BIOGRAPHICAL SKETCH

NAME: Manoj T. Duraisingh

eRA COMMONS USER NAME (credential, e.g., agency login): MDURAISINGH

POSITION TITLE: John LaPorte Given Professor of Immunology and Infectious Diseases

EDUCATION/TRAINING

Institution and Location	Degree	Completion Date	Field of Study
University of Oxford, Oxford, UK	B.A. (Hons)	06/1993	Biochemistry
London School of Hygiene & Tropical Med.	M.Sc.	08/1994	Mol. Parasitology
London School of Hygiene & Tropical Med.	Ph.D.	08/1999	Mol. Parasitology
Walter & Eliza Hall Institute, Melbourne	Post-doc	08/2002	Mol. Parasitology

A. Personal Statement

I am a Professor in the Department of Immunology and Infectious Diseases at the Harvard T.H. Chan School of Public Health, where I have successfully established an independent research program studying the biology and pathology of host-parasite interactions in malaria. In my laboratory, we are currently applying a combination of molecular, cellular, and genetic approaches toward the study of the molecular interactions between human malaria parasites, Plasmodium spp., and the host red blood cell. We are defining the essential molecular parasite and host determinants required for invasion and intracellular survival by this important human pathogen.

Areas of particular interest are: genetic analyses of parasite and host determinants of infection and virulence, with a focus on parasite motility, egress and invasion from host cells; the epigenetic control of virulence gene expression in P. falciparum; the biology of other human malaria parasites- *P. vivax*, *P. knowlesi* and *Babesia* spp., and their development as tractable in vitro model systems for genetic analyses.

B. Positions and Honors

Positions and employment

1999 – 2002 Postdoctoral Research Fellow, Walter and Eliza Hall Institute, Melbourne, Australia
2002 – 2010 Assistant Professor, Harvard School of Public Health, Boston, MA
2010 – 2014 Associate Professor, Harvard School of Public Health, Boston, MA
2014-present Professor, Harvard T.H. Chan School of Public Health, Boston, MA
2011-present Associate Member, Broad Institute, Cambridge, MA

Other Experience and Professional Memberships

2002-present Member, American Society of Tropical Medicine & Hygiene
2012-2018 NIH Peer Review Committee: Pathogenic Eukaryotes, permanent member

Honors

1999 – 2001 Wellcome Trust Advanced Training Fellowship
2004, 2009 W. F. Milton Award
2006 –2013 Burroughs Wellcome New Investigator in the Pathogenesis of Infectious Disease
2009 – 2013 Burke Global Health Fellowship
2011 – 2014 President, ACMCIP Subgroup, American Society of Tropical Medicine & Hygiene

C. Contribution to Science

1. Signal transduction regulating red blood cell invasion and asexual proliferation in Plasmodium falciparum

The recently acquired Plasmodium genomes provide a plethora of targets, but due to the genetic intractability of the parasite, it has hitherto been difficult to identify and functionally analyze essential genes in the clinically relevant blood-stage of the parasite. We have developed and applied a method for inducible protein expression for knockdown of signal transduction genes involved in the processes of egress and invasion. Using this system, we have found several P. falciparum genes that regulate egress from the red blood cell and invasion into new red blood cells: a plant-like calcium-dependent protein kinase, PfCDPK5, required for egress from the red blood cell, a DOC2 protein regulating invasion and microneme exocytosis, and parasite calcineurin as essential for parasite attachment during invasion. We are now annotating

essential genes in the asexual cell-cycle using this approach and have identified a key regulator of DNA replication through multiple rounds of schizogony, and exploiting increasingly powerful new conditional systems for the simultaneous validation of essentiality and determination of function. With Dr. Marc-Jan Gubbels (Boston College) we have studied the roles of orthologous signal transduction proteins in the related apicomplexan parasite, Toxoplasma gondii, feeding our interest in the comparative biology of apicomplexans.

- Dvorin, JD, Martyn, DC, Patel, SD, Collins, CR, Hopp, C, Grimley, JS, Bright, T, Westenberger, S, Winzeler, E, Blackman, MJ, Baker, DA, Wandless TJ, **Duraisingh, MT**. Knockdown of Plasmodium falciparum calcium-dependent protein kinase reveals essential step for egress. Science, 328:910-2, 2010. PMCID: PMC3109083
- Farrell A, Thirugnanam S, Lorestani A, Dvorin JD, Eidell KP, Anderson-White BR, **Duraisingh MT#**, Marth GT# and Gubbels MJ#. A DOC2 protein identified by mutational profiling is essential for apicomplexan parasite exocytosis. Science, 335:218-21 2012. (# equal corresponding author). PMCID: PMC3354045
- Paul AS, Saha S, Engelberg K, Jiang RH, Coleman BI, Kosber AL, Chen CT, Ganter M, Espy N, Gilberger TW, Gubbels MJ, **Duraisingh MT**. Parasite calcineurin regulates host cell recognition and attachment by apicomplexans. Cell Host Microbe, 18:49-60, 2015. PMCID: PMC4506782
- Ganter M, Goldberg JM, Dvorin JD, Paulo JA, King JG, Tripathi AK, Paul AS, Yang J, Coppens I, Jiang RH, Elsworth B, Baker DA, Dinglasan RR, Gygi SP, **Duraisingh MT**. Plasmodium falciparum CRK4 directs continuous rounds of DNA replication during schizogony. Nature Microbiology, 2:17017, 2017. PMCID: PMC5328244

