HIV Pathogenesis Programme

2010 Scientific Retreat

Programme and Abstracts

19-22 October
2010

HIV Pathogenesis Programme, Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, 719 Umbilo Road, Congella, Durban 4001, South Africa

HPP
HIV Pathogenesis Programme
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine

Contents

1. The HIV Pathogenesis Programme

History of the programme

In 1999, Professor Philip Goulder of the University of Oxford received funding from the Elizabeth Glazier Paediatric AIDS Foundation to study the immunopathogenesis of HIV in children. In collaboration with Professor Hoosen (Jerry) Coovadia they established a small but highly productive Cellular Immunology Laboratory in the Department of Paediatrics and Child Health, under the direction of Professor Jerry Coovadia.

During this time, the field of cellular immunology was being transformed with the development of new methodologies to study CTL and T-helper cells. In partnership with Professor Bruce Walker from Partners AIDS Research Centre Boston, USA, the transfer of this new technology made it possible to study HIV-1 clade C virus infection. Similar studies and techniques were being undertaken and used at all three sites (Boston, Oxford and Durban), but the key difference is that the Durban site was, and is, at the heart of the epidemic.

Dr. Photini Kiepiela was the first locally appointed Director of the HIV Pathogenesis Programme of the Doris Duke Medical Research Institute, and under her leadership and despite limited facilities; the "infant" HPP unit produced many publications from the start up work undertaken. After further funding was obtained from the Doris Duke Charitable Foundation, the laboratory was able to expand and develop. This additional funding allowed the laboratory to continue its valuable work with much better resources.

In July 2003, the Doris Duke Medical Research Institute was completed and the research lab moved into the new premises housing a state-of-the-art laboratory, a boardroom with conference facilities and new offices (180m2). Since that time HPP has grown considerably in size to 82 personnel encompassing local and visiting scientists, students, clinicians, nurses, counsellors, administration and support staff. There are now four clinic sites where studies are undertaken and patients recruited. Professor Thumbi Ndung'u is the Scientific Director of the unit. He and his team at have published extensively in the fields of HIV molecular virology and immunology.

Currently the HPP boasts excellent facilities on a par with international research laboratories. These include 17,000 square feet of BL1, BL2 and BL2+ research and office space, 1 LSRII flow cytometer, 2 FACSCalibur flow cytometers, 14 biosafety cabinets, liquid nitrogen storage facility, 10 PCR thermocyclers, cell culture facilities, core facilities for quantitation of HIV viral load and CD4 cell counts, warm rooms, cold rooms, wireless internet, videoconferencing, and sufficient office space for students, as well as space for visiting scientists.

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2. HPP 2010 Retreat Venue

Mondazur at San Lameer is located on the beautiful South Coast of South Africa's KwaZulu-Natal Province. Mondazur is just off the N2 highway, 140 km or 90 minutes south from Durban and minutes away from the popular resort town Margate.

Physical Address:

Lower South Coast Main Road (R61),
Southbroom, KwaZulu Natal

GPS Coordinates:

- S: 30° 56' 35.0"
- E: 30° 17' 22.49"

Emergency contact numbers

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Police
10111
Ambulance/ Emergency Medical
10177 OR **083 911**
Margate Ambulance
039 312 1000
AA Breakdowns
080 001 0101
Call to Go
0824625770/0727868921

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3. Welcome from Prof Ndung'u , Scientific Director, HPP

On behalf of HPP faculty, staff and students, it is my great pleasure to welcome you to scenic KwaZulu-Natal for our biennial retreat. Over the past few years, our programme has experienced significant growth in size and in the scope of our research, training and community engagement activities. We now have 5 locally based researchers with independent grant funding, and over the last 3 years, we have graduated 1 PhD and 3 masters degree students and we have co-authored over 30 publications in peer-reviewed journals, with an increasing number of our students and fellows as lead authors. Many HPP students, fellows and staff have had opportunities to train locally or internationally. We have also hosted students and fellows from within and outside Africa. We have contributed to several local and global initiatives to enhance knowledge generation and build capacity in HIV/AIDS and TB research. We are very grateful to the many funders who have made our programme a tremendous success.

We remain deeply aware of the devastating impact of the dual epidemics of HIV/AIDS and TB in our province, country and continent. Consequently, we remain focused on research and capacity building for the development of efficacious HIV/AIDS and TB vaccines and in contributing to the successful and optimal utilization of currently available biomedical technologies. We are grateful that you have all taken time off your busy schedules to join in assessing and evaluating our programme, and helping us to plan for the future. We hope that you will enjoy the time we spend together in this beautiful retreat venue and ask that you use your expertise and experience to help us to come out with a renewed sense of purpose, commitment and dedication as we together stand up to HIV/AIDS and TB. We look forward to your contribution and to building new collaborative initiatives until we win this fight. *Yes, we can!*

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4. Schedule

Day of Departure: Tuesday 19th October

14:00	Depart from Medical school from Glastonbury road
14:00-16:00	Travel to Mondazur on the south coast of KwaZulu Natal www.mondazur.com
16:00-17:30	Check-in at Mondazur Resort, room allocation

Evening of Arrival: Tuesday 19th October

18:00-19:15	**Gala Dinner & introduction of our guests**

Opening Address and Overview from faculty

19:30-19:40	**Welcome: Thumbi Ndung'u/Bruce Walker**
19:40-19:50	William Carr
19:50-20:00	Victoria Kasprowicz

Keynote speaker

20.00 – 20.45	**Laurie Glimcher**
21:00	Free Time

Day One: Wednesday 20th October

6:45-7:45 **Breakfast**

Session One: Clinical and Public Health HIV (Chair: Bruce Walker/David Bangsberg)

08:00-08:10	**Introduction**	**Bruce Walker/David Bangsberg**
08:10-08:20	Acute Infection	Mammekwa Makgoro
08:20-08:30	Sinikithemba & PEHSS	Manjeetha Jaggernath
08:30-08:40	Masibambisane	Huub Gelderblom Katherine Johnston
08:40-09.15	Discussion	
09:15-09.30	**Break**	

Session Two: Adaptive Immunity (Chair: Hendrik Streeck and Wendy Burgers)

09:30-09:40	**Introduction**	**Hendrik/Wendy**
09:40-09:50		Christina Chang
09:50-10:00		Christina Thobakgale
10:00-10:10		Shivan Chetty
10.10-10.20		Dshanta Naicker
10.20-11.00	Discussion	
11:00-11:30	**Tea Break**	

11.30-13.00 **Poster Session 1**

12.00 – 13.00 **Masibambisane investigators meeting**

13:00-14:00 **Lunch**

Session Three: Viral Genetics and humoral immunity (Chair: Dennis Burton and Lynn Morris)

14:00-14:10	**Introduction**	**Lynn Morris/Dennis Burton**
14:10-14:20		Derseree Archary
14:20-14:30		Jennifer Giandhari
14:30-14:40		Kamini Gounder
14.40-14.50		Jaclyn Wright
14:50- 15.20	**Session Discussion**	

15.20 – 15.30	**Short Break**	
15:30 - 16:00	**Keynote speaker:**	**Sharon Lewin**
16.00 – 16.30	**Tea Break**	

16.30-18.00: **Meet the investigator /Round Table Discussion session**

18:00-19:00	**Free time**
19:00-21:00	**Dinner, Fun and Games**

Day Two: Thursday 21st October

6:45-7.45 **Breakfast**

Session Four: Innate Immunity (Chair : Darrel Irvine, Marcus Altfeld)

08.00-08:10	**Introduction**	**Darrel Irvine, Marcus Altfeld**
08:10-08:20		Vivek Naranbhai
08:20-08:30		Marianne Mureithi
08:30-08:40		Bongiwe Ndlovu
08:40-09.10		**Discussion**
09.10-09.30	**Break**	

Session Five: Host Genetics and Host Restriction Factors (Mary Carrington and Arup Chakraborty)

09:30-09:40	**Introduction**	**Mary Carrington/Arup Chakraborty**
09:40-09:50		Paradise Madlala
09:50-10:00		Kavidha Reddy
10:10-10:20		Ravesh Singh
10.20-10.30		Veron Ramsuran
10.30-11.00	**Discussion**	
11:00- 11.30	**Tea break**	
11:00-11:30	**Keynote speaker**	**Walter Jaoko**
11:30-13:00	**Posters session II**	

13:00-14:00	**Lunch**	
14:00-17:00	**Team building Beach activities**	

Session Six: Clinic Staff (Chair: Fundisiwe Chonco)

18:30-:18:40	**Introduction**	Fundi Chonco
18:40-18:50		Mammekwa Mokgoro/Manjeetha
18:50-19:00		Clinic nurse 1/sister Jabu
19.00-19.10		Clinic Patient
19:10-19:30	**Discussion**	
20:00-24:00	**Social gala Dinner/dance**	

Day Three: Friday 22nd of October

6:45-8:30	**Breakfast**

Strategising for the next phase of HPP's growth

08:30-09:15	**Reflecting on the strengths and weaknesses of HPP, External view**	Jo-Anne Passmore/Lynn Morris/Vincent Marconi
09:15-10:00	**Responses**	Thumbi Ndung'u, Bruce Walker, Phillip Goulder, Marcus Altfeld
10:00-10:30	**Tea**	
10:30-11:30	**Faculty meeting/Networking**	Chair: Thumbi Ndung'u
11:30-12:00	**Group Photo**	
12:00-13:00	**Check-out, prepare to depart**	
13:00-14:00	**Lunch**	
14:00: 16:00	**Bus departs Mondazur to return to NRM**	

Abstracts

5. ABSTRACTS FOR ORAL PRESENTATIONS

Tuesday Evening, 19th October 2010

Professor Thumbi Ndung'u: Thumbi Ndung'u, B.V.M., PhD is an Associate Professor in HIV/AIDS Research and Director of the HIV Pathogenesis Programme at the Doris Duke Medical Research Institute, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa. He holds the South African Research Chair in Systems Biology of HIV/AIDS. He was until recently Co-Chair of the Young and Early Career Investigators Committee (YECIC) of the Global HIV Vaccine Enterprise. He graduated with a degree in Veterinary Medicine from the University of Nairobi, Kenya, and obtained a PhD in Biological Sciences in Public Health from Harvard University. He is a past recipient of the Edgar Haber award (Harvard University) and the Vice-Chancellor's research award (University of KwaZulu-Natal). Dr Ndung'u's research interests are host-virus interactions, antiviral immune responses and biomedical interventions applicable to resource-limited settings. He has published widely in HIV-1 virology and immunology. At the HPP he is leading a multidisciplinary team of researchers working in the fields of HIV and TB pathogenesis and vaccine development and has a particular interest in capacity building for biomedical research in Africa.
Outside of the laboratory, he enjoys soccer, baseball, tennis and theatre.

Dr. William Carr: Dr. William Carr is an Instructor in Medicine at the Ragon Institute of MGH (Massachusetts General Hospital), Harvard and MIT in Boston, USA, and has a joint appointment a Senior Lecturer in the Department of Paediatrics at the University of KwaZulu-Natal. Since 2006 he has initiated a research program on innate immunity within the HIV Pathogenesis Programme. The mission of his research group is to do good, cutting-edge science and build capacity in South Africa through teamwork. Dr. Carr received his Doctorate of Veterinary Medicine (D.V.M.) degree in 1992 and a Ph.D. in immunology in 2004 from Stanford University where he trained in immunogenetics with mentorship from Prof. Peter Parham. Dr. Carr's research interest are in understanding the genetic basis of naturally occurring innate immune mechanisms underlying prevention of HIV-1 transmission and protection from HIV-1 disease progression among South Africans. To address these questions he has initiated studies with cohorts of exposed, uninfected individuals (mother-child cohorts and adult cohorts), cohorts of acutely infected infants and adults, as well as cohorts of chronically infected adults in South Africa. His work is supported by grants from the National Institutes of Health (NIH), the Ragon Institute, MGH, and SHARP (South African AIDS Research Platform). He currently is supervising postgraduate students (2 Masters students, 1 Honours student, and 1 Durban University of Technology - DUT student) at UKZN and has 2 research technicians. To address his scientific questions he uses genetic typing and sequencing, molecular biology approaches (recombinant proteins, reporter constructs and qRT-PCR), multiparametric (LSRII) flow cytometry, and cellular immunology techniques as well as *in vitro* virus inhibition assays. The aim of his work is to develop novel strategies for HIV vaccine development that exploit mechanisms of naturally occurring immunity. William enjoys cooking, learning to speak Spanish, swimming and wine collecting.

Victoria Kasprowicz: Victoria Kasprowicz, M.Biochem, Ph.D., is an Instructor in Medicine at Harvard Medical School and Honorary Senior Lecturer at the University of Kwazulu-Natal's Nelson R. Mandela School of Medicine. Her research focuses on the immune response to Mycobacterium tuberculosis (MTB) in the context of the HIV epidemic in Durban, South Africa. Her studies aim to identify the correlates of protective MTB immunity, guide the design of novel immunodiagnostics, and understand the mechanisms leading to an accelerated course of both diseases in the setting of HIV-MTB co-infection. She is playing a key role in developing the MTB Immunology Program at the newly created KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH) and is the Interim-Director of the K-RITH Training Program. K-RITH is a result of a groundbreaking partnership between the Howard Hughes Medical Institute (HHMI) and the University of KwaZulu-Natal in South Africa. The aim of this initiative if to establish an international research center focused on making major scientific contributions to the worldwide effort to control the devastating co-epidemic of tuberculosis and HIV, and on training a new generation of scientists in Africa. Victoria enjoys photography and the saxophone.

