J Vect Borne Dis 43, September 2006, pp. 144–146

Human chitotriosidase helps *Plasmodium falciparum* in the *Anopheles* midgut

M. Di Luca[a], R. Romi[a], F. Severini[a], L . Toma[a], M. Musumeci [a], A.M. Fausto[b], M. Mazzini[b], G. Gambellini[b] & S. Musumeci[c]

[a]*Istituto Superiore di Sanità, Department of Infectious, Parasitic and Immune-mediated Diseases;*[b] *Department of Environmental Sciences and Interdepartmental Centre of Electron Microscopy, University of Tuscia, Viterbo, Italy;* [c]*Department of Pharmacology, Obstetrics and Gynecology, Pediatrics, University of Sassari, and Institute of Population Genetics, CNR, Alghero, Sassari, Italy*

Key words Chitotriosidase – peritrophic matrix – malaria – *Plasmodium falciparum*

High levels of plasma chitotriosidase (Chit) represents a marker of macrophage activation in human malaria infection[1]. *Plasmodium falciparum* during its maturation cycle in the *Anopheles* midgut produces an analogue of Chit for the digestion of peritrophic matrix (PM)[2]. During development in the *Anopheles* midgut, the invasive ookinete, penetrates the PM secreting focally Chit. Then it invades the midgut wall where it settles down as an oocyst producing several thousands of sporozoites that complete the cycle when reaching the salivary glands[2]. If PM penetration depends on matrix disruption and destruction, Chit present in the blood meal from malaria patients could help the parasite in this function. We tested this hypothesis in a "Membrane feeding assay". Batches of 30 mosquito females, placed in different containers, were fed either on whole blood of healthy donors or on blood, where Chit from different sources was added. After 16, 20 and 24 h blood-fed mosquitoes were anesthetised with CO_2 and dissected to extract the midgut. Intact midguts were immediately fixed in formaldehyde, stained with toluidine blue and then observed with Zeiss Axiophot optical microscopy. For transmission electron microscopy (TEM), ultrathin sections (60–70 nm thick) were collected on copper grids, stained with uranyl acetate and lead citrate, and observed with a JEOL 1200 EX II electron microscope.

Morphological examination of the control midgut, fed with blood of a healthy donor revealed a fully developed PM by 16 h (Fig. 1). It appears at the light microscope level as a light brownish layer between the interstitial epithelium and the alimentary bolus. The PM structure was recognised as an amorphous and laminated structure segregating the blood meal. The PM enclosed and surrounded the entire blood meal just above the epithelium. The PM increased its thickness at 20 h and decreased at 24 h, in the midgut obtained from *Anopheles* (Fig. 1), when the insects were fed with the blood of a healthy donor. The natural colour (dark) can be attributed to the presence of haeme bound to the matrix and to haeme precipitates clearly visible between the erythrocytes and the PM after the blood meal. PM formation was loosed in midgut of *Anopheles* fed with blood of a healthy donor added with plasma of malaria patients containing 0.68 and 0.95 mU/ml of Chit. Also the PM in the midgut of *Anopheles* fed with the blood of a healthy donor added with plasma of Gaucher dis-

