1

ASSOCIATIONS BETWEEN PROTECTION FROM MALARIA AND ANTIBODIES TO KNOWN AND PREDICTED MEROZOITE ANTIGENS

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Antibodies play an important role in protective immunity against *Plasmodium falciparum* in humans. Merozoite antigens are likely to be important, but the major targets mediating protection have not been clearly identified. Very few of the large number of merozoite antigens have been studied as targets of human immunity, and few prospective cohort studies have compared responses to a multitude of antigens. In this study we aimed to assess the acquisition of antibodies and protective associations for most merozoite antigens that are regarded as potentially important targets. We screened 139 recombinant proteins that were either known or predicted to be *P. falciparum* merozoite antigens located on the merozoite surface or in apical organelles. After assessment of antigen quality and immunoreactivity, 75 proteins were tested for antibody responses using plasma from a prospective cohort of 206 school-aged children resident in Papua New Guinea. For each antigen, we assessed the acquisition of antibodies to merozoite antigens by examining associations with age, exposure, and active infection, and we prospectively examined associations between antibodies and protective immunity. Antibody responses to almost all merozoite antigens were associated with reduced risk of malaria. However, the strength of protective associations varied substantially between antigen-specific responses, which may reflect their significance as targets of protective immunity. Protection from malaria is likely to result from a combination of responses to different antigens. Examining this, we found that responses to specific combinations of antigens were most strongly associated with protection, which supports the strategy of including multiple antigens in a vaccine. These findings have important implications for understanding and evaluating human immunity, and for the selection of specific candidate antigens for vaccine development.

2

EARLY PRODUCTION OF HIGH AVIDITY ANTIBODIES TO FULL-LENGTH VAR2CSA DURING PREGNANCY CORRELATES WITH ABSENCE OF PLACENTAL MALARIA

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Pregnant women, especially primigravidae, are at risk of *Plasmodium falciparum* malaria because infected-erythrocytes (IE) sequester in the placenta causing placental malaria (PM). Sequestration is mediated by VAR2CSA on IE that binds to chondroitin sulfate A (CSA) on placental cells. In the placenta, IE induce inflammation and monocyte infiltration that increases the risk of maternal anemia and poor birth outcomes. Antibodies (Ab) to VAR2CSA can block sequestration and have been associated with improved pregnancy outcomes. However, a direct link between Ab levels to VAR2CSA and clearance of parasites from the placenta resulting in absence of PM at delivery has not been reported. The role of high avidity Ab in clearance of placental IE is also unknown. The goal of this study was to identify IgG responses to full-length VAR2CSA (FV2) that correlate with absence of PM. Using the bead-based multi-analyte profiling assay, Ab levels to FV2 in 89 women living in high and low transmission areas in Cameroon were measured using samples collected during the course of pregnancy. The percentage of high avidity Ab to FV2 (i.e., percent Ab bound in the presence of 3M NH_4SCN) was determined. In the high transmission area, the level of Ab to FV2 ($p=0.0047$) and the percentage of high avidity FV2 Ab ($p=0.0009$) were significantly higher in women without PM than those with PM. Further, women with moderate FV2 Ab levels in the 5-6th and 7-8th month had a 2.3 (95% CI, 1.0-4.9) and 2.0 times (95% CI, 1.0-3.9), respectively, reduced risk of PM at delivery. Also, women who had ≥35% of high avidity Ab to FV2 at 5-6th month had a 7.6-fold lower risk of PM ($p=0.0013$, 95% CI: 1.2-50.0). In contrast, no difference was found in women living in the low transmission area. Differences between the two study sites show that frequent malaria infections are required to develop protective Ab. In conclusion, early production of Ab to FV2, especially those with high avidity, are associated with absence of PM.

3

MOTHER AND NEONATE DISTINCT IMMUNOGLOBULIN G: A NEW APPROACH USING PROTEOMICS FOR NEONATAL SEROLOGICAL DIAGNOSIS

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This study provides for the first time a way to distinguish neonatal from maternal antibodies and to measure specific antibodies synthesized by a newborn. In the context of malaria, the knowledge of firstly acquired antibody responses against *Plasmodium falciparum* is essential for orientating the choice of appropriate vaccine strategies. Nevertheless, as maternal antibodies are transferred to the fetus during pregnancy, shared maternal and neonatal antibodies are present in the infant's plasma during his first months of life. We propose a technique of differential detection and dosage, in newborn plasma, of immunoglobulin G of mother and child, by a proteomic approach. This method relies on the allelic polymorphism of the IgG3 that corresponds to thirteen G3m allotypes located on the constant domains of the heavy chains. Peptide sequences encompassing G3m discriminatory amino acids, aimed at identifying the greatest number of G3m allotypes, were defined. Preliminary experiments were done on a series of controlled mixtures of plasma samples from individuals homozygous for distinct G3m allotypes, as determined by a classical haemagglutination-inhibition method: total IgG3 were purified using affinity chromatography before being digested by a combination of proteases; resulting peptides were separated by nano-HPLC and allotype-specific peptides were successfully detected by mass spectrometry. A label-free approach using the nano-HPLC retention times and peak intensity of the peptides gave semi-quantitative information showing a significant correlation with the artificial allotypes-mix ratio. Validation of the proteomic approach was made on total IgG3 purified from plasma

samples of one mother and her baby drawn quarterly from birth to nine months. The concomitant serological determination of the father's Gm allotypes allowed determining unambiguously the G3m allotypes of the infant. The possibility of quantifying neo-synthesized total IgG3 in infant, offered by this new method, may be extended to specific IgG3 elaborated in response to pathogens. It will allow improving knowledge on the acquisition of anti-malarial natural immunity in infancy. In a wider perspective, this approach represents a promising diagnostic tool for vertically-transmitted diseases.