Wednesday, 20th October2010

SESSION 1: Oral presentations

Mammekwa Mokgoro: I'm a research clinician in charge of the Prince Mshiyeni site & its satellite clinics. I also co-ordinate the Acute HIV study at HPP. I am "what you see is what you get" kind of person. I enjoy reading, travelling, hiking, and spending quality time with my loved ones in my free time.

Manjeetha Jaggernath: I joined the Nelson R. Mandela school of Medicine in 1999 and qualified in 2004 with a Bachelor of Medicine and Bachelor of Surgery degree. I completed my Internship and Community service in 2005&2006 respectively. Thereafter I worked as a Senior Medical Officer at a Government Community health centre until June 2010 when I joined HPP. My role at HPP is that of Research Clinician at the St. Mary's and MCords hospital sites and I am in charge of the smooth running of those sites as well as liaising with the HPP base at DDMRI. In my spare time I enjoy reading fiction novels/ watching movies.

Huub C Gelderblom: Huub Gelderblom earned his MD and PhD from the University of Amsterdam, and his MPH from New York University. He is currently employed by the University of Oxford, as a Clinical Research Fellow / Project Manager stationed at the HIV Pathogenesis Programme in Durban, KwaZulu-Natal, South Africa. With HIV prevalences in antenatal clinics >40%, KwaZulu-Natal is the epicenter of the worldwide HIV epidemic. Huub is leading a team setting up a cohort study that focuses on (1) HIV prevention, and (2) the psychosocial, virological and immunological aspects of HIV acquisition and pathogenesis. Huub is a co-author of 11 peer-reviewed articles, and co-author of the Dutch National Guidelines for the treatment of chronic hepatitis C virus infection. Huub has worked in The Netherlands, Tanzania, The US, Namibia, Uganda, and South Africa.

"During an internship in a rural hospital in Tanzania in 2000-2001 I was deeply impressed by the devastating impact of infectious diseases in the developing world. While in Tanzania and later during my clinical training in Amsterdam, I realized that most decisions that determine the burden of HIV/AIDS, malaria, tuberculosis and other diseases of poverty are made outside the hospital. In 2006, during my residency in Medical Microbiology, I reasoned that rather than becoming a medical specialist in a hospital, I could be more effective as a manager in a global public health setting, applying my skills as a physician, scientist, and master of public health to the design, implementation and evaluation of sustainable disease control and prevention programs, and the overall improvement of health systems in developing countries. In the clinical setting I treated one patient at a time, but as a manager in a public health program I will be able to prevent disease and increase access to treatment on a larger scale. That is why I have switched from clinical medicine to public health." Huub likes Italian cuisine, music, swimming, books, ruminating, and traveling the world.

Katherine Johnston: Katherine Johnston BSc CompSci Hons Economics, is the Senior Data Manager at the HIV Pathogenesis Programme. She is a developer and data manager with an interest in the HIV medical research field and has spent 10 years in the field of medical informatics developing patient record systems, databases and data systems used both in patient care and medical research. She is working on the data setup of current and new studies establishing data collection tools, databases and study documentation. Her aims for HPP Data Management are to set up clear data collection standards for the unit and improve the accessibility and quality of the data collected both at the clinical sites and within the HPP laboratory. On a personal level, Katherine enjoys gaming, swimming and baking and is expecting her first baby at the end of 2010.

SESSION 2: Oral Presentations

Christina Chang : Poster 1

Prospective study on patients with Cryptococcal meningits and HIV coinfection – death, deterioration and immune restoration disease.

Chang CC, Lim A, Omarjee S, Elliott JH, Gosnell BI, Carr WH, Moosa Y, Ndung'u T, Lewin SR, French MA.

Cryptococcal meningitis (CM) is the leading cause of adult meningitis in many parts of Africa. 30-50% of patients experience neurological deterioration (ND) even after initiating adequate antifungal and ART. We have prospectively enrolled 94 HIV seropositive, ART-naive patients experiencing their first episode of CM in an on-going study and followed those starting ART for 24 weeks to investigate the various causes of ND with a focus on the incidence and immunopathogenesis of CM-associated immune restoration disease (CM-IRD). 78 patients were commenced on ART, of whom 14 died following ART commencement. Eighteen ND events were reported with 7 episodes deemed due to possible or probable CM-IRD. Patients demonstrated

good virological response and immunological response over 6 months with >90% adherence to ART.

Effector T cells were enumerated in whole blood and cerebrospinal fluid (CSF) by staining for the chemokine receptors CXCR3 and CCR5. Antigen-responsive CD4 T cells (CD25+CD134+) were quantified in whole blood cultures stimulated with cryptococcal mannoprotein (CMP). T cell responses to phytohaemagglutinin (PHA) were also assessed. In whole blood, CCR5/CXCR3 double-positive and CCR5 single-positive cells were more frequent in the CD8 T-cell subset than the CD4 T-cell subset before ART. There was an increase in CXCR3+ CD4 T-cells and a decrease in double-positive CD8 T-cells with immune reconstitution. CD8 T-cells predominated in CSF, with double-positive cells increasing during antifungal treatment and concurrent rise in expression of CXCR3 on CD4 T-cells. During immune reconstitution over 24 weeks, there was an increase in the frequency of PHA-responsive CD4 T-cells, but not in CMP-responsive CD4 T-cells in the blood.

Christina is a Melbourne-trained infectious diseases physician, 'banished' to deep dark Africa by Professors Martyn French and Sharon Lewin to set up a new clinical cohort so as to explore cryptococcal associated immune restoration disease. Christina is (secretly) only interested in South African music and travel prospects, but has to dabble in some science to justify her stay. She is immensely grateful for her many undeserved opportunities and thanks the staff at HPP and UKZN for their warm welcome, friendship and laughter.

Christina Thobakgale: Poster 2

HIV-Specific CD8+ T cell Responses in Infected Infants Enrolled on a Study of Early HAART and Structured Treatment Interruption

Christina F Thobakgale[1], Danni Ramduth[1], Nompumelelo Mkhwanazi[1], Andrew Prendergast[3], Hayley Crawford[3], Hendrik Streeck[2], Claudia Molina[3], Julia Prado[3], Alasdair Leslie[3], Zenele Mncube[1], Lungile Maphumulo[1], Sharon Reddy[1], Eshia Moodley[1], Chantal de Pierres[1], Wendy Mphatshwe[1], Natasha Blankenberg[1], Ayanda Cengimbo[1], Krista Dong[1], Fundisiwe Chonco[1], Prakash Jeena[1], Raziya Bobat[1], Gupreet Kindra[1], Gareth Tudor-Williams[4], Photini Kiepiela[1], Hoosen Coovadia[1], Bruce D Walker[1,2,5], Marcus Altfeld[2], Thumbi Ndung'u[1] and Philip J R Goulder[1,3]

[1]HIV Pathogenesis Programme, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, South Africa,
[2]Ragon Institute of MGH, MIT and Harvard University, Charlestown, MA, USA, [3]Department of Paediatrics, Peter Medawar Building for Pathogen Research, University of Oxford, UK, [4]Department of Paediatrics, Division of Medicine, Imperial College, London, UK [5]Howard Hughes Medical Institute, Chevy Chase, Maryland, USA.

The manifestation of HIV-1 infection is different in children and adults. Most of the children who acquire HIV perinatally progress to disease within the first two years of life, while adults can remain asymptomatic for up to ten years. However, a small minority group of children can control the virus for years in the absence of antiretroviral therapy. We investigated whether the age at the time of infection, specificity and functionality of the generated CD8+ T cell responses, genetic make up and the maternal immune responses to HIV, influenced disease progression in the child. We found that the majority of in-utero infected infants mounted CD8+ T cell responses from the first days of life, the specificity of the initial response in acutely infected infants was directed towards Env and Rev proteins and CD4+ T cell responses were minimal during infancy. Slow progression to disease in the child was associated with possession of one of the protective HLA-B alleles by either the mother or the child, targeting of Gag epitopes presented by the

protective HLA-B alleles and generation of polyfunctional CD8+ T cell responses. The ability of infants to induce CD8+ T cell responses early in life is encouraging for vaccine interventions. The differences in the specificity of the initial responses between adults and children, insufficient priming of these responses as a result of minimal CD4+ T cell help during infancy and possession of non-protective HLA alleles shared between mother and child, may explain the rapid disease progression generally noted in most infants. However, slow progression to disease in the minority group of children may be attributed to functional capacity of the CD8+ T cells generated by the child, mediation by protective HLA alleles, acquisition of low fitness viruses from the mother or *de novo* attenuation of the virus by the child's own immune responses.

Obtained her Masters degree in Biochemistry at the University of Natal in 2003 before joining HPP. Graduated with a BSc and BSc Honours degrees in Microbiology from the University of the North prior to that. Started working as a Research assistant at HPP in 2003, a year later was awarded a Doris Duke scholarship to study for a PHD in Paediatric HIV Immunology. Currently completing a 5 year PHD programme, thesis write up complete and awaiting examination. Research focus has been to understand HIV pathogenesis in children and to understand mechanisms for differences in disease outcome in children following acute infection. Future plans are to undertake Post-doctoral fellowship focusing on Innate immunity in acute HIV infection. Hobbies include cooking, music and travelling.

Shivan Chetty: Poster 3

Highly differential soluble and T cell cytokine profiles specific to different states of HIV/TB co- and mono-infection

Shivan Chetty[1,2], Filippos Porichis[3], Pamla Govender[1], Mona Pillay[1], Bruce D. Walker[3], Thumbi Ndungu[1],Daniel E. Kaufmann[3], Victoria O. Kasprowicz[1,2]

[1] HIV Pathogenesis Programme, University of KwaZulu-Natal, Durban, [2]KwaZulu-Natal Research Institute for TB and HIV (K-RITH), KwaZulu-Natal, Durban,[3]The Ragon Institute of MGH,MIT and Harvard, Charlestown, Boston, USA

Using Luminex, we analysed plasma levels of IFNy, IL-2, TNFα, IL-10, IL-6 and IL-13 in 23 HIV+/TB active , 23 HIV+/LTBI , 23 HIV+/TB absent and 9 HIV-/TB active individuals. We found elevated levels of IL-6 ($p<0.0001$), IFNy ($p=0.014$), TNFα ($p=0.0027$) and IL-13($p=0.0389$) in HIV+/TB active individuals as compared to HIV+/LTBI individuals. We subsequently performed 10 colour intracellular cytokine staining on isolated PBMCs from 13 individuals per aforementioned HIV+ categories. We measured IFNy, TNFα, IL-2, IL-17, IL-10 and IL-21 levels in response to SEB, GAG and the TB RD-1 antigens. Interestingly, CD4+ T cells from HIV+/TB active individuals released significantly higher levels of IL-21($p=0.0131$) and IL-17 ($p=0.0093$) but lower levels of Th1 cytokines when compared to HIV+/LTBI and HIV+/TB absent individuals stimulated with SEB. Conversely higher levels of TNFα ($p=0.0248$) and IL-2 ($p=0.0127$) but not IFNy ($p=0.683$) were seen in CD8+ T cells from HIV+/TB absent individuals as compared to the other groups. Overall both Th1 and Th2 cytokines seem to be elevated in the plasma of HIV+/TB active individuals whilst T cells from HIV+/TB active individuals exhibit impaired ability to produce Th1(IL-2, TNFα) and Th17(IL-17, IL-21) cytokines in comparison to HIV+/LTBI and HIV+/TB absent individuals.

I am currently a Virology Master's student working with the TB immunology group within HPP. I completed my undergraduate degree in biomedical science and have also worked on projects focusing on malaria and congenital eye disease. My current research entails looking at plasma cytokine levels, its polyfunctionality and characterizing the effects of IL-10 in HIV/TB co- and mono-infection. My hobbies include; football, hiking, rock and blues guitar and reading.

Dshanta Naicker: Poster 4

Effects and Mechanisms of IL-10 promoter variants on outcome of chronic HIV-1 infection

Dshanta D. Naicker, Bingxia Wang, Elena Losina, Jennifer Zupkosky, Tomoyuki Hongo, Shabashini Reddy, Karen Bishop, Fundisiwe Chonco, Philip J.R. Goulder, Bruce D. Walker, Daniel E. Kaufmann, Thumbi Ndung'u

Interleukin-10 (IL-10) is a powerful immunoregulatory cytokine. IL-10 promoter polymorphisms have previously shown to affect HIV-1 susceptibility and pathogenesis. However, the underlying mechanisms are poorly understood. We investigated the relationship between IL-10 promoter variants, plasma IL-10 levels, the breadth and magnitude of HIV-1-specific CTL immune response and markers of disease outcome in chronically HIV-1-infected individuals.

We used TaqMan assays to genotype two IL-10 promoter single nucleotide polymorphisms (SNPs) in 451 antiretroviral naïve individuals chronically infected with HIV-1 subtype. Baseline plasma IL-10 levels were measured using Luminex technology for 112 individuals. Viral load, CD4 cell counts and HIV-1- specific IFN-γ ELISpots were performed at baseline. Three hundred individuals were followed longitudinally and the rate of CD4 count decrease measured over a median of 25 months of follow-up.