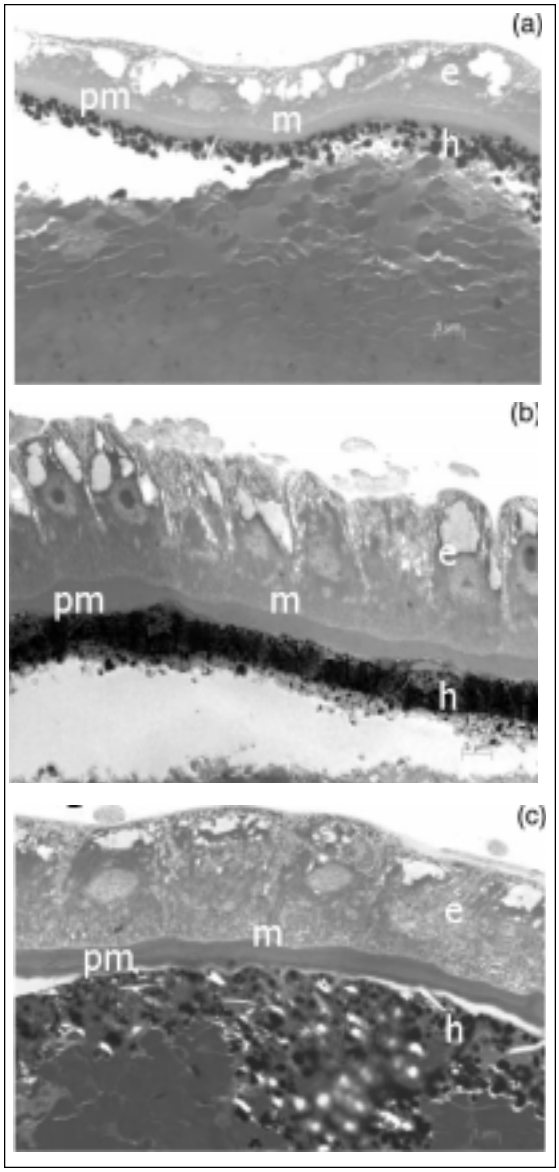

Fig. 1: Morphological examination of the midgut from *Anopheles* fed with the blood of healthy donor revealed a fully developed PM by 16 h (a), which increased its thickness at 20 h and decreased at 24 h (b & c); pm–peritrophic matrix; m–microvilli; h–haeme; e–epithelium

ease patients containing 5.2 and 10.4 mU/ml of Chit was markedly reduced in thickness. However, at these levels of Chit activities the structures of PM appear conserved. Only in the samples of blood donor enriched with commercial *P. falciparum* Chitinase (PfCHT1), 100 and 2000 mU/ml, produced by New England Biolabs, Inc, USA, the PM appear clearly disrupted and the haeme precipitates reach the epithelium. The thickness of PM examined at 20 h after blood meal was furtherly reduced in all samples, but in the sample enriched with PfCHT1 the PM was not visible, substituted by digested erythrocytes and haeme precipitates. The alterations of PM thickness were visible in all samples containing variable levels of Chit activity at 24 h after blood meal. The structure of PM was disrupted showing the passage of red cells and precipitates of haeme through the residual membrane, in a progression proportional to the Chit activity of blood meal. In Fig. 2 the progression or regression of PM in the presence of Chit, as measured on the microscopic section of stomach (MP/epithelium) is graphically reported. In fact in the midgut of *Anopheles* fed with blood of malaria patients and of Gaucher patients the PM formation was measurable after 16 h, but clearly reduced at 20–24 h in a degree proportional to Chit contents. These alterations were clearly documented at TEM at 16, 20 and 24 h. Now most of haeme bound to the PM is associated with enormous number of small electron dense granules and the residual PM is full of erythrocytes and haeme precipitates, also if the PM structurally could appear conserved (Fig. 3).

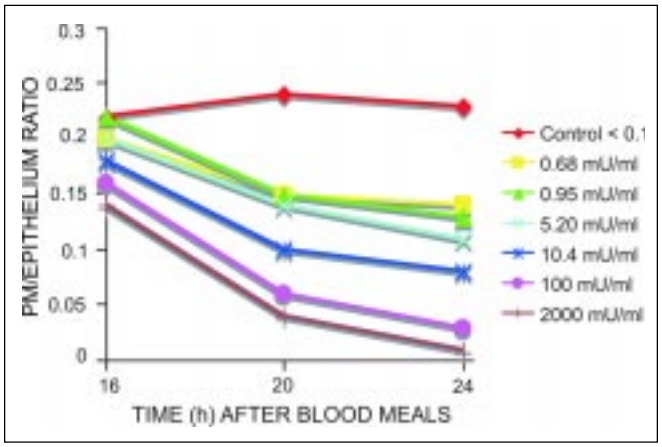

Fig. 2: PM/epithelium ratio measured on the optical images at 16, 20, 24 h. The values are the mean of ten measurements in different samples