2. Plasmodium knowlesi: human adaptation and the development of in vitro culture and genetic systems

Most human P. knowlesi infections are characterized by low blood-stage parasite densities, consistent with zoonotic transmission, however, a few occasional high parasite density infections can result in severe disease, raising the concern that P. knowlesi may be adapting to growth in humans. We have identified shifting of tropism for red blood cells of different age as a potential mechanism for a zoonotic infection to increase growth and virulence in humans. We have established an in vitro culture of P. knowlesi in human red blood cells, as a second in vitro model for Plasmodium parasites in addition to P. falciparum in human red blood cells, over which it demonstrates superior genetics, opening the door for comparative studies, as well as forward genetics.

- Lim, C, Hansen, E, DeSimone, T, Moreno, Y, Unnoson, K, Bei, AK, Brugnara, C Buckee, CO, **Duraisingh MT**. Expansion of host cellular niche can drive adaptation of a zoonotic malaria parasite to humans. Nature Communications, 4:1638, 2013. PMCID: PMC3762474
- Grüring C, Moon RW, Lim C, Holder AA, Blackman MJ, **Duraisingh MT**. Human red blood cell-adapted Plasmodium knowlesi parasites: a new model system for malaria research. Cellular Microbiology, 16, 612-20, 2014. PMCID: PMC4004062
- Assefa SA, Lim C, Preston MD, Nair M, Duffy CW, Goldberg JE, Neafsey D, Divis PC, Clark TG, **Duraisingh MT**, Conway DJ, Pain A and Singh, B. Mosaic genomic substructure and selection in the zoonotic malaria parasite Plasmodium knowlesi. Proceedings of the National Academy of Science, 112, 13027-32, 2015. PMCID: PMC4620865
- Dankwa S, Lim C, Bei AK, Jiang RH, Abshire JR, Patel SD, Goldberg JM, Moreno Y, Kono M, Niles JC, **Duraisingh MT**. Ancient human sialic acid variant restricts an emerging zoonotic malaria parasite. Nature Communications, 7, 11187, 2016. PMCID: PMC4822025

3. In vitro functional analysis of red blood cell determinants of malaria infection

The contribution of the red blood cell to host-parasite interactions in malaria has largely been neglected, due to the genetic intractability of the red blood cell in vitro as it is a terminally differentiated enucleated cell. We have developed a novel and robust genetic approach for the in vitro functional analysis of human red blood cell determinants of malaria infection. We target genes in nucleated hematopoietic stem cell precursors, followed by in vitro differentiation to mature red blood cells. Through the generation of knockdowns using shRNA, we have used this system to demonstrate the strain-dependent importance of the major surface glycoprotein glycophorin A, a receptor for the P. falciparum vaccine candidate ligand EBA-175. Similarly, we

have used a knockdown approach to demonstrate the requirement of the basigin receptor for invasion and glycophorin C for rosetting. We developed and executed a forward genetic screen of red blood cell determinants and identified the human blood group CD55 as essential for P. falciparum invasion. The genetic accessibility of the red blood cell in vitro allows us to explore numerous host-parasite interactions at the molecular and cellular levels.

- Kanjee U, Gruring C, Chaand M, Lin KM, Egan E, Manzo J, Jones PL, Yu T, Barker R Jr, Weekes MP, **Duraisingh MT**. CRISPR/Cas9 knockouts reveal genetic interactions between strain-transcendent erythrocyte determinants of *Plasmodium falciparum* invasion. *Proc. Natl Acad Sci* 114(44):9356-9365, 2017. PMCID:PMC5676921
- Bei, AK, Brugnara, C and **Duraisingh MT.** *In vitro* reverse genetic analysis of a human erythrocyte determinant of *Plasmodium falciparum* invasion. *Journal of Infectious Diseases,* 202:1722-7, 2010. PMCID: PMC3107553
- Crosnier C, Bustamante LY, Bartholdson JY, Bei AK, Theron M, Uchikawa M, Ndir O, Mboup S, Kwiatkowski D, **Duraisingh MT**, Rayner JC, Wright GJ. BASIGIN is a PfRh5 receptor essential for erythrocyte invasion by *Plasmodium falciparum. Nature* Nov 9. doi: 10.1038/nature10606. PMCID: PMC3245779
- Egan ES, Jiang RHY, Moechtar MA, Barteneva NS, Weekes MP, Nobre LV, Gygi SP, Paulo JA, Frantzreb C, Tani Y, Takahashi J, Watanabe S, Goldberg J, Paul AS, Brugnara C, Root DE, Wiegand RC, Doench JG, **Duraisingh MT**. A forward genetic screen identifies erythrocyte CD55 as essential for *Plasmodium falciparum* invasion. *Science*, 348:711-4, 2015. PMCID:PMC4465434