The allele frequencies for the -1082G and -592A variants were 0.3 and 0.34 respectively. IL-10 levels were significantly higher in the -1082GG group than in the combined AA/AG group (p=0.0009). The 592AA genotype significantly correlated with higher breadth of IFN-γ CTL responses compared to the CC and CA (p= 0.002 and 0.004 respectively). There was a significant correlation between the rate of CD4 decline and IL-10 592 genotype (p= 0.0496), with -592AA having the least change in CD4 cells per year.

IL-10 variants correlate with IL-10 plasma levels and CD4 T cell decline during chronic HIV-1 infection. IL-10 promoter variants may influence the rate of HIV-1 disease progression by regulating plasma IL-10 levels which in turn may affect the breadth of HIV-1-specific CD8 responses.

I have been with HPP since 2006, when I began my Masters under the supervision of Prof Ndung'u. Thus far, I have completed my Masters and have now undertaken a PhD. My short term goal is to complete my PhD by the end of next year. I was fortunate to be given the opportunity to present my data, on the role of IL-10 polymorphisms in HIV pathogenesis, at the IAS 2010 in Vienna and in the future I hope to become a well established scientist in the field of HIV. I enjoy playing and listening to music, baking, swimming and dancing.

SESSION 3: Oral Presentations

Derseree Archary: Poster 5

Autologous neutralizing antibodies in chronic HIV-1 infection

[1]Derseree Archary, [2]Rong Rong, [2]Saikut Boliar, [2]Cynthia A. Derdeyn & [1]Thumbi Ndung'u

[1]HIV Pathogenesis Programme, Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa.
[2]Department of Pathology and Laboratory Medicine, Emory Vaccine Centre at Yerkes National Primate Research Centre, Atlanta, Georgia

HIV-1 envelope (Env) gp160 is an attractive vaccine target despite all its structural complexity. The flexibility, mutability and genetic diversity of the Env across multiple HIV subtypes and within individuals, is one of the most significant obstacles to the development of a vaccine. The rationale for this study was to elucidate whether broad nAb responses are associated with control in slow progressors versus progressors. Here, we report on autologous neutralizing antibodies (nAbs) to plasma-derived envelopes generated by single genome amplification. The cloned amplicons were generated into pseudoviruses for single round reporter assays in chronically infected slow-progressors and progressors. An average of 2 clones per time-point were tested from study entry and exit timepoints over a period of 19.5 months. Slow progressors (n=4) were immunologically (p=0.7) and virologically (p=1.0) similar at study entry and exit. However, the progressors (n=4) had significantly higher CD4 (p=0.03) and lower viral loads (p=0.03) at entry compared to exit. Progressors had a highly significant nAb IC50 titre when entry virus was challenged against the exit nAbs (p= 0.0004) compared to the contemporaneous responses. Contemporaneous nAb responses in both groups were not different. Slow progressors did not show any significant or potent nAb activity in any of the challenge assays. Progressors maintained sustained and maturing antibody responses in contrast to slow-progressors. In summary, although NAbs do not protect against disease pathogenesis, broad and potent nAbs are generated in progressive chronic infection. Slow-progressors may exploit mechanisms other than nAbs in order to delay progression to disease.

I have been a research scientist/student in the Department of Paediatrics and Child Health for the past 13 years. I completed my masters in medical science then went on to do a business degree after which I decided that I really want to stay in science. Babies however super-ceded any notion of doing a PhD, so once the diaper and tantrum stage had passed I decided to commit myself to a PhD in 2007.
Pet subject: Antibody research and humoral immunity
Likes: Baking, cooking, playing cricket "like a girl" according to my sons, girly soccer, reading and playing thabla- oh eating too, also I have to mention, I love my kids too (Thashir- 9, and Shayur-6). I love entertaining guests and having a good laugh.
Other interests: Astrology, palmistry and anything mysterious.

Jennifer Giandhari: Poster 6

The Role of Protease Cleavage Sites in Viral Fitness and Drug Resistance in HIV-1 Subtype C

J Giandhari[1], TN Green [1], H Sunpath[2], Y Moosa[3], B Gosnell[3], T Ndung'u[1], ML Gordon[1]

[1]HIV Pathogenesis Programme, Department of Molecular Virology, University of KwaZulu-Natal, Durban, South Africa; [2]Department of Medicine, McCord Hospital, Durban, South Africa; [3]Infectious Disease Unit, University of KwaZulu-Natal, Durban, South Africa

There is an increasing number of patients failing second line highly active antiretroviral therapy in South Africa, where HIV-1 subtype C predominates. Mutations at *gag* cleavage sites (CS) have been found to correlate with resistance mutations in *protease* (PR). Therefore, it is important to collect data on subtype C *protease* and *gag* sequences as these mutations may affect the efficacy of protease inhibitor containing drug regimens. Resistance genotyping of 30 subtype C infected second-line (AZT, DDI, Kaletra) failures was done. The *gag* genes from these isolates were also sequenced. In addition, *gag* genes of 30 HIV subtype C infected first-line (D4T, 3TC, NVP/EFV) failures were also sequenced. Other control groups included subtype B HAART-naïve, subtype C HAART-naïve and M group HAART-naïve sequences that were downloaded from the Los Alamos Sequence Database. Correlation between *gag* and *protease* mutations was measured using Spearman rank-order correlation.

Of the 30 second-line failures genotyped, 16 had resistance mutations in PR. The most frequent major mutations were: I54V/L, M46I, V82A, I84V. Other accessory mutations were: L10F/I/V L76V, A71V, and T74S. In the second-line failures known *gag* CS mutations associated with resistance were A431V and G381S/N. Most second-line failures that did not have any mutations in PR harboured mutations in *gag*. Several positive significant correlations between *gag* and *protease* mutations were noted: A431V with V82A-M46I-I54V/L-L76V-L10F/I/V; P453L with I84V; and L449P/F with I84V. These findings emphasize the need for further investigation of *gag* mutations and their contribution to the evolution of HIV resistance to PIs.

I have spent two great years at HPP undertaking a Masters degree. Research at HPP has given me the opportunity to travel and come into contact with leading scientists in this field thereby providing me with a broader understanding of the topic. My short term goal is to graduate with my Masters degree within a year from now and my long term goal is to become a pioneer in HIV research specifically focusing on drug resistance and bioinformatics. My hobbies include listening to music, cooking, reading and spending time with my family, friends and pets.

Kamini Gounder: Poster 7

Gag adaptation to immune pressure during primary HIV-1 subtype C infection

Kamini Gounder[1], Mopo Leshwedi[1], Jaclyn Wright[1], Mary van der Stok[1], Mammekwa Mokgoro[1], Manjeetha Jaggernath[1], Philip Goulder[1,2,3], Bruce D. Walker[4] and Thumbi Ndung'u[1]

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Recent studies suggest that in a majority of HIV-1 heterosexual transmission cases, only a single or very limited number of genetic variants are established in the recipient. However, infection is followed by host immune pressure, which induces viral diversification and impacts on viral fitness. HIV-1 Gag is an early target of cytotoxic T cell immune responses, some of which may be associated with control of viral replication. The precise Gag-directed immune responses, immune escape pathways and associated fitness implications during primary HIV-1 subtype C infection have not been described. Specifically, and consistent with our recent findings that some protective HLA-B alleles are associated with reduced Gag-Protease replicative capacity during chronic HIV-1 infection, we hypothesize that some HLA-B-mediated control involves driving the virus to a less fit state while other protective alleles may have multiple mechanisms of viral control. We are using single genome amplification and sequencing of *gag* sequences from longitudinal plasma samples from 25 HIV-1 subtypes C (HIV-1C) acutely infected subjects. In addition, Gag-directed gamma interferon (IFN-γ) immune responses have been measured using HIV-1C consensus overlapping peptides, and Gag-Protease viral replicative capacity will be analyzed. Thus far, a total of 128 full length gag sequences from 11 subjects have been amplified, sequenced and systematically analyzed. Preliminary data suggests rapid evolution of the plasma RNA virus. We hope to better understand the dynamics of immune responses, viral adaption and and clinical consequences of these interactions during primary HIV-1 subtype C infection. These studies may shed light on better approaches for HIV-1 vaccine design.

I joined HPP about a year ago as a Post Doctoral Fellow with Prof. Thumbi Ndung'u as my mentor. We are currently working on understanding the dynamics of immune responses, viral adaption and clinical consequences of these interactions during primary HIV-1 subtype C infection using the SGA approach. I did my undergraduate (BSc) and BSc Honours in Biotechnology at the University of KwaZulu Natal and then did a Masters in Medical Microbiology working on a project entitled 'Rapid diagnosis of TB meningitis'. My PhD (at the University of the Free State) involved high throughput genome sequencing and bioinformatic analysis of bacteria from extreme environments using the latest 454 pyrosequencing technology. When not working in science, I enjoy reading, shopping and doing crafts.

Jaclyn K. Wright: Poster 8

Gag-Protease-Mediated Replication Capacity in HIV-1 Subtype C Chronic Infection: Associations with HLA Type and Clinical Parameters

Jaclyn K. Wright[1], Zabrina L. Brumme[2,3], Jonathan M. Carlson[4], David Heckerman[4], Carl M. Kadie[4], Chanson J. Brumme[3], Bingxia Wang[5], Elena Losina[5], Toshiyuki Miura[6], Fundisiwe Chonco[1], Mary van der Stok[1], Zenele Mncube[1], Karen Bishop[1], Philip J.R. Goulder[1,7,8], Bruce D. Walker[1,8,9], Mark A. Brockman[2,3], Thumbi Ndung'u[1,8]

1. HIV Pathogenesis Programme, Doris Duke Medical Research Institute, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa. 2. Simon Fraser University, Burnaby, Canada. 3. BC Centre for Excellence in HIV/AIDS, Vancouver, Canada. 4. eScience Group, Microsoft Research, Redmond, Washington, United States of America. 5. Program in HIV Outcomes Research, Massachusetts General Hospital, Boston, Massachusetts, United States of America. 6. Institute of Medical Science, University of Tokyo, Tokyo, Japan. 7. Department of Paediatrics, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. 8. Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard University, Boston, Massachusetts, United States of America. 9. Howard Hughes Medical Institute, Chevy Chase, Maryland, USA.

The mechanisms underlying HIV-1 control by protective HLA class I alleles are not fully understood and could involve selection of escape mutations in functionally important Gag epitopes, resulting in fitness costs. This study was undertaken to investigate at a population level the impact of HLA-mediated immune pressure in Gag on viral fitness and its influence on HIV-1 pathogenesis. Replication capacities of 406 recombinant viruses encoding plasma-derived Gag-Protease from patients chronically infected with HIV-1 subtype C were assayed in an HIV-1-inducible green fluorescent protein reporter cell line. Viral replication capacity varied significantly with respect to the specific HLA-B alleles expressed by the patient and protective HLA-B alleles, most notably HLA-B*81, were associated with lower replication capacity. HLA-associated mutations at low entropy sites were associated with lower replication capacity, especially HLA-B*81-associated 186S in the TL9 epitope. Most mutations linked to alterations in replication capacity in the conserved p24 region decreased replication capacity while most in the highly variable p17 region increased replication capacity. Replication capacity also correlated positively with baseline viral load and negatively with baseline CD4 count, but not with subsequent rate of CD4 decline. In conclusion, there is evidence that protective HLA alleles, in particular HLA-B*81, significantly influence Gag-Protease function by driving sequence changes in Gag, and that conserved regions of Gag should be included in a vaccine aiming to drive HIV-1 towards a less fit state. However, long-term clinical benefit of immune-driven fitness costs is uncertain given lack of correlation with longitudinal markers of disease progression.

I am a PhD student in the HIV Pathogenesis Programme at the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa. I obtained a Masters in Medical Science degree (cancer/cell biology research) from the University of KwaZulu-Natal in 2008. My current research project, entitled "Impact of Immune-Driven Sequence in HIV-1 Subtype C Gag-Protease on Viral Fitness and Disease Progression", is aimed at understanding better HLA-mediated mechanisms of immune pressure and viral adaptation in response to this pressure. My other interests include oil painting and piano.

Thursday, *21st October2010*

SESSION 4: ORAL PRESENTATIONS

Vivek Naranbhai: Poster 9

Natural Killer Cell Activation and Function Correlate with Protection from HIV Acquisition in CAPRISA004: Clues to the Next Preventive technology?

Vivek Naranbhai, Marcus Altfeld, Lise Werner, Sengeziwe Sibeko, Quarraisha Abdool Karim, Salim S Abdool Karim and William H Carr.

Background:Defining the correlates of protection from HIV acquisition is crucial to the development of a preventive HIV vaccine or microbicide. Natural Killer (NK) cells have been implicated in providing protection from HIV-1 acquisition, however the mechanisms remain elusive. We hypothesized that the degree of NK cell frequency, activation and function correlate with susceptibility to HIV-acquisition.

Methods: We recruited women at high-risk for HIV acquisition, participating in CAPRISA004, a phase IIb, double-blinded RCT of 1% Tenofovir gel in South Africa. Using cryopreserved PBMC, we evaluated NK cell phenotypes, activation (CD69 and HLA-DR), activating receptor expression (KIR and CD38) and effector functions (degranulation-CD107a, cytokine secretion-IFN-γ and proliferation-Ki-67) by multiparametric flow cytometry. We compared HIV exposed uninfected (EU) women at the timepoint they reported the highest sexual activity (n=36), to women who acquired HIV at the timepoint most recently prior to HIV acquisition (n=44) using two-tailed Mann-Whitney tests. Samples were evaluated blinded to cohort group. We used logistic regression to model the risk of HIV acquisition based on markers and receptors for NK cell activation (CD69, HLA-DR and CD38).

