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

Jack S. Richards1, Thangavelu U. Arumugam2, Linda Reiling1, Freya J. Fowkes1, Julie Healer1, Anthony N. Hodder3, Robin F. Anders4, Satoru Takeo2, Paul R. Gibson1, Jennifer K. Thompson3, David L. Narum5, Chetan E. Chitnis2, Nadia Cross1, Christine Langer1, Peter M. Siba7, Christopher L. King4, Ivo Mueller2, Motomi Torii2, Brendan S. Crabb1, Alan F. Cowman3, Takafumi Tsuboi2, James G. Beeson1

1Burnet Institute, Melbourne, Australia, 2Ehime University, Matsuyama, Japan, 3Walter and Eliza Hall Institute, Parkville, Australia, 4LaTrobe University, Bundoora, Australia, 5National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, 6International Centre for Genetic Engineering and Biotechnology, New Delhi, India, 7Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, 8Case Western Reserve University, Cleveland, OH, United States, 9Ehime University Graduate School of Medicine, Toon, Japan

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.

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

Celia Dechavanne1, François Guillonneau2, Laila Sago2, Prisca Lévy1, Virginie Salnot1, Evelyne Guittard1, François Ehrenmann4, Cédric Broussard2, Philippe Chafey3, Aignès Le Port4, Marie-Paule Lefranc5, Jean-Michel Dugoujon1, Patrick Mayeux6, Florence Migtot-Nabias1

1Institut de Recherche pour le Développement and Université Paris Descartes, Paris, France, 2Plate-forme protéomique de l’Université Paris Descartes, Paris, France, 3Centre National de la Recherche Scientifique and Université Paul Sabatier, Toulouse, France, 4Centre National de la Recherche Scientifique and Université Montpellier 2, Montpellier, France, 5Institut Cochin and Institut National de la Santé et de la Recherche Médicale, Paris, France, 6Plate-forme protéomique de l’Université Paris Descartes and Institut Cochin, Paris, France

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.

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

Peter D. Crompton¹, Xiaolin Tan², Philip L. Felgner¹, Kim C. Williamson³⁴

¹National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, ²University of California, Irvine, Irvine, CA, United States, ³National Institute of Allergy and Infectious Diseases, National Institutes of Health and Loyola University Chicago, Bethesda, MD, United States

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 extracellular 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 Pfs48/45 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.

ASSOCIATION OF HLA ALLELES WITH PLASMODIUM FALCIPARUM SEVERITY IN MALIAN CHILDREN

Kirsten E. Lyke¹, Marcelo A. Fernández-Viña², Kai Cao³, Jill Hollenbach⁴, Drissa Coulibaly⁵, Abdoulaye K. Kone⁶, Ando Guindo⁵, Laura A. Burdett⁷, Robert J. Hartzman⁸, Angela R. Wahl⁹, William H. Hildebrand⁴, Ogobara K. Doumbo⁵, Christopher V. Plowe⁵, Marcelo B. Sztein¹

¹Center for Vaccine Development, University of Maryland, Baltimore, MD, United States, ²M.D. Anderson Cancer Center, Houston, TX, United States, ³Comprehensive Transplant Center, Cedars-Sinai Health System, Los Angeles, CA, United States, ⁴Center for Genetics, Children’s Hospital Oakland Research Institute, Oakland, CA, United States, ⁵Malaria Research and Training Centre, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Bamako, Mali, ⁶Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States, ⁷C.W. Bill Young DoD Marrow Donor Program, Naval Medical Research Institute/Georgetown University, Kensington, MD, United States, ⁸Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, ⁹The Center for Vaccine Development, University of Maryland School of Medicine and the Howard Hughes Medical Institute, Baltimore, MD, United States

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: gfs=0.2031 vs. gfs=0.1064, OR=3.17, P=0.004, CI [1.94-5.19]) and (A*33:01: gfs=0.0781 vs. gfs=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