CYTOKINE RESPONSES TO *PLASMODIUM FALCIPARUM* MALARIA PEPTIDES IN KENYAN ADULTS LIVING IN A SPORADIC MALARIA TRANSMISSION AREA

BY

KISIA LILY

A THESIS SUBMITTED TO THE SCHOOL OF MEDICINE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN IMMUNOLOGY, DEPARTMENT OF IMMUNOLOGY, MOI UNIVERSITY

NOVEMBER, 2009

ABSTRACT

Studies have shown that cytokine responses are important in protection from *P.falciparum* malaria infection and disease. However, convincing T-cell correlates of protection have not been demonstrated in naturally exposed populations. Recent studies have shown that highland malaria transmission can be characterized as seasonal or sporadic, a situation that could lead to differences in the host immune response to malaria in highland populations. This study was undertaken to describe cytokine responses to various *P.falciparum* malaria peptides in a sporadic malaria transmission area of Western Kenya by use of the cytometric bead assay. The results were compared with those obtained from standard ELISA especially in measuring the levels of IFN-γ. To determine cytokine responses to *P. falciparum* malaria peptides in Kenyan adults living in a sporadic malaria transmission area by using the cytometric bead assay. The study was conducted on 29 randomly selected healthy adults living in Kipsamoite village in Nandi North District of Western Kenya during the month of August 2007 after a prolonged period of low malaria transmission. PBMC's were isolated from whole blood by density gradient centrifugation then challenged with malaria peptides/mitogen for 120hrs under CO_2 (5%) and humid conditions. Supernatants were collected and stored at -80°C. The Cytometric bead assay was used to determine the levels of IL-2, IL-6, IL-8, IL-10, IL-12, IFN-γ, TNF-α and RANTES while standard ELISA was used to determine the levels of IFN-γ in the supernatants. Descriptive statistics were used to determine the geometric means, ranges and frequencies of cytokines produced to the malaria peptides tested. Spearman's rank correlation was used to determine the correlations in the levels of IFN-γ to various malaria peptides between standard ELISA and the cytometric bead assay. Informed consent was sought from the participants and they were free to withdraw at any time. Ethical approval to conduct the study was obtained from Moi University Institutional Review Committee, KEMRI Ethical Review Committee and University of Minnesota Institutional Review Board.

The data from this study showed high IL-8 (36-947pg/ml) and moderate IL-6 (0-17.11pg/ml) and RANTES (0.3-9.03pg/ml) levels in response to CSP-22, CSP-35, LSA-1 T3, LSA-1 T4, LSA-1 94, TRAP-TR539, TRAP-TP6 and AMA-1 (n=29) to *P. falciparum* malaria peptides. TNF-α, IFN-γ, IL-12, IL-10 and IL-2 levels were <1.5pg/ml. The frequencies of positive cytokine responses were between 17-56% for IL-8, IL-6, IFN-γ and RANTES and <4% for IL-12, IL-10, IL-2 and TNF-α to the same peptides. On comparing the cytometric bead assay to standard ELISA, the data indicated that there were poor correlations ($rho<0.5$, $p>0.1$) between the two assays in measuring the levels of IFN-γ to all peptides tested except to AMA-1P where the correlation was weak but significant ($rho<0.46$, $p>0.02$) (n=25). This study concluded that IL-8 responses to TRAP-TP6, LSA-1T3, LSA-1 94 and LSA-1 T4, IL-6 responses to LSA-1 T3 and RANTES responses to LSA-1 T4 were higher and more frequent than cytokine responses to other peptides. Though at low levels, IFN-γ responses were frequent. It was also concluded that standard ELISA and the cytometric bead assay are not comparable in measuring the levels of IFN-γ to malaria peptides tested.