Results: Exposed uninfected women had significantly lower levels of baseline NK cell activation (especially on KIR negative NK cells), lower levels of baseline and stimulant-induced NK cell degranulation, higher levels of NK cell proliferation, and markedly higher levels of CD38 on NK cells (%NK cells CD38pos: 46.9 vs. 68,7%) than women who acquired HIV. In a multivariate logistic regression model of receptors predictive of acquisition, for every 10% increase in proportion CD38pos NK cells, the risk of HIV acquisition decreased by 40.4% (p=0.0021). No difference was found between the Tenofovir gel and placebo arms in this regard. Furthermore, these results were not explained by a more mature NK cell phenotype (CD57pos) or HSV-2 serostatus.

Conclusions: Our data suggest that activation and inflammation may drive HIV acquisition; however, increased CD38 expression on NK cells appears to play a protective role. Thus, these results provide new insights on the role of NK cells in resistance to HIV-1 acquisition. Study of prevention technologies that dampen inflammation or exploit NK cells through CD38, a receptor that mediates NK cell activation and trafficking, may be warranted.

I am a graduate student at HPP. After completing his MBChB training at the NRM School of Medicine in 2009, he upgraded his MMedSci to a PhD and his graduate focusses on the role of Natural Killer cells in HIV acquisition and control. Aside from assiduously avoiding haircuts, during this year Vivek also co-ordinates a suite of basic science ancillary studies to CAPRISA004, a phase IIb placebo-controlled double-blinded RCT of 1% Tenofovir gel. These studies, known as the TRAPS studies focus on discovering correlates of protection in CAPRISA004.
He's looking forward to a career in translational infectious disease research, and returns to the clinic in 2011 to complete his internship. Vivek enjoys reading, gardening and hiking in the KZN mountains.

Marianne Mureithi : Poster 10

Assessment of the impact of HIV-1 infection on the ability to monocytes to respond to MTb and MTb-encoded TLR ligands

Marianne W. MUREITHI [1, 2], Danielle N POOLE [1, 2], Kewreshini NAIDOO[1, 2] Saleha OMARJEE [2], Zenele MNCUBE [2], Thumbi NDUNG'U[1] and Marcus ALTFELD[1, 2]

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Background: HIV-1 / MTB (*Mycobacterium tuberculosis*) co-infection represents a major public health threat globally, especially in sub-Saharan Africa where approximately 1/3 of all TB cases can be directly attributed to infection with HIV. HIV-1-encoded TLR7/8 ligands and TLR ligands encoded by products of microbial translocation have been implicated in inducing and sustaining immune activation in infected individuals. Monocyte function is also impaired in HIV-1-infected individuals, reducing their ability to respond to MTB, rendering them more susceptible to the development of active TB.
Objective: To examine the impact of pre-exposure of monocytes to HIV-1 encoded TLR8 ligands on their ability to respond to subsequent stimulation with microbial TLR2/4 ligands and in addition, to assess the impact of HIV-1 infection on the ability of monocytes to respond to MTb and MTb-encoded TLR ligands in a prospective followed HIV-1 infected cohort.
Method: Phenotypic and functional characteristics of primary monocytes from a prospective HIV-1 infected cohort were studied to characterize monocytes activation following stimulation with ligands for TLR2, TLR4 and TLR8, including chemically inactivated HIV-1 and BCG. HLA-DR, CD83, and CD86 were used to study activation and maturation of the monocytes. TNF-α and IL-12 cytokine production was quantified by intracellular cytokine staining using flow cytometry.
Results: The exposure of monocytes to HIV-1 or HIV-1-derived TLR8 ligands sensitized these cells for TLR4 stimulation, resulting in a significant higher response to LPS compared to cells that were not pre-stimulated with TLR8 ligands or HIV-1. The exposure of primary monocytes from HIV-1 infected individuals to BCG and ligands for TLR2, TLR4 and TLR8 resulted in significant higher activation in individuals with low CD4 counts of <200 and higher TNF-α and IL-12 production compared to the individuals with higher CD4 counts of >350.
Conclusion: Monocyte dysfunction play an important role in the modulation of immune function in HIV-1 infection rendering the HIV-1 infected individuals more susceptible to develop active TB.

I received my undergraduate degree from the University of Essex in 2004 and PhD from the University of Bristol in the United Kingdom in 2008. Am currently working as a Fogarty Postdoctorial fellow both at the Ragon Institute of MGH, MIT and Harvard and the HIV-1 Pathogenesis Program at the Doris Duke Medical Research Institute in Durban investigating the immunological mechanisms in macrophages and NK cells that are responsible for the increased risks of MTB infection and TB disease progression in HIV-1 infected individuals, even before their immune system is compromised to levels at which other opportunistic infections occur. I enjoy travelling, watching Friends, Family Guy and Glee on TV, listening to John Mayer and other cheesy boyband music, and spending time with family, friends and God.

Bongiwe Ndlovu: Poster 11

THE USE OF DRIED BLOOD SPOTS FOR THE DETERMINATION OF KILLER IMMUNOGLOBULIN-LIKE RECEPTOR GENE REPERTIORES AND HLA AMONG SOUTH AFRICANS

Ndlovu BG[1], Danaviah S[3], Moodley E[1], Bland R[3], Viljoen J[3], Newell M-L[3], Ndung'u T[1], Gao X[4], Carrington M[4] and Carr WH[1,2]

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Specific KIR/HLA genotypes have been reported to play a major role in HIV-1 pathogenesis, however the integrity of genetic studies can be compromised by the yield and the quality of DNA obtained from venous blood samples. The purpose of this study was to evaluate the use of dried blood spots (DBS) for killer immunoglobulin-like receptor (KIR) gene genotyping, IL-10 genotyping, and high resolution human leukocyte antigen (HLA) typing with whole genome amplification (WGA). We used DBS samples previously collected from 21 adult South Africans and stored for at least 8 years prior to our analysis. Previously collected whole blood samples from these same individuals were used as the gold standard. With a subset of 9 DBS samples we compared genomic DNA (gDNA) extraction by 3 commercially available kits. The Qiagen QIAamp DNA mini kit yielded the most optimal DNA yield and quality ($p<0.001$; t-test). Similarly this kit also yielded the most optimal results for PCR-based detection of a house-keeping gene, HLA-DBR1 (p,0.001; McNamara's test). Using sequence specific primer (SSP)-PCR for KIR genotyping of WGA-derived gDNA (wgaDNA) from DBS, we found that the sensitivity and specificity was 100% for the detection of specific number of KIR genes. Furthermore, using TaqMan® SNP Genotyping Assay kit with Taqman primers and probes, we found that IL-10 genotyping for SNP 592 and 1082 was most reliable using gDNA isolated with the QIAamp DNA mini kit ($p< 0.001$). However, HLA genotyping of wgaDNA produced allelic dropouts and increased homozygousity. However, high resolution HLA typing from DBS-derived gDNA without WGA did not differ from the gold standard. In conclusion, we found that that Qiagen QIAamp DNA mini kit was a robust method for DNA isolation from DBS with subsequent genetic analysis of KIR, HLA and IL-10 genes. We

also found that WGA produced a template suitable for reliable KIR genotyping but not for high resolution HLA genotyping.

SESSION 5: Oral Presentations

Paradise Madlala: Poster 12

Association of Polymorphisms in the Lens Epithelium Derived Growth Factor/p75 Gene (*PSIP1*) with Susceptibility to HIV-1 Infection and Disease Progression

Paradise Madlala[1,3], Rik Gijsbers[5], Frauke Christ[5], Anneleen Hombrouck[5], Lise Werner[2], Koleka Mlisana[2], Salim S. Abdool Karim[2], Cheryl A. Winkler[4], Zeger Debyser[5] and Thumbi Ndung'u[1,2]

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[4]Laboratory of Genomic Diversity, Science Applications International Corporation-Frederick, National Cancer Institute-Frederick, Frederick, Maryland, United States of America
[5]Molecular Medicine, Katholieke Universiteit Leuven, B-3000 Leuven, Flanders, Belgium

Background: Lens epithelium derived growth factor p75 (LEDGF/p75) is an interaction partner of human immunodeficiency virus type 1 (HIV-1) integrase (IN) and targets HIV-1 integration into active genes. LEDGF/p75 is encoded by *PC4 or SFRS1 interacting protein 1 (PSIP1)* gene. We investigated the role of polymorphisms in the *PSIP1* gene in susceptibility to HIV-1 infection and disease progression in black South Africans.
Methods: Integrase binding domain (IBD) of LEDGF/p75 was resequenced in 126 participants, four haplotype tagging SNPs (rs2277191, rs1033056, rs12339417, rs10283923) and one exonic SNP (Q472L, rs61744944) identified by sequencing were genotyped in 195 HIV-1 seronegative, 52 primary infected and 403 chronically infected individuals using TaqMan assays. LEDGF/p75 expression was quantified by real-time RT PCR. AlphaScreen assay was performed to determine the impact of the rs61744944 (Q472L) SNP on the interaction with HIV-1 IN.
Results: rs2277191A was more frequent among seropositives (p = 0.06, Fisher's exact test), associated with higher likelihood of HIV-1 acquisition (RH = 4.69, p=0.08; Cox model) and rapid disease progression (RH = 5.98, p=0.04; Cox model). rs12339417C was associated with slower rate of $CD4^+$ T cell decline (p = 0.02; mixed-effects model) and lower levels of LEDGF/p75 mRNA (p=0.004, GEE model). Seroconverters had higher preinfection levels of LEDGF/p75 mRNA (p=0.005, Mann Whitney test) but reduced once seroconverted (p=0.02, Mann Whitney test). LEDGF/p75 Q472L mutation showed approximately 2-fold increase in affinity for HIV-1 integrase.
Conclusions: Genetic variants of *PSIP1* may affect HIV-1 outcomes. Further studies are needed to better understand the mechanisms underlying the observed effects.

I am a PhD student at HPP, University of KwaZulu-Natal. My short term goal is to finish my PhD degree and project, entitled "association of genetic polymorphisms of select HIV-1 replication cellular cofactors with HIV-1 infection and disease progression, by end of October 2010. My future goal is to become an expert in HIV-1 replication cofactors. I enjoy singing in my church choir group and reading novels.

Kavidha Reddy: Poster 13

APOBEC3G Expression is Dysregulated in Primary HIV-1 Infection and a Polymorphic Variant Influences CD4+ T Cell Counts and Plasma Viral Load

Kavidha Reddy,[1] Cheryl Winkler,[2] Lise Werner,[3] Koleka Mlisana,[3] Salim Abdool Karim,[3] Thumbi Ndung'u,[1,3,*] and the CAPRISA Acute Infection Study Team

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APOBEC3G is a cellular cytosine deaminase with potent antiviral effects. In the absence of HIV-1Vif, APOBEC3G inhibits the virus by inducing hypermutations on viral DNA, among other mechanisms of action. We investigated the association of *APOBEC3G* mRNA levels and *APOBEC3G* genetic variants on HIV-1 susceptibility, and early disease pathogenesis using viral load and CD4+ T cell counts as outcomes. The study group was 245 South African females at high risk for HIV-1C infection. Quantitative real-time PCR was used to measure the *APOBEC3G* expression in HIV-ve and HIV+ve samples during primary infection. *APOBEC3G* variants were identified by DNA re-sequencing and TaqMan genotyping. We found no correlation between *APOBEC3G* expression levels and plasma viral loads (r=0.053, p=0.596) or CD4+ T cell counts (r=0.030, p=0.762) in 32 seroconverters. However, *APOBEC3G* expression levels were significantly higher in HIV-ve individuals compared to HIV+ve individuals (p<0.0001), including matched pre- and post infection samples from the same individuals (n=13, p<0.0001). Twenty-five single nucleotide polymorphisms (SNPs), nine of which were novel, were identified within *APOBEC3G* by re-sequencing followed by genotyping of 168 individuals. The *H186R* mutation, a codon changing variant in exon 4, was associated with high viral loads (p=0.0097) and decreased CD4+ T cell levels (p=0.0081). These data suggest that *APOBEC3G* transcription is rapidly downregulated upon HIV-1 infection. During primary infection, *APOBEC3G* expression levels in PBMCs do not correlate with viral loads or CD4+ T cell counts. However, structural variation of *APOBEC3G* may significantly affect early HIV-1 pathogenesis, although the mechanism remains unclear and warrants further investigation.

My interest in HIV began in 2006 as a pre-doctoral research fellow under the mentorship and supervision of Prof. Thumbi Ndung'u. I subsequently registered for a PhD in 2008. My research focuses on the role of an antiviral host enzyme APOBEC3G, in acute and early HIV infection in South Africa. This host factor has previously been shown in vitro to restrict HIV replication and play a significant role in resistance to HIV infection and in early virus replication. I aim to complete my PhD next year and thereafter hope to expand my research efforts and become an established scientist in this field of research. When I am not thinking about science I enjoy reading a good book, baking, beading, decoupage and anything that is creative.