4

DECREASED HUMAN ANTIBODY RESPONSE AGAINST *PLASMODIUM FALCIPARUM* ANTIGENS EXPRESSED IN BOTH GAMETOCYTES AND GAMETES

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The role of natural immunity in malaria transmission is complex, but critical to disease control efforts. Development of the sexual stages of the *Plasmodium* parasite that are required for transmission begins in RBCs in the human host. After maturation gametocytes circulate for several days before being cleared by the human host if not taken up in a blood meal by a mosquito. In the mosquito midgut the gametocytes emerge from the RBC as extracelluar gametes which fertilize and begin sporogonic development. The surface of the extracellular gamete is a target for malaria transmission-blocking antibodies and four antigens (Pfs230, Pfs48/45, Pfs25 and Pfs28) have been identified and are being developed as vaccine candidates. Pfs25 and Pfs28 are only translated in the mosquito, but Pfs230 and Pf48/45 are expressed in the gametocyte and therefore exposed to the human immune response. To examine antibody production against antigens expressed on sexual stages, proteomic data from gametocytes and gametes was incorporated into the analysis of the data from a recombinant *P. falciparum* protein microarray probed with plasma from 220 individuals before and after the malaria season in Mali. The results indicate that antibodies against antigens represented on the array that are expressed in gametocytes or both gametocytes and gametes, including Pfs230 and Pfs48/45, increased with age and from the beginning to end of the malaria season. This finding is consistent with exposure to sexual stage parasites during the course of the season, which could boost a transmission-blocking vaccine. Interestingly, analysis of immunogenic antigens indicated that there was a significantly stronger antibody response against antigens expressed in gametocytes, than those expressed in both gametocytes and gametes. This decreased response against antigens expressed in gametes was evident at the both the start and end of the season ($p<0.008$ and $p<0.0002$, respectively) and suggests a bias against antigens that could interfere with malaria transmission.

5

ANTIGEN-SPECIFIC MEMORY B CELL DETECTION USING A FLOW CYTOMETRY BASED ASSAY

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Infant B cell development and memory formation is poorly understood because B cell frequency in peripheral blood is low and there is limited sample volume of blood that can be obtained from infants. To address this limitation, we have developed a flow cytometry based assay capable of detecting antigen specific memory B cells from small volumes of peripheral blood. We first developed and validated this assay using tetanus (TT) and diphtheria (DT) vaccine responses. The flow-based assay is 1.5 - 4 times more sensitive at detecting TT and DT-specific memory B cells at higher frequencies compared to the traditional B cell ELISPOT assay. Moreover, because this assay can be multiplexed, a total of 10^6 PBMC are consumed for TT and DT specific memory B cell detection with the flow based assay compared with $2x10^6$ PBMC needed for the DT B cell ELISPOT alone (10^6 PBMC are needed for each additional antigen tested by ELISPOT). We have applied this technology to cord blood mononuclear cells and successfully detected TT and DT-specific memory B cells in neonates whose Kenyan mothers were vaccinated during pregnancy. We have recently expanded this assay to detect malaria antigen-specific memory B cells. Specifically we were able to detect MSP1, MSP3, but not AMA1 specific memory B cells from 5 Kenyan adults and 1 Kenyan child (age 28 months) with known past malaria infection. 1 Kenyan child (age 28 months, same region) with no evidence of past malaria infections (no T cell responses to MSP1 and no antibody recognition of multiple malaria antigens by serology) had no detectable malaria antigen-specific memory B cells using the flow assay. Additionally, we were able to detect MSP1, MSP3, but not AMA1 specific memory B cells from a neonate whose Kenyan mother had evidence of malaria during pregnancy, indicative of fetal priming to malaria antigens. These data suggest that this flow based assay will be a valuable tool to overcoming major constraints and furthering our understanding of the development of human infant B cell immunity.

6

ASSOCIATION OF HLA ALLELES WITH *PLASMODIUM FALCIPARUM* SEVERITY IN MALIAN CHILDREN

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Pre-erythrocytic immunity to *Plasmodium falciparum* malaria is likely to be mediated by T cell recognition of malaria epitopes presented on infected host cells via class I and II major histocompatibility complex (MHC) antigens. To test for associations of HLA alleles with disease severity, we performed high resolution typing of HLA class I and II loci and compared the distributions of alleles of HLA-A, -B, -C and DRB1 loci in 359 Malian children of Dogon ethnicity with uncomplicated or severe malaria. We observed that alleles A*30:01 and A*33:01 had higher frequency in the group of patients with cerebral disease compared to patients with uncomplicated disease (A*30:01: gf=0.2031 vs. gf=0.1064, OR=3.17, P=0.004, CI [1.94-5.19]) and (A*33:01: gf=0.0781 vs. gf=0.0266, 4.21, P = 0.005, CI [1.89-9.84]), respectively. The A*30:01 and A*33:01 alleles share some sequence motifs and A*30:01 appears to have a unique peptide binding repertoire compared to other A*30 group alleles. Computer algorithms predicted malaria peptides derived from Liver Stage Antigens 1 and 3 (LSA-1 and LSA-3), Merozoite Surface Protein 1 (MSP-1) and Thrombospondin-related Anonymous Protein (TRAP) with strong

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