 10 replicates (10 ovary pairs) in each case. *Significantly higher compared to control and sham injected; **significant higher compared to all other experimental mosquito groups ($P < 0.05$).

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Email: info@egsz.org.

المؤتمر الدولى الخامس عشر
للجمعية المصرية الألمانية لعلم الحيوان
كلية البنات – جامعة عين شمس
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مَلْنَنَة حُبَيبات السِيفادِكْس بواسطة البعوضة الناقلة للملاريا، أنُوفِيلِيس جَامْبيا: تأثير وجبة الدم، وميكانيكية التكاليف التناسلية

أشرف محمد أحمد

قسم علم الحيوان، كلية العلوم، جامعة المنيا، المنيا، مصر.

تُعد الملْنَنَة هى الإستجابة المناعية الأساسية لقتل طفيل الملاريا فى معيِ البعوض المماِنع له. هذه الدراسة توضح هذه الإستجابة المناعية ضد نموذج حبَيْبات السِيفادِكْس فى بعوضة أنُوفِيلس جامبِيا، والحساسة لهذا الطفيل، وذلك بعد ٢٠ ساعة من إدخال الحبَيْبات فى الحيِز الدموى لمنطقة الصدر. و بالتالى، تم دراسة عمليتى الموت المبَرمج، أبُوبتُوزيز، و الاضمحلال فى الحويصلات البيضية المتنامية فى المبيض، وذلك كميكانيكيتان للتكلُفة التناسلية، و الناتجة عن هذه الإستجابة المناعية. و لذلك تم إدخال واحدة من نوعين مختلفين من الحبَيْبات، إما سالبة الشحنة CM C-25 أو المتعادِلة CM G-25 فى الحيِز الدموى لصدر البعوضة. لوحظ أن الملْنَنَة كانت قوية نسبيا ضد حبَيْبات G-25 فى البعوض المغْتذى على محلول السكر، و التى أصبحت أقوى نسبيا بعد وجبة الدم. على الجانب الآخر، كانت الملْنَنَة ضعيفة نسبيا ضد حبَيْبات C-25 فى البعوض المغتذى على السكر و أيضا المغتذى على الدم. كما توضح هذه الدراسة أيضا أن الملْنَنَة تسببت فى تزايد عمليتى الموت المبَرمج والاضمحلال للحُويصلات البيضية المتنامية، كعاملين يؤديان إلى تَثْبيط عملية التَبْويض. فقد وجِد أن متوسط نسبة الاضمحلال فى الحويصلات البيضية المتنامية قد ارتفع نوعيا فى مبايض البعوض المزروع فيه حبَيْبات C-25 مقارنة بتلك فى مبايض كل من بعوض التجربة الضابطة و ذلك المحقون بمحلول البعوض الفسيولوچى APS. فى حين أنها كانت أقل نوعيا من تلك فى مبايض البعوض المزروع فيه حبَيْبات G-25، و الذى أظهر زيادة نوعية فى اضمحلال الحويصلات البيضية المتنامية مقارنة بتلك فى كل بعوض المعاملات الأخرى فى هذه الدراسة. و لهذا، فإن ملْنَنَة الحبَيْبات، و اضمحلال الحُويصلات البيضية المتنامية والمرتبط بهذه الإستجابة، يبدوان أنهما يعتمدان على نوع الحبَيْبات المزروعة. فمن ناحية، يعتبر هذا التباين إنعكاسا للتباين فى قُدرات الجهاز المناعى ضد أنواع طفيلات الملاريا المختلفة فى مجتمع البعوض الطبيعى. و من ناحية أخرى، فإنه يحِد من الإستراتيچيات الحديثة والتى تَهدف إلى استخدام المقاومة-المناعية لطفيل الملاريا.