Ravesh Singh: Poster 14

Association of TRIM22 with Type 1 Interferon Response and Viral Control during Primary HIV-1 Infection

Ravesh Singh,[1] Gaurav Gaiha,[2] Lise Werner,[3] Kevin McKim,[2] Koleka Mlisana,[3] Jeremy Luban,[4] Bruce D. Walker,[1,2,5] Salim S. Abdool Karim,[3] Abraham L. Brass,[2] Thumbi Ndung'u,[1,2,3*] and the CAPRISA Acute Infection Study Team

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Type 1 interferons (IFN-1) induce the expression of the Tri-partite interaction motif (TRIM) family of E3 ligases but the contribution of these antiviral factors to HIV pathogenesis is not completely understood. We hypothesized that increased expression of select IFN-1 and TRIM isoforms is associated with significantly lower likelihood of HIV-1 acquisition and viral control during primary HIV-1 infection. We measured IFN-α, IFN-β, myxovirus resistance protein A (MxA), huTRIM5α and TRIM22 messenger RNA (mRNA) levels in peripheral blood mononuclear cells (PBMCs) of high risk, HIV-1 uninfected participants, and HIV-1 positive study participants. Samples were available from 32 uninfected subjects, and 28 infected persons, all within one year of infection. HIV-1 positive participants had higher levels of IFN-β ($p=0.0005$), MxA ($p=0.007$) and TRIM22 ($p=0.01$) and lower levels of huTRIM5α ($p<0.001$) compared to HIV-1 negative participants. TRIM22 but not huTRIM5α correlated positively with IFN-1 (IFN-α, IFN-β and MxA) (all $p<0.0001$). In a multivariate model, increased MxA expression showed a significant positive association with viral load ($p=0.0418$). Furthermore, TRIM22 but not huTRIM5α, IFN-α, IFN-β or MxA showed a negative correlation with plasma viral load ($p=0.0307$) and positive correlation with $CD4^+$ T cell counts ($p=0.0281$). *In vitro* studies revealed that HIV infection induced TRIM22 expression in PBMCs obtained from HIV-negative donors. Stable TRIM22 knockdown resulted in increased HIV-1 particle release and replication in Jurkat reporter cells. Collectively, these data suggest concordance between IFN-1 and TRIM22 but not huTRIM5α expression in PBMCs, and that TRIM22 likely acts as an antiviral effector *in vivo*.

I am a PhD student currently in my third year of study at HPP. The title of my PhD is "Regulation of TRIM E3 Ligases and Cyclophilin A and the Impact on HIV-1 Replication and Pathogenesis".

Veron Ramsuran: Poster 15

Duffy-Null-Associated Neutropenia Influences HIV-1 Infection Rate in High-Risk South African Black Women

Veron Ramsuran, Hemant Kulkarni, Weijing He, Koleka Mlisana, Edwina J. Wright, Lise Werner, John Castiblanco-Quinche, Rahul Dhanda, Matthew J. Dolan, Weihua Guan, Robin A. Weiss, Robert A. Clark, Salim S. Abdool Karim, Sunil K. Ahuja, Thumbi Ndung'u

Apart from the prior observation in the Multicenter AIDS Cohort Study that Caucasian men who resisted HIV-1 infection had higher blood neutrophil and CD8+ T-cell counts than those who became infected, the influence of levels of peripheral blood cells on HIV risk is unknown. We evaluated this question in a prospective study of black South African high-risk women, including commercial sex workers. Pre-seroconversion neutrophil counts in women who subsequently seroconverted were significantly lower, whereas platelet counts were higher, compared with those who remained HIV-negative. Comprising 27% of the cohort, subjects with initial neutrophil counts of <2,500 cells/mm^3 had a ~3-fold greater risk of acquiring HIV. In genome-wide association analyses, an African-specific polymorphism (rs2814778) in the promoter region of Duffy Antigen Receptor for Chemokines (*DARC −46T>C*) was highly predictive of neutrophil counts (P_d=7.9x10^{-11}); a statistically significant association for platelet counts was not detected. Consistent with the prevailing viewpoint that the *DARC −46C/C* genotype that imparts the Duffy-null state on erythrocytes is a strong determinant of ethnic neutropenia of African ancestry, only *DARC −46C/C*-bearing study participants had initial neutrophil counts of <2,500 cells/mm^3. Before or after adjustment for platelet counts and population admixture, the risk and rate of acquiring HIV were four-fold (P=0.007) or three-fold (P=0.005) higher, respectively, in those with the trait of Duffy-null-associated low neutrophil counts compared with all other study participants. Because of the high prevalence of this trait among persons of African ancestry, it may be a major contributor to the dynamics of the HIV epidemic in Africa.

I am a PhD student under the supervision of Prof Thumbi Ndung'u and Prof Sunil Ahuja. The title of my dissertation is "Genetic/epigenetic determinants in chemokines and chemokine receptor genes that influence HIV susceptibility in a cohort of high-risk women from South Africa". My goal is to submit my thesis for examination in the next few weeks. Very recently I am became a proud father of a beautiful baby girl Naradi Gopi Ramsuran. Apart from writing up my thesis, Naradi steals all my time. Future plans for me entails working in research perhaps a Post Doctorate….offers welcome ☺

6. ABSTRACTS FOR POSTERS

Susan Bryan: 1a

Modelling longitudinally measured HIV outcome biomarkers with immune genetic parameters

Candidate: Susan Bryan
Supervisor: Prof. Henry G. Mwambi
Co-Supervisor: Dr Shaun Ramroop

Objective: It is clear that modelling and understanding HIV infection data that is generated from a longitudinal measurement process is complicated. Apart from the complexity of individual to individual variability the outcome is multi-dimensional. This type of data needs methods such as joint modelling and their modifications in order to fully understand the problem.
Methods: This involves doing multiple outcome modelling, analysis and inference. The analysis inherently combines both linear and non-linear mixed models for longitudinal data. An additional complexity or problem is that of missing or incomplete data requiring a clear understanding of missing data frameworks and mechanisms. New innovations and advancements in longitudinal data methods include mixed effects selection models, mixed effects pattern-mixture models (adding shared random parameters to combine the measurement process, missing data mechanisms with individual to individual variability or heterogeneity and mixed effects hybrid models (which combines both selection and pattern properties in one framework). These techniques all require Joint Modelling of more than one outcome or response (observed and unobserved) in addition to allowing for dependence on measured covariates such as the quality of care and other intervention strategies which may include combined therapies.
Results/Conclusion: The current work will seek to exploit novel longitudinal data methods mentioned above in order to efficiently model longitudinally measured HIV outcome biomarkers with immune genetic parameters using the Sinikithemba study.

I am currently doing my Masters in Statistics. I am registered with the University of Kwa-Zulu Natal on the Pietermaritzburg campus, under the supervision of Professor Mwambi and Dr Ramroop. My majors at an undergraduate level included Statistics and Psychology, with an Honours in Statistics. My other interests include Show Jumping, which I do at a competitive level.

Eshia Moodley: Poster 1b

TIM-3 contributes to T cell exhaustion in HIV-1 clade C infected adults and children

Eshia Moodley[1], Fundi Chonco[1], Mathieu Angin [2], Fang Wen[2], Marcus Altfeld[1,2],Hoosen M. Coovadia[1], Photini Kiepiela[1], Bruce Walker[1,2,4], Philip Goulder[1,2,3],Thumbi Ndung'u[1,2] and Marylyn Addo[1,2]

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Background: Inhibitory receptors such as Tim-3 (T cell immunoglobulin and mucin domain containing molecule) play a crucial role in regulating T cell function during chronic viral infections. A recent study in HIV-1 clade B infected adults suggested that Tim-3 expression on T cells was increased in chronic HIV infection and correlated with markers of HIV disease progression. Tim-3 expressing T cells exhibited an "exhausted" phenotype with impaired functionality and blockade of Tim-3 resulted in restoration of T cell effector activity such as cytokine secretion and proliferation. To date no studies have been performed to investigate the role of Tim-3 in HIV-1 infected children and no data is available in the context of HIV-1 clade C infection. In this study we therefore aimed to assess Tim-3 expression profiles in HIV-1 clade C infected children and adults and to investigate its impact on markers of disease progression.
Methods: We studied 10 clade C infected adults, 20 perinatally infected children and adult and pediatric HIV negative control subjects. Expression of the inhibitory molecules Tim-3 and PD-1 on CD4+ and CD8+ T cells and monocytes was determined by multiparameter flow cytometric assays. FMO (fluorescence minus one) controls were utilized for optimal gating and positive results were analyzed above background. Data was analyzed using FLOWJO and GRAPHPAD Prism software.
Results: Tim-3 expression levels were found to be elevated on T cells from HIV-1 clade C infected adults and children as compared to HIV-1 uninfected controls.
Similar to findings in HIV-1 clade B infection, expression of Tim-3 was higher on CD8 compared to CD4 T cells, but Tim-3 expression levels on CD4 and CD8 T cells were positively correlated ($r=0.45$; $p=0.028$). In adults Tim-3 expression on T cells showed a negative correlation with CD4 T cell count and CD4 percentage as described before. In untreated perinatally infected children expression of Tim-3 ranged from 1-51 (mean 22%) on CD4 and 18-77 (mean 44%) on CD8 T cells. Tim-3 expression on T cells was highest in infants under 6 months and children that required initiation of ART and appeared to be associated with high viral loads. Tim-3 expression on CD4 and CD8 T cells correlated with expression of PD-1. In the children who were followed longitudinally, Tim-3 expression levels on T cells were maintained at high levels and tracked with HIV viral load.
Conclusions: We here demonstrate that the expression of the inhibitory molecule Tim-3 on T cells is elevated in adult and pediatric HIV-1 clade C infection. Its high expression on T cells from perinatally infected children suggests that Tim-3 contributes to T cell exhaustion in children, with highest expression levels in the age group of < 6 months, where HIV-1 specific T cell immunity is of lowest magnitude and breadth. Tim-3 in concert with other inhibitory molecules such as PD-1 may therefore contribute to the failure to control viral load and to accelerate disease progression in this vulnerable pediatric patient population.

Eshia Moodley: *I have the benefit of been part of our prestigious HPP research collaboration for over six years. My research interests have grown as with technology, knowledge and supervision within this field. My current gratification lies in the completion of my PhD investigation by contributing to the evolving knowledge in the determinants of protective immunity within HIV infected Mother-Child Pairs. My future goal is to watch my children be part of an HIV wise/controlled/free generation. My hobbies lie in the challenge of balancing a healthy life and a continual smile within each facet of life (GOD, family, academia, friends and business).*

Nasreen Ismail: poster 1c

Characterization of HLA-B*4201-Restricted Responses in HIV-1 Clade C Infection

Nasreen Ismail [1], Hoosen Coovadia [1], Bruce Walker [1,3], Philip Goulder [1,2,3], Thumbi Ndung'u [1]

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3. Partners AIDS Research Center, Massachusetts General Hospital, Charlestown, MA 02129

HLA-B*4201 is a common HLA allele in the Zulu/Xhosa population in South Africa and it has been associated with relative control of HIV-1 viral load in some studies. However, all the specific epitopes presented by this HLA protein have not been identified. Additionally, the mechanisms by which protective HLA class I molecules influence HIV-1 viral load are poorly understood and this has important implications for rational vaccine design. Here, we optimally defined seven HLA-B*4201- restricted cytotoxic T lymphocyte epitopes from an analysis of 34 HLA-B*4201 positive, therapy naïve, clade C infected subjects using epitope-specific T cell lines and HLA-mismatched B-cell lines by interferon-gamma intracellular cytokine staining. Two of the epitopes were Gag p24 variants, viz GPGHKARVL and GPSHKARVL. The other epitopes optimally defined were EPKDREPLTSL in Gag, LPPIVAKEI in Integrase, HPKVSSEVHI in Vif, FPRPWLHGL in Vpr and GPKVKQWPL in Reverse transcriptase. Analysis of 464 chronically infected HIV-1 positive patients revealed that HLA-B*4201-positive individuals were more likely to make IFN-γ ELISPOT responses to the Gag overlapping peptide 25 (OLP 25) than HLA-B*4201 negative patients ($p < 0.0001$, Fisher's exact test). HLA-B*4201 positive patients were more likely to have immune-driven mutations in this peptide (p value <0.0001, Fisher's exact test). We have optimally defined 7 novel epitopes targeted by HLA-B*4201 which may assist in studies of HLA-driven HIV pathogenesis and vaccine design. We have demonstrated that HL-B*4201 selects for escape mutations within Gag OLP 25.

*I was previously employed in the Department of Medical Microbiology and have experience working in the M. tuberculosis and Sexually Transmitted Infections field. I am currently enrolled as a Masters student and my topic involves the Characterization of HLA-B*4201 epitopes in Clade C HIV-1 Infection. I am near the end of the project and I am in the process of putting together a thesis. My hobbies include reading and baking. My immediate goal is to complete the current degree. My more distant goals are to have a more direct impact on the HIV epidemic.*

Manishka Jagwanth: Poster 1d

Unusual Monoclonal Antibody Reactivity Revealed by KIR2DL1/S1 Staining Patterns Among South Africans

Manishka Jagwanth[1], Vivek Naranbhai[1,2] and William Carr[2,3]

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Studies have shown monoclonal antibodies to display unusual reactivity to KIR2DL3 resulting from polymorphisms in the amino acid sequence. Unusual staining for KIR2DL1 and KIR2DS1 has also been observed in donors from the Sinikithemba (SK) cohort, suggesting that the antibodies employed have different specificities for KIR2DL1 and KIR2DS1. Here we aim to determine the basis of unusual antibody staining patterns for KIR2DL1/S1 using donors with predetermined genotypes, and hypothesise that differences in KIR2DL1/2DS1 staining patterns between different antibodies are due to variation in amino acid sequences. Amino acid alignments of all KIR2DL1/KIR2DS1 allotypes were performed to identify polymorphisms in the extracellular domains. The KIR2DL1 amino acid alignment revealed polymorphisms of interest in positions 138, 177 and 205 in the D2 domain for several subtypes. Staining will be performed on a buffy coat sample with a known genotype to validate initial staining patterns. Following this, PBMC from chronically HIV-infected donors from the SK cohort representative of the different staining patterns seen with anti- KIR2DL1/KIR2DS1 (HP-3E4) and anti-KIR2DL1 (143211) antibodies will be used. Panels were designed to define NK cells, as well as to exclude KIR2DS4, KIR2DL2 and KIR2DL3 as some monoclonal antibodies are able to cross-react with several KIRs. We present results of flow cytometry data collected and analysed with an LSRII using FlowJo analysis software. We anticipate that this study will provide valuable information for selection of the most appropriate, commercially available reagents for the analysis of NK cells that express KIR variants endemic among South Africans.