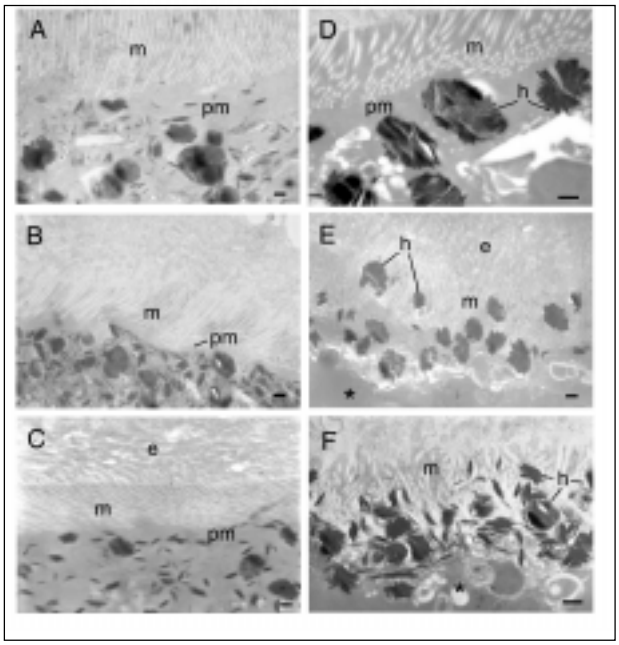

Fig. 3: TEM micrographs of midgut sections from *An. stephensi* female mosquitoes at 16 h (A, D), 20 h (B, E) and 24 h (C, F) after blood feeding. Scale bar = 500 nm; A, B & C: mosquitoes fed with the blood of healthy donors. PM is well formed showing an electrondense laminated structure interposed between the microvilli (m) of midgut epithelial cells (e) and the blood meal. During the digestion of blood the PM appears structurally conserved and continues to maintain a barrier function. h: heme precipitates; D, E & F: mosquitoes fed with blood containing 0.95 mU/ml Chit from malaria patient. PM is partly conserved and appears completely filled by haeme precipitates (h) that in some areas directly contact microvilli (m) of epithelial cells; *blood meal

Our results confirm the hypothesis that Chit contained in blood of malaria patients could help *P. falciparum* to complete its cycle in the *Anopheles* midgut and to produce a bigger number of oocysts/sporozoites. This could balance the different genetic protection in humans conferred by the heterozygous Hb beta (S) gene, which seems to be associated with an increasing effect on *P. falciparum* transmission from humans to mosquitoes[3]. In fact if in European regions, where Chit has became redundant for an inactive polymorphism present in 5–6% of homozygous and in 35–45% of heterozygous individuals[4], it may have contributed to the rapid eradication of malaria, together with the use of DDT and of land reclamation[5,6]; on the contrary in African regions, where the inactive polymorphism is absent for the precarious environmental conditions, the high levels of plasma Chit of malaria individuals, may contribute to the persistence of malaria endemicity. In these countries only a genetic change and improved environmental conditions could ward off malaria.

References

1. Barone R, Simpore J, Malaguarnera L, Pignatelli S, Musumeci S. Plasma chitotriosidase activity in acute *Plasmodium falciparum* malaria. *Clin Chim Acta* 2003; *331:* 79–85.

2. Huber M, Cabib E, Miller LH. Malaria parasite chitinase and penetration of the mosquito peritrophic membrane. *Proc Natl Acad Sci USA* 1991; *88:* 2807–10.

3. Robert V, Tchuinkam T, Mulder B, Bodo JM, Verhave JP, Carnevale P, Nagel RL. Effect of the sickle cell trait status of gametocyte carriers of *Plasmodium falciparum* on infectivity to anophelines. *Am J Trop Med Hyg* 1996; *54:* 111–3.

4. Malaguarnera L, Simpore J, Prodi DA, Angius A, Sassu A, Persico I, Barone R, Musumeci S. A 24-bp duplication in exon 10 of human chitotriosidase gene from the sub-Saharan to the Mediterranean area: role of parasitic diseases and environmental conditions. *Genes Immun* 2003; *4:* 570–4.

5. de Zulueta J. Malaria and ecosystems: from prehistory to posteradication. *Parassitologia* 1994; *36:* 7–15.

6. de Zulueta J. The end of malaria in Europe: an eradication of the disease by control measures. *Parassitologia* 1998; *40:* 245–6.

Corresponding author: Prof. Salvatore Musumeci, Department of Pharmacology, Gynaecology/Obstetrics, Pediatrics, University of Sassari and Institute of Population Genetics, CNR, Alghero, Italy
E-mail: smusumeci@tiscalinet.it

Received: 24 January 2006 *Accepted in revised form:* 12 July 2006