4. Ligand-receptors for Plasmodium falciparum erythrocyte invasion that mediate host cell tropism

We seek to elucidate the mechanism by which malaria parasites penetrate host red blood cells, the first step in the establishment of a blood-stage infection. Plasmodium spp. parasites belong to the phylum Apicomplexa because of the presence of specialized apical organelles, which play a critical role in the invasion of host cells. To initiate the asexual replication cycle, the short-lived Plasmodium merozoite (the invasive form) must rapidly recognize and attach to a red cell surface. Our research has focused on several areas towards understanding the phenotypic significance of P. falciparum parasites using alternative invasion ligands belonging to members of the PfRh and PfEBA family for invasion, in both laboratory and field isolates. We are testing the roles of variant expression and sequence polymorphism of invasion ligands in the evasion of the immune system and/or attachment to polymorphic receptors, particularly in the context of field-based studies. We have also carried out a functional analysis of the cytoplasmic domains of PfRh parasite ligands involved in red blood cell invasion to mediate host cell tropism.

- Dvorin J, Bei AK, Coleman B, **Duraisingh MT**. Functional Diversification Between Two Members of a P. falciparum Merozoite Invasion Ligand Family is Determined by the Cytoplasmic Domain, Molecular Microbiology 75, 990-1006, 2010. PMCID: PMC3627358
- Desimone T, Jennings CV, Bei AK, Coleman B, Comeaux C, Refour P, Triglia T, Stubbs J, Cowman AF, **Duraisingh MT**. Cooperativity between P. falciparum adhesive proteins for erythrocyte invasion. Molecular Microbiology, 72, 578-89, 2009. PMCID: PMC2891881
- **Duraisingh MT**, Maier A, Triglia T, Cowman AF. The erythrocyte binding antigen-175 functions in invasion of human erythrocytes by Plasmodium falciparum utilizing sialic-acid-dependent and –independent pathways. Proceedings of the National Academy of Sciences. 100, 4796-801, 2003.
- **Duraisingh MT**, Triglia T, Ralph S, Rayner JC, Barnwell JW, MacFadden GI, Cowman AF. Phenotypic variation of Plasmodium falciparum merozoite proteins directs receptor targeting for invasion of human erythrocytes. EMBO J 22. 1047-57, 2003.

5. Epigenetic regulation of antigenic variation and sexual development in Plasmodium parasites

Eukaryotic pathogens have developed varied mechanisms for evading the immune system and for persistence within their hosts. High frequency variation of adhesins appears to be a common strategy used by different organisms, including the malaria parasite P. falciparum. We have been studying the role of epigenetic regulators in the control of the fundamental parasitic processes of the clinical blood-stage of malaria. We find that specific regulators (sirtuin histone deacetylases) regulate alternative sets of virulence

genes. Study of a specific histone deacetylase (PfHda2) has shown that in contrast to all previously studied epigenetic regulators uniquely controls both antigenic variation and gametocyte conversion. We have conducted a molecular analysis of the switching on and off in expression of the variantly expressed virulence genes, involved in various pathogenic processes, such as invasion (PfRh4) and cytoadherence (var genes) in P. falciparum, and have shown these to be epigenetic phenomena.

- Coleman BI, Skillman KS, Jiang RHY, Childs LM, Altenhofen LM, Ganter M, Leung Y, Goldowitz I, Kafsack BFC, Marti M, Llinas M, Buckee CO, **Duraisingh MT**. A Plasmodium falciparum Histone Deacetylase Regulates Antigenic Variation and Gametocyte Conversion. Cell Host Microbe, 16, 177-86, 2014. PMCID: PMC4188636
- Coleman, BI, Ribacke U, Manary, M, Bei, AK, Winzeler, EA, Wirth DF, **Duraisingh MT**. Nuclear repositioning precedes promoter accessibility and is linked to the switching frequency of a Plasmodium falciparum invasion gene. Cell Host and Microbe, 12:739-50, 2012. PMCID: PMC4066821
- Merrick CJ, Huttenhower C, Buckee CO, Amambua-Ngwa A, Gomez-Escobar N, Walther M, Conway DJ, **Duraisingh MT**. Epigenetic dysregulation of virulence gene expression in severe malaria. Journal of Infectious Dieases 205:1593-600, 2012. PMCID: PMC3415821
- Merrick CJ and **Duraisingh MT**. PfSir2: an unusual sirtuin with dual histone deacetylase and ADP-ribosylase activity. Eukaryotic Cell 6, 2081-2091, 2007. PMCID: PMC2168398