I completed my BSc (majors in Microbiology and Genetics) at UKZN (PMB campus), and then went on to do my honours in Gentics . Currently, I am working at CAPRISA (Fellow/Research assistant to Dr Vivek Naranbhai), and am part of the Innate Immunology research group. I am looking forward to starting my MSc studies in the near future. In my spare time, I enjoy cooking, jogging on the beach, spending time with friends and family, and playing the piano.

Serron Wilson: Poster 1e

Impact of Gag p2/p7^{NC} cleavage site polymorphisms on HIV-1 subtype C viral fitness

Serron Wilson
Supervisor: Dr M Gordon

A significant feature of HIV-1 subtype C is its many naturally occurring polymorphisms. In particular, the cleavage sites of the Gag precursor are significantly more polymorphic in subtype C than in subtype B, with the p2/p7 cleavage site being the most polymorphic. Previous studies on Gag cleavage site mutations indicate that variation at cleavage sites can have a significant influence on viral characteristics including drug resistance and increased rate of cleavage of Gag. This study aims to determine the effect of genotypic variability at the p2/p7 Gag cleavage site on cleavage rate and replication capacity. The p2/p7 region from subtype C drug naive sequences was downloaded from the Los Alamos Sequence database. Three dimensional models of the peptide sequence at the cleavage site have been generated using Swiss-Model at Expasy to predict the secondary structure followed by Chimera generation of the models. The binding affinity between Protease and the peptide sequences will be estimated using the docking software Molegro and then ranked in order of affinity. Variants from each group of high, medium and low affinity binding for Proteases will be generated and characterised in terms of replication capacity and Gag cleavage rate. Preliminary results have revealed a hydrophobic region at the scissile bond, with a moderately conserved "IMM" motif at the P2, P1 and P1' positions. However, in comparison, positions P3-P5 were found to be more variable. The results obtained will demonstrate whether HIV-1 subtype C viral fitness is influenced by Gag p2/p7 cleavage site polymorphisms.

I attained my Bachelors degree in Biochemistry and Chemistry at UKZN PMB and continued with Biochemistry for honours. My honours project was on the subject of enzymes involved in pathogenesis of African Animal Trypanosomosis. I joined HPP in February 2010 to begin Masters in Medical Science with Dr Michelle Gordon. My short term goal is to finish my degree in 2011, followed by extensive travel. My long term goal is to obtain a PhD and become an accomplished researcher in the field of HIV. Personal activities I enjoy are running, breeding of German Shepherds and horse riding.

Ngomu Akeem Akilimali: Poster 1f

Leukocyte Immunoglobulin (Ig)-Like Receptors (LILRs) in Mtb/HIV co-infection

Ngomu Akeem Akilimali[1, 2], Shivan Chetty[1], Jessica Michelle[1], Pamla Govender[1], Mona Pillay[1], Bruce D. Walker[3], Thumbi Ndungu[1], Victoria O. Kasprowicz[1, 2, 3]

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Leukocyte Immunoglobulin-Like Receptors (LILRs) are a family of immunoregulatory cell surface receptors that may be activating (LILRA) or inhibitory (LILRB) depending on the nature of their

transmembrane and cytoplasmic domains. They are expressed on cells of both myeloid and lymphoid lineage and may influence signalling pathways of both the innate and adaptive immune systems. We aim to investigate the expression profile of LILRs on immune cells (CD4, CD8, monocyte/macrophages, DCs and NK cells) in healthy individuals and then compare this to individuals with both latent and active TB disease. The impact of HIV co-infection will also be assessed. A total of 50 individuals from the following categories will be used: Absent of TB/HIV-; Active TB/HIV-; Latent TB/HIV; Active TB/ HIV+; Latent TB/HIV+; Absent of TB/HIV+. LILR expression profiles will be assessed by Flow cytometry on whole blood using the six available antibodies (LILRA2, LILRB1, LILRB4, LILRB2, LILRB3 and LILRA2), and qPCR on sorted cell populations using primer sets for all 11 LILR family members. We hypothesize that MTB and HIV infection will alter LILR receptor expression profiles compared to that observed in healthy individuals. This may be indicative of a role in receptor-mediated immune cell functional modulation. We are currently working on qPCR-primer optimization on genomic DNA.

I am an MMEDSC student under the supervision of Dr. Victoria Kasprowicz. I completed a Bachelor of Science degree (BSc-AG) in Genetics at the University of KwaZulu-Natal (UKZN) Pietermatzburg campus. My Masters Research project is title: "The Role of Leukocyte Immunoglobulin (Ig)-Like Receptors (LILRs) in Mtb/HIV co-infection". Throughout the four years of my degree I worked as a research assistance for school of Zoology (UKZN), school of Botany (UKZN) and the University Animal House. During this period I gained experience in administrative work, laboratory, field experiment and handling of research animals. I enjoy fixing computers, Church community, sound engineering, relaxing in the park and at the beach, running, reading and playing computer games.

Nompumelelo Prudence Mkhwanazi: Poster 1g

Immunodominant HIV-1-Specific HLA-B- and HLA-C-Restricted CD8+ T-cells do not differ in Polyfunctionality

Nompumelelo Mkhwanazi[a], Christina F. Thobakgale[a,] Mary van der Stok[a], Shabashini Reddy[a], Zenele Mncube[a], Fundisiwe Chonco[a], Bruce D. Walker[a,b,c], Marcus Altfeld[a,b], Philip J.R. Goulder[a,d], Thumbi Ndung'u[a,b*]

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HIV-1 specific HLA-B-restricted CD8+ T-cell responses differ from HLA-C-restricted responses in antiviral effectiveness. To investigate possible reasons for these differences, we characterized the frequency and polyfunctionality of immmunodominant HLA-B*57/B5801- and HLA-Cw*07-

restricted CD8+ T-cells occurring concurrently in nine study subjects assessing IFN-γ, TNF-α, IL-2, MIP-1β, and CD107a by flow cytometry and analyzed sequence variation in targeted epitopes. HLA-B*57/5801 and HLA-Cw*07 restricted CD8+ T-cells did not differ significantly in polyfunctionality (p=0.84). Possession of three or more functions correlated positively with CD4+ T-cell counts (r=0.85; p=0.006) and monofunctional CD8+ T-cells inversely correlated with CD4 cell counts (r=-0.79; p=0.05). There were no differences in polyfunctionality of CD8+ T-cells specific to wildtype versus mutated epitopes. These results suggest that loss of polyfunctionality and increase in monofunctional HIV-1-specific CD8+ T-cells are associated with disease progression independent of restricting HLA allele. Furthermore, sequence variation does not appear to significantly impact CD8+ T-cell polyfunctionality in chronic HIV-1 infection.

I have just submitted my Master's thesis for examination in August at University of KwaZulu-Natal. I obtained BSc (biochemistry and microbiology) in University of KwaZulu-Natal (PMB). In 2004, I have obtained BSc Honours in Biochemistry in UKZN. I have worked in HIV Pathogenesis Programme since 2005 as a research assistant. I have co-authored 10 peer-reviewed journal articles papers since I joined HPP. I have presented most of the work done here in HPP in local and international meetings. In 2010, I have published the first paper as the first author in Virology, I am now looking forward to starting a new PhD project. I love reading newspapers, inspirational books and I like discussing issue around our society, watching soccer and news. I also love spending time with God.

Vanessa Naidoo: Poster 1h

Gag-Protease Sequence Variation and Fitness Influence on HIV-1 Mother to Child Transmission and Paediatric Disease Progression

Supervisor: Professor Thumbi Ndung'u

Mother to child transmission (MTCT) and paediatric disease progression of HIV-1 are influenced by viral and host factors. We hypothesize that immune driven viral fitness and sequence variation within the functionally essential HIV-1 Gag and protease proteins may be important determinants of MTCT and paediatric disease progression. The study involves 46 HIV-1 subtype C infected infants and their transmitting mothers, with a control group of 62 HIV-1 infected non-transmitting mothers. Gag-protease from viruses infecting these patients were amplified. The amplicons were then cotransfected along with NL4-3ΔGag-Protease into a CEM-GXR cell line to generate chimeric viruses. One hundred and forty-five recombinant viruses have been generated thus far. Viral replicative capacities of recombinant viruses will be measured using an HIV-1-inducible green fluorescent expressing cell line. We expect to find that viruses which infect infants in HIV-1 positive mother-infant pairs are more fit than those that infect their mothers and that Gag-protease-driven viral fitness is a determinant of the rate of disease progression in HIV-1 infected infants. We expect to find that transmitting mothers are infected with viruses that have a higher fitness, that there is a correlation between the fitness of viruses infecting the mother and child and the HLA type of the mother and the child respectively. The results of this study may have important implications for the design of vaccines that interrupt MTCT or paediatric HIV-1 disease progression and may help provide novel information on the mechanisms of paediatric HIV-1 pathogenesis.

I am a Masters student and I am currently in my second year. I studied BSc in Biomedical Sciences as part of my undergraduate studies from 2004-2006 at the University of KwaZulu Natal. I studied honours in Molecular Medicine and Medical Biochemistry at the University of Witwatersrand in 2007. Thereafter I have worked as a technician in a pathology laboratory before I started my masters in 2009. I am really fascinated and passionate about HIV research and I plan to study further and continue working in this field. My hobbies include reading novels, walks on the beach, attending concerts, listening to music and swimming.

Mbali Nkabinde: Poster 1i

Optimization of quantitative RT-PCR assays for KIR gene expression analysis

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The Killer Immunoglobulin-like Receptors (KIR) are inhibitory and stimulatory receptors found on Natural Killer (NK) cells. NK cells are cells of the innate immune response that act as the host's first line of defense against infections. We aim to determine relative frequency of KIR expression in 20 healthy adults. Using SYBR green reagent and quantitative RT-PCR methods we quantified relative KIR expression in PBMC's using Roche LC480 machine. Here we present the progress in optimizing the detection of KIR gene expression; for the genes *GAPDH, β-actin, KIR2DL2*, *KIR2DL4*, *KIR2DS1*, *KIR3DL1* and *CD56* RT-PCR conditions on the LC480 machine were optimized. *CD56* was used as a NK cell marker. The PCR conditions for *KIR2DL1, KIR2DL3, KIR2DL5, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL2, KIR3DL3,* and *KIR3DS1* are still being optimized. *GAPDH* and *β-actin* were used as positive controls. To make the above results more reliable, the PCR products were also run on a 2% Agarose gel and one specific product was obtained, and then it will be sequenced. To determine what KIR genes were present in the donors, KIR genotyping was done. The samples that we used were PBMC samples from 4 individuals from a cohort of healthy donors. We conclude that this technology can be used to assess KIR expression in whole blood and dried blood spots, therefore we will attempt to quantify KIR expression using dried blood spots samples and whole blood samples.

I am currently doing my internship at HPP under the work-intergrated programme from the Durban University of Technology. I love watching soccer and cricket. On my spare time I do karate lessons. My goal next year is to do an Honours Degree in Medical Microbiology.

Henrik Kloverpris: Poster 2a

HLA-B*4201-restricted Inter- and Intra-Protein-Specific Differences in Anti-HIV CD8$^+$ T-Cell Epitope Efficacy

Henrik N. Kloverpris, Catherine K. Koofhethile, Rebecca Payne, Philippa Matthews and Philip Goulder

In order to improve T cell based vaccines against HIV, it is crucial to identify and better characterise the CD8+ T cell responses most effective in suppressing viral replication. A large number of CD8+ T-cell epitopes are yet to be defined. In this study, we focused on the prevalent HLA-B*4201 allele expressed in ~20% of the individuals in subSaharan African populations. We used a statistical approach to identify, from 410 overlapping 18mer peptides (OLPs), 18 OLPs containing epitopes presented by B*4201, of whom only 6 epitopes have been unequivocally defined previously. We used peptide HLA-B*4201 tetramers as a novel approach to define the following 4 novel epitopes, Gag RM9 p17 (RPGGKKHYM), IM9 Int (IIKDYGKQM), HI10 Vif (HPKVSSEVHI) and IL9 Env (IPRRIRQGL). To assess efficacy of these distinct HLA-B*4201-restricted responses, we first compared viral setpint in B*4201-positive responders to each epitope, with viral setpoint in the non-responders. In all the 5 B*4201-restricted Gag epitopes, response to the epitope tended to be associated with lower viral setpoint; the opposite was the case with respect to the Acc-Reg and Envelope-specific responses. We next compared this criterion for efficacy with the ability of different CD8+ T-cell responses to drive the selection of escape mutants. In particular we focused on two Gag epitopes and show that the B*4201-RM9-specific response selects escape mutants, whereas the B*0702-RM9 response does not; and the B*0702-GL9 (GPSHKARVL) response selects escape mutants whereas the B*4201-GL9 response does not. These data demonstrate the importance of detailed characterization of the HIV-specific CD8+ T-cell response to better understand what epitopes are likely to prove important to incorporate into an effective HIV vaccine.

