

Parasite-derived lipids as putative toxins in *Plasmodium vivax* malaria ?L D Wanasekara^{1*}, R Carter², K N Mendis³ and N D Karunaweera¹¹ Department of Parasitology, Faculty of Medicine, Colombo 8, Sri Lanka² Department of Parasite Biology, ICAPB, University of Edinburgh, UK³ World Health Organization, Geneva, Switzerland

Paroxysms are prominent clinical episodes in *P. vivax* malaria. It is speculated that red blood cells infected with schizonts, the mature blood stage of the malaria parasite, rupture and release toxin(s) during paroxysms. It has been shown that the active fraction of paroxysm plasma was rich in lipids (Sudan III test). This study was carried out to identify the parasite derived factors present in plasma obtained from *P. vivax* patients during paroxysm (paroxysm plasma).

Paroxysm plasma and *P. vivax* schizont extract were heat inactivated and extracted with organic solvents, acetone, 95% ethanol, petroleum ether and diethyl ether. This resulted in 5 fractions of paroxysm plasma and *P. vivax* schizont extract. Residues prepared from these fractions were tested in a cell aggregation assay to check their ability to induce aggregate formation of white blood cells which occurs in the presence of paroxysm plasma. Paroxysm plasma, heat inactivated paroxysm plasma, normal human plasma and extracts of uninfected red blood cells were tested as controls. Results showed that, active parasite factors were present in lipid fractions of heat inactivated paroxysm plasma and freeze-thawed extracts of *P. vivax* schizonts. However, the activity of lipid fractions of paroxysm plasma and schizont extract which was not significant when tested on their own in the cell aggregation assay was enhanced significantly when reconstituted with the recombinant human cytokines rh TNF- α , rh GM-CSF, rh IL-10 and rh IL-6. As such, fraction one and fraction four of the five lipid fractions of paroxysm plasma as well as the schizont extract induced significant levels of cell aggregation, mean % Relative Cell Aggregation Index=36.06 \pm 17.26 & 9.62 \pm 1.42 and 23.13 \pm 14.90 & 27.62 \pm 3.38 respectively. Thin layer chromatography showed that the five lipids fractions of paroxysm plasma as well as the schizont extract contained cholesterol and triglycerides, lecithin, unidentified lipids, phospholipids and a residual pellet with sphingolipids respectively. In the *P. vivax* schizont extract, the more prominent effect was seen in the phospholipid-containing fraction 4. By contrast, the greatest activity from the paroxysm plasma was in the fraction 1 (which contains cholesterol and triglycerides) which is speculated to contain active putative malaria toxins of *P. vivax* infections.

Acknowledgement: Financial assistance by Wellcome Trust, United Kingdom.

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