Complete List of Published Work in MyBibliography (135 publications):
http://www.ncbi.nlm.nih.gov/sites/myncbi/manoj.duraisingh.1/bibliography/41150379/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research

NIH 2U19AI089688-09 (PI: Rathod, Role: Leader in Infection Biology) 07/01/17-03/31/24
Malaria Evolution in South Asia
This is a formal collaboration with the South Asia ICEMR directed by Dr. Pradipsinh K. Rathod Dr. Duraisingh will assume the role of Pathogenesis (Project 3).

NIH R01AI140751 (PI: Duraisingh) 07/01/18-06/30/23
Elucidating ligand-receptor interactions required for Plasmodium vivax blood-stage infection
This project proposes to use genetic screens to identify receptors on the red blood cell surface and the parasite molecules used for cellular entry through these receptors.

NIH 1R01AI138551 (PI: Duraisingh) 02/20/18-01/31/23
Functional analysis of epigenetic regulators of malaria blood-stage proliferation and transmission
This project will target the Plasmodium falciparum histone deacetylase molecules that are essential for growth of the parasite in the blood, the stage of infection responsible for both virulence and transmission of the parasite. We will define the mechanisms by which these epigenetic regulator proteins function to provide a rational biological basis for antimalarial therapies.

NIH/NHLBI 5R01HL139337-02 (PI: Duraisingh) 04/20/17-01/31/21
Functional analysis of red blood cell determinants of *Plasmodium* invasion
In this project, we hypothesize that the identification and analysis of essential interactions between the host red blood cell and malaria parasite will provide ideal candidates for the development of novel therapeutics and will greatly inform vaccine development.

NIH R21AI139973 (PI: Duraisingh) 07/01/18-06/30/20
Forward genetic overexpression screens to identify parasite determinants of antimalarial susceptibility
We aim to develop a novel genetic approach in malaria parasites that will identify molecular determinants in

the Plasmodium genome that can influence susceptibility to specific antimalarial compounds. Knowledge of the molecular basis of antimalarial activity will inform parasite drug development.

NIH/NIAID 5R21AI128480-02 (PI: Duraisingh) 12/15/16-11/30/18
Chemical genetic screening to identify synergistic inhibitors of malaria parasite cell cycle regulators
The goal of this project is to establish a novel experimental strategy, combining chemical and genetic approaches, to identify lead compounds for malaria drug development. We will target PfCRK4, an essential protein that we recently identified that possesses many features ideal for a drug target in malaria parasites.

Completed research (last three years)
NIH/NIAID 5R21AI126154-02 (PI: Duraisingh) 06/01/16-11/30/18
Functional analysis of *Plasmodium vivax* drug resistance polymorphisms
This project aims to understand the impact of changes in putative drug-resistance transporters of *P. vivax* by exploiting the closely related *Plasmodium knowlesi* parasite as a powerful model system for genetics.

NIH/NIAID 5R21AI126889-02 (PI: Duraisingh) 06/02/16-05/31/18
Genetic screens for erythrocyte determinants of protein trafficking in malaria parasites
This project will use forward genetics to identify critical host red blood cell molecules required trafficking of proteins to the red blood cell by malaria parasites.

Bill and Melinda Gates Foundation GCE-Phase II OPP1035276 (PI: Duraisingh) 06/01/14-05/31/17(NCE)
Targeting erythrocyte determinants of malaria sexual development
In this proposal we will identify erythrocyte genes that are essential for the sexual development of the malaria parasite *P. falciparum*, that encode proteins that can be targeted by small molecules for inhibition.

NIH/NIAID R01 AI091787-07 (PI: Duraisingh) 05/01/11-04/30/17(NCE)
Functional analysis of erythrocyte determinants of malaria infection
This grant focuses on the analysis of host red blood cell proteins in the process of *P. falciparum* invasion, developing and using *in vitro* genetic analysis of human blood group genes.

Bill and Melinda Gates Foundation - OPP1023594 (PI: Duraisingh) 11/17/10-10/31/16
***In vitro* culture of blood-stage Plasmodium *vivax* parasites**
The goal of this project is the establishment of continuous *in vitro* culture of blood-stage *P. vivax*