Work - Identification of new CTL epitopes in HIV using a novel approach of peptide-MHC class I tetramers. Novel responses, as well as known responses, are then examined with respect to; 1) selection of viral escape mutants, 2) kinetic of epitope processing and presentation, 3) Viral inhibition, 4) differences in the immunophenotype and other functional assays.
Position - Phd student Dec 2007-Dec 2010
Hobbies - Football, Tennis, Golf, Sailing, Beers, Wine, Music

Emily Adland : Poster 2b

HLA-A*7401-associated immune control of C-clade HIV-1 infection independent of HLA-B*5703 linkage disequilibrium.

HLA-B alleles have the greatest impact of HLA class I alleles on viral set point in HIV-1 infection. HLA-B*5703 is the best-characterised example of this with its favourable control of viraemia. The impact of HLA-A alleles can therefore potentially be obscured by linkage disequilibrium with an HLA-B allele. We here describe the association of HLA-A*7401 with low viral setpoint in 3 subSaharan African populations, in Durban, Bloemfontein and Gaborone, independent of the effect of linkage disequilibrium between HLA-A*7401 and HLA-B*5703. We then used two approaches to identifying HLA-A*7401-restricted CD8+ T-cell epitopes through which this effect

on viral setpoint might be mediated. First, sequence analysis identified six sites of HIV polymorphism associated with HLA-A*7401, in Gag, Pol and Nef. Using a predicted peptide-binding motif for HLA-A*7401, we identified putative A*7401-binding epitopes that incorporated the sites of HLA-A*7401-associated polymorphism and tested their ability to bind A*7401. The second approach we adopted to defining optimal A*7401 epitopes was to identify overlapping 18mer peptides (one in Gag, one in Rev) specifically recognized by HIV-1 C clade-infected study subjects expressing HLA-A*7401. Using these two approaches we have successfully defined 3 novel epitopes restricted by A*7401 in Gag, Pol and Rev and have identified a further 4 putative epitopes based on sites of polymorphism associated with HLA-A*7401. Three of these A*7401-associated mutants are predicted to revert to wild type following transmission to an HLA mismatched host suggesting a fitness cost is imposed by these mutations. These studies support the hypothesis that certain HLA-A alleles may function in tandem with HLA-B alleles to control HIV-1 viraemia and highlights the potential contribution an HLA-A allele may make in this control.

I graduated from The University of Wales, Aberystwyth in 2005 with a BSc(Hons) in Equine Science. From here I worked as Research Technician in the Institute of Cancer Studies at Birmingham University where my research interests were focussed around Epstein-Barr Virus infections of epithelial and NK/T cells. I moved to Oxford in 2009 to join Prof. Philip Goulder's lab as a Research Assistant where my current research interests include the effects of HLA-A on HIV-1 viraemia. I wish to continue my research here in Oxford and register for a DPhil in Prof. Philip Goulder's lab. Interests outside of work include competing my horses in showjumping, dressage and endurance riding and socialising at the local pub!!

Catherine Koofhethile: Poster 2c

Rapid generation of MHC Class I Tetramers

Koofhethile C. K., Kloverpris H., Buus S. and P. Goulder.

MHC tetramers are complexes of MHC Class I or II glycoproteins with peptide bound to the fluorescently labelled streptavidin molecule. These complexes have been extensively used in immunological studies especially the direct enumeration and characterisation of antigen-specific CD8+ and CD4+ T cells. We have generated an array of these reagents using two approaches. First, we have followed the method described by Altman *et al*, 1996. A 15 amino acid recognition tag is engineered on to the carboxyl terminus of the extracellular $\alpha 3$ domain of the heavy chain of the MHC I gene to allow it to be enzymatically labelled with biotin and bound to streptavidin. The α (heavy) and $\beta 2$ microglobulin (light) chain proteins are then expressed and purified, and finally refolded with the peptide to form the peptide-MHC I monomer. The monomers are exchanged from the folding buffer into the biotinylation buffer and then isolated from other proteins by Fast protein liquid chromatograph (FPLC). Finally, the MHC tetramers are generated by the binding of monomers to fluorescently labeled straptavidin molecules. The second approach of tetramer production described by Leisner *et al* 2008, is based on the *in vitro* refolding of the $\beta 2$ microglobulin chain with the peptide and the correctly oxidised, *in vivo* biotinylated MHC Class I heavy chain and tetramerisation with Streptavidin - all in one pot reaction without the need for purification steps. The heavy and light chains used in this approach

are readily generated at the Laboratories of Experimental Immunology in Denmark (Ferre *et al* 2003). The advantages of the latter approach are that the method is simple, fast and cost effective in that a tetramer is produced within 2 days with very limited effort and hands on time.

I was born and brought up in Botswana. I graduated from the University of Surrey, UK in 2005 with a BSc (Hon) in Medical Microbiology. I went back to Botswana to work for Botswana Harvard Partnership Laboratory as a Research Assistant/Forgaty Fellow for 2 years. I then came back to the UK to read for a Masters Degree in Immunology at the University of Birmingham. Upon completion in 2008, I joined Prof Goulder's Laboratory at Oxford University as a Research Assistant. This is where it all started to happen for me as my interests in HIV research grew stronger. In addition to sample processing and running ELISPOT assays, I have spent some time on a number of projects including sequencing of the HIV B and C clade viruses for a number of cohorts. The most exciting and challenging project that I have spent most time on is the 'Tetramer synthesis' project which has not only been fruitful after a good number of months, but has also taught me a lot about myself as a researcher, the patience and determination one needs in order to achieve a goal. My plan is to continue on studying CD8 T cell responses by doing a PhD at the University of KwaZulu Natal in collaboration with Prof Goulder's group. Apart from the love of science, I love shopping and if I were the head of the lab, all lab coats would be pink!

Dr Joris Hemelaar: Poster 2d

Application of 454 sequencing in mother-to-child transmission studies to investigate virus transmission and within-host viral evolution in mother and child

Joris Hemelaar[1], Christina F. Thobakgale[2], Christian L. Boutwell[3], Todd M. Allen[3], Thumbi Ndung'u[2], Bruce D. Walker[2,3,4], Philip Goulder[2,5]

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HIV sequence variability and evolution play key roles in HIV immune control and escape. Ultra-deep sequencing allows for the simultaneous sequencing of many hundreds of viral species in a given sample, affording the detection of low-frequency variants. Applied to mother-to-child transmission of HIV, this approach will enable the accurate and efficient identification of transmitted/founder viruses and the determination of multiplicity of infection. The influence of the stage at which transmission took place (intra-uterine, intra-partum, post-partum/breastfeeding) on founder virus characteristics and the multiplicity of infection can thus be determined. HIV evolution and immune pressures in acute as well as established infection can be examined in more detail than previously possible, examining the whole length of the genome for reversion of transmitted mutations, new escape mutations and compensatory mutations. Patterns of evolution will be compared between mothers and their children, between acute and chronic paediatric infection as well as between fast and slow progressor children.

Born in The Netherlands, Joris completed his BSc and MSc in Molecular Biology and Genetics at Leiden University. He went on to do a DPhil (PhD) in Molecular Immunology, focusing on antigen presentation in HTLV-1 infection, with Prof Sir Andrew McMichael FRS at Oxford University. This was followed by a post-doc with Prof Hidde Ploegh at the Harvard Medical School, where he used a proteomics approach to identify enzymes interacting with ubiquitin-like modifiers. He then returned to Oxford to obtain his BM BCh medical degree. After working as a doctor in London for two years, he again returned to Oxford in 2009 to start his specialty training in Obstetrics and Gynaecology. During his medical training and early years as a medical doctor he conducted a global molecular epidemiology study of HIV with the World Health Organisation determining the global distribution of HIV subtypes and recombinants. In his current position as an Academic Clinical Fellow he conducts research alongside his clinical training, focusing on HIV evolution and T cell immunology in the context of mother-to-child transmission of HIV.

Anne G Kasmar: Poster 2e

CD1b tetramers detect human glycolipid-specific T cells

Anne G Kasmar[1], Tan-Yun Cheng[1], Marie Turner[2], Chetan Seshadri[3], Andre Schiefner[4], Ravi Kalathur[5], Ian A Wilson[5], John Shires[6], John Altman[7] and DB Moody[1]

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6 Emory Vaccine Center
7 NIH tetramer facility, Emory University

αβ T cells were previously thought to solely recognize peptides. Studies over the last decade show that CD1 molecules present lipid, glycolipid and lipopeptide antigens to T cells. Investigation of CD1 function during infection *in vivo* has been dominated by the study of one major population, CD1d-restricted Natural Killer T (NKT) cells; however, NKT cells are only one part of the larger family of CD1-restricted T cells that recognize CD1a, CD1b or CD1c. *In vitro* study of CD1b-restricted T cell clones demonstrates proliferation, cytolysis and release of interferon-gamma in response to lipid ligands presented by CD1b. These studies raise the possibility that CD1b-restricted T cells play a role in natural *in vivo* host immune responses. We developed glucose monomycolate-loaded CD1b tetramers and used them to identify mycolate-specific T cells in the peripheral blood of a patient infected with *Mycobacterium tuberculosis*. Future work at KRiTH will investigate the impact of HIV co-infection on the frequency, phenotype and function of mycolate-specific T cells isolated from the peripheral blood and infected tissues.

Anne Kasmar MD MSc is a physician-scientist who studies human immunity to tuberculosis with an emphasis on T cell responses to glycolipids. She obtained her BA in Comparative Literature from Brown University, her MD from the University of California San Francisco and her master's in the Immunology of Infectious Diseases from the London School of Hygiene and Tropical Medicine. She completed internship, residency, chief residency and infectious diseases fellowship at the Massachusetts General Hospital and is currently a postdoctoral fellow in the laboratory of D. Branch Moody at Harvard Medical School. She has been working on TB-related projects spanning

epidemiology, public health, clinical care and basic investigation for the past twelve years. She loves travel, yoga and is really looking forward to working together at KRiTH

Denis Chopera: Poster 2f

Impact of B*57/B*5801 associated CTL escape and compensatory mutations on HIV-1 subtype C viral fitness

D.R. Chopera[1], Z. Woodman[1], D.P. Martin[1], D. Assis de Rosa[2], K. Mlisana[3], S. Abdool Karim[3], C.M. Gray[2], T. Allen[4], C. Williamson[1]

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Background: Individuals infected with viruses carrying B*57/B*5801-associated CTL escape mutations, A146X and T242N have been shown to have higher CD4+ counts and lower viremia, presumably due to fitness cost incurred by escape. This study investigated whether the reversion following transmission affected viral fitness, as measured by replication and infectivity.
Methods: The gag genes, from a B*57/5801-negative participant infected with transmitted escape (A146X and T242N), were amplified from samples collected in early infection and at 2.5 years post infection. Amplicons were cloned into a subtype C backbone. Site-directed mutagenesis was used to revert the compensatory mutations to determine their effect on viral infectivity. Viral stocks were generated by transfection of 293T cells. Replication within PBMCs was determined by p24 production over 21 days. Infectivity was determined by infecting TZM-bl cells and measuring luciferase activity at 48 hr postinfection. Cyclophilin A dependency of the mutants was determined by measuring infectivity in 5 µM of cyclosporine A (cyclophilin A inhibitor).
Results: A B*57/B*5801-negative participant was infected with a virus carrying B*57/B*5801-associated escape (A146P and T242N) and compensatory (H219Q, I223V and M228I) mutations, which reverted to wildtype at 146 and 242 sites by two years postinfection. Contrary to expectation, replication kinetics showed that the transmitted escape virus harbouring the escape mutations was fitter than the reverted virus at two years postinfection, in the presence of compensatory mutations. We investigated the mechanism affecting fitness in infectivity assays using TZM-bl cells. We found that reversion of the compensatory mutations did not affect infectivity, except for H219Q. This mutation was found to act in combination with T242N to make the virus cyclophilin A independent.
Conclusions: The data may explain why viral loads do not always increase following reversion of the T242N mutation *in vivo*. The specific combination of escape and/or compensatory mutations may be a determinant of viral fitness.

My name is Denis Rutendo Chopera. I am currently a postdoctoral fellow in the Division of Medical Virology, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town. I enjoy playing tennis and watching formula 1 racing.

Filippos Porichis: Poster 2g

Clinical and immunological determinants of HIV-specific CD4 T cell responsiveness to PD-1 blockade

Filippos Porichis[1], Douglas S. Kwon[1], Daniel P. Tighe[1], Jennifer Zupkosky[1], Ashley McMullen[1],David F. Pavlik[1], , Daniel G. Kavanagh[1], Gordon J. Freeman[2], Bruce D. Walker[1] and Daniel E. Kaufmann[1]

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Defining the impairment of HIV-specific Thelper functions mediated by Programmed death–1 (PD-1) is crucial to understand the contribution of this molecule to defective viral control and to determine the therapeutic potential of interventions targeting this pathway. We set to describe the relationships between disease stage, PD-1 expression, and reversibility of PD-1 mediated impairment of HIV-specific CD4 T cells. We measured expression of IFN-γ, IL-2, IL-13 at the mRNA and protein levels in CD8-depleted PBMC stimulated with a Gag peptide pool in the presence of isotype control antibody or anti-PD-L1. PD-L1 blockade significantly enhanced secretion of IFN-γ IL-2 and IL-13. The effect of PD-L1 blockade was correlated with markers of disease progression and was more potent in viremic individuals than in ARV treated and elite controllers. Blockade of the PD-1 pathway restored functions in both PD-1-intermediate and PD-1-high sorted CD4 T cell subsets. Phenotypic analysis on the same subsets showed a markedly lower expression of CD160 and CD244 on PD-1 expressing HIV-specific CD4 T cells as compared to CD8 T cells. Thus we have shown that blockade of the PD-1 pathway restores both Th-1 and Th-2 cytokine secretion by HIV-specific CD4 T cells. These data show that PD-1 impairs HIV-specific Thelper responses both by limiting expansion of these cells and by inhibiting effector functions of multiple differentiated Thelper subsets.

Raised in a small island in Greece. I took my Bachelors and Masters in the UK. I went back to Greece for my PhD and joined the Ragon Institute in 2008 as a research fellow. I am working on Immunoregulatory networks in HIV infection with main interest on T cell exhaustion and the PD-1 molecule. I like football (soccer), sailing, windsurfing and pipetting.

Anne-Sophie Dugast: Poster 2g

Elite Controllers elicit HIV-specific antibodies that in the absence of neutralizing activity can efficiently mediate ADCC

Anne-Sophie Dugast, Caitlin Barkume, Sarah Ruberman and Galit Alter

Ragon Institute of MGH, MIT and Harvard Building 149, 13th street, Charlestown 02149

Introduction While a great deal of work has been performed over the past 27 years on the role of neutralizing antibodies in HIV infection, new data from the RV144 Thai trial suggest that non-neutralizing antibodies may have conferred a modest level of protection against HIV infection. However the properties of these types of non-neutralizing antibodies are poorly understood, and could potentially provide critical information regarding the key protective mechanisms that if enhanced by vaccination could provide protection from infection. Interestingly, durable control of HIV is associated with an enrichment of innate immune recruiting, but not neutralizing, antibodies. Thus we hypothesized that defining the antiviral properties of innate immune recruiting antibodies in Elite Controllers (ECs), who spontaneously control HIV to undetectable levels in the absence of antiretroviral therapy, may provide critical clues regarding the protective mechanisms that could potentially be mediated by these humoral immune responses.

Methods Plasma IgG was purified from 15 Elite Controllers, 15 chronic treated, 15 chronic untreated and 15 HIV-negative controls. NK cells, Monocytes and CD4 T cells were purified from HIV-negative individuals. The CD4 T cells were infected with JRCSF at a 0.01 MOI and co-incubated with effector cells in the presence or absence of antibodies from the patients. The ability of IgGs to inhibit viral replication was determined by p24 ELISA. Anti-gp120 antibodies binding and neutralizing titers were performed by ELISA and a TZMBL cell based assay against a tier 1 and tier 2 viruses, SF162 and JRCSF.

Results Our results show that antibodies from ECs are able to more potently recruit NK cells as well as monocytes to mediate robust ADCC compared to antibodies from chronic patients ($p=0.001$). Furthermore, ECs have elevated gp120-binding titers compared to antibodies from chronic treated patients ($p=0.035$), that correlate with the level of ADCC inducing antibodies ($r^2=0.62$, $p=0.0014$), but not neutralizing activity.

Conclusion Overall, antibodies from ECs elicit high titers of anti-gp120 specific antibodies, that in the absence of neutralization, have additional properties to efficiently recruit innate immune effector functions to control viral replication and prevent disease progression. Defining the mechanisms by which ECs induce such potent innate immune recruiting antibodies may provide critical information for vaccine design.

I am a post doc fellow working with Galit Alter since the past 2 years. The aim of my project is to characterize the role of protective non-neutralizing cytolytic antibodies and their capacity to recruit innate immune effector cells in natural control of HIV-1 infection. I strongly focus my efforts on understanding how antibodies may provide specificity to the innate immune system for virally infected cells using samples from well-defined cohorts.

Mattieu Angin: Poster 2h

Successful expansion of functional human regulatory T-cells from individuals with HIV-1 infection.

Mathieu Angin1, Ashley Rezai1, Melanie King1, Alicia Piechocka-Trocha1, Ildiko Toth1, Bruce D. Walker1, 2, Marylyn M. Addo1, 2
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In the context of HIV-1 infection**,** the exact impact of regulatory T cells (Tregs) on HIV pathogenesis remains poorly understood. Natural CD4+CD25+Foxp3+ Tregs represent 5-7% of CD4+ T cells in healthy donors, however in untreated HIV-1 seropositive individuals absolute CD4+ Treg numbers decrease during disease progression with the overall loss of CD4 T cells, making this population remarkably hard to study. In the present study, we describe an approach to expand functional regulatory T cells from HIV-1 positive individuals.
Peripheral CD4+CD25+CD127lo natural Tregs and CD4+CD25-CD127+ conventional T cells were isolated from the peripheral blood of individuals with chronic untreated HIV-1 infection, Elite controllers and healthy donors using a FacsAria cell sorter. The Tregs were stimulated with anti-CD3/CD28 microbeads and cultured in the presence of IL-2. Frequency and phenotype of the expanded Tregs and conventional T cells were measured by flow cytometry. The suppressive function was assessed by standard flow-based proliferation assays using CFSE dilution of microbead-activated PBMCs in the presence or absence of expanded Tregs.
Sorted CD4+CD25+CD127lo Tregs from HIV-1 negative and positive donors were succesfuly expanded, with a mean fold change of 49 at day 7. The expanded Tregs expressed high levels of Foxp3, CTLA4 and Helios compared to the conventional T cells ($p<0.05$). Expanded CD4+ conventional T cells showed a phenotype of cytokine-secreting non suppressive T cells while the expanded Tregs showed an activated Tregs phenotype.
More importantly, the expanded Tregs were able to suppress activated PBMCs *in vitro*. Detailed analyses on HIV-1 infectability of these expanded Tregs are ongoing.

Our data show that CD4+CD25+CD127lo Tregs isolated from HIV-1 positive individuals can be successfully isolated and expanded *ex vivo*, while maintaning high expression of published Tregs markers, and their suppressive function. This approach holds great potential for more detailed studies on the role of regulatory T cells in the setting of HIV-1 infection, especially in situations where specimen are scarce (e.g pediatric samples, tissues).

I did my PhD in my hometown : Nantes, France, where I evaluated the induction of tolerance in transplantation in a primate model and the induction of CD8+ Treg in a rat model after costimulation blockade. In early 2009, I joined Marylyn Addo's lab at the Ragon Institute of MGH, MIT and Harvard in Boston to study the impact of regulatory T-cells on HIV infection and immunopathogenesis.

Zaza Ndhlovu

Mosaic HIV-1 Gag Vaccines Present both Clade B and Clade C HIV-specific epitopes to Human T cells.

Background: Polyvalent "mosaic" HIV immunogenes offers a potential solution for generating vaccines that can elicit immune responses against genetically diverse viruses. However, it is unclear whether key CD8 T cell epitopes are processed and presented from these synthetic antigens and recognized by epitope-specific
human T cells.
Methods: Here we tested the ability of mosaic HIV immunogens expressed by recombinant, replication incompetent adenovirus serotype 26 vectors to process and present major HIV clade B and clade C CD8 T cell epitopes in human cells. A bivalent mosaic vaccine expressing HIV Gag sequences was used to transduce PBMC from 11 HIV-1-infected individuals from the US and 10 HIV-1-infected individuals from South Africa, and intracellular cytokine staining together with tetramer staining was used to assess the ability to stimulate pre-existing memory responses compared to natural clade B and C vectors.
Results: Mosaic Gag vaccines presented all 7 clade B epitopes tested in 11 US subjects and all 5 clade C epitopes tested in 10 South African subjects. Overall, the magnitude of cytokines induced by stimulation with mosaic vaccines was comparable to clade B and clade C antigens tested, but the mosaic vaccines elicited greater cross-clade recognition.
Conclusions: Our studies demonstrate that mosaic vaccines express major clade B and clade C epitopes in human cells, and support the evaluation of mosaic HIV-1 vaccines in humans.

Position: Post-Doctoral Fellow with Bruce Walker
Research: Determine whether a unique subset of HIV-specific CTL is present in HIV Elite Controllers, and whether this subset mediates antiviral functions My other area of research is "mosaic" HIV vaccines. I 'll be presenting a poster on this topic
Hobbies: Soccer, Travel/ site seeing, photography and fatherhood

David Shasha (MD): *An infectious disease specialist from Hadassah-Hebrew University medical center, Jerusalem, Israel. Working as a post-doc at Bruce Walker's lab. Working on CTLs functionality during acute HIV infection, and on characterization of CTLs function in patient with HLA alleles associated with bad prognosis.*

Hobbies: SCUBA diving

7. HPP Staff Biosketches

Michelle Gordon, PhD: *Michelle Gordon is a Virologist and Lecturer in the Department of Virology at the Nelson R Mandela School of Medicine, UKZN, as well as a Senior Scientist at the HIV Pathogenesis Programme (HPP). She currently supervises 4 Masters' students at HPP and is involved in many of the research projects. Her research focuses mainly on HIV ARV drug resistance. She also has extensive bioinformatics experience and has co-authored several papers on the characterization of HIV-1 subtype C and is also a co-author on several bioinformatics papers.*

Pedzisai Gaza: *My name is Pedzisai Gaza, am the new P.A to Director Prof Ndungu. Joined the institute on 01/10/2010. Am married, and my hobbies are cooking and reading novels.*

Keshni Hiramen: *I am the Laboratory Manager (Hasso Plattner Research Laboratory) and P2+ laboratory (Africa Centre). I am responsible for procurement in the laboratory and overseeing the smooth running of the laboratory. My hobbies include reading.*

Busisiwe Mlotshwa: *I function as the laboratory manager for the HIV Pathogenesis Programme. This means I have oversight of all laboratory operational activities, including staff and student training and research projects. I coordinate activities between HPP and its collaborators supervise the laboratory staff to ensure effective running of the lab and maintain a quality assurance program in collaboration with the quality coordinator.*
In my personal time, I enjoy watching formula one racing; participate in various types of sports, both in and out-doors; read good books and am an enthusiastic board game player.

Sharon Reddy: *I have been at HPP since 2004. I am currently managing the Flow Core as well as the sample inventories. My hobbies include gardening and sewing.*

Stanley Carries: *Stanley Carries is a BMedSci (Honours) graduate from the University of Natal. He is currently the Data Capturer at HPP. He has been involved in the Clinical / Medical Research field for the past seven years.*

Nagavelli Padayachi: *I am employed by the University of Kwazulu-Natal in the capacity as a Senior Technician. I have an MSc (Virology) that I obtained in 2007. I am presently involved in the Adult tropism (Naïve and Failures) project that Ashika Singh had undertaken and used for her PhD degree. I also train Medical students in basic molecular techniques for eg.PCR as well as facilitating to the undergraduate students as part of my service to UKZN. I enjoy playing Table tennis, keeping fit, gardening and trying out new cookery recipes.*

Simbarashe Mabwe: *Research assistant HPP Immunology Core. Interests: Immunology and proteomics. I love the teamwork and capacity building at HPP; currently involved in TQM, healthy & Safety, and Evacuation. I love jokes, and volleyball is my game.*

Tresha Moodley: *I am a Lab Assistant. I have been working at HPP for the last 11 years. I do preparation of Media and stocking up of Consumables and reagents in the lab.*
My primary responsibility is the reception of samples. My hobbies are reading, shopping and socializing

Raveshni Durgiah: *Currently, I am a research assistant at HPP. I completed my masters in Medical Biochemistry at UKZN, Mycotoxin Research Unit. I enjoy taking long walks on the beach. And, I am also trained in the beautiful Indian classical dance form, Bharatanatyam.*

Nothemba Nontala: *I joined HPP since October 2008. I am a currently working as a lab assistant, involved in the general maintenance, stock taking, waste management and media preparation at the Hasso Plattner lab. I am also involved in the Health and Safety committee to ensure the safety of scientists in the lab. Previously work experience involved TB research at the Medical Microbiology department at UKZN. When I am not working, I enjoy watching soccer, socializing, cooking and spending time with my family.*

Mary van der Stok: *I have a Master's in medical science. I am currently working in the virology core processing CD4's and viral loads. I am also an administrator of the HPP Laboratory Information Management System.*

Lungile Maphumulo: *I am Lungile Maphumulo. I am a Research Technologist,working in the HPP,Viro Core lab.We do testing like CD4's ,Viral load,Ampliscreen as well as Western blot. In my spare time I enjoy jogging, reading and cooking.*

Mercy Tendani Raphalalani: *I'm Mercy Tendani Raphalalani. I'm part of HPP working under immunology core as a research assistant where I'm primarily responsible for conducting processes such as ficolling PBMC's, ELISPOT ASSAY. I have a BSc (Hons) in Microbiology and my hobbies and interest are listening to music, reading and travelling.*

Kewreshini Naidoo: *I joined HPP earlier this year and am working with Dr Marianne Mureithi as a research assistant. I am currently completing my MSc in Microbiology. I enjoy reading and am a sports enthusiast.*

Mona Pillay: *My name is Mona Pillay. I have obtained a BSc. degree in Microbial Biotechnology and a Honours degree in Medical Science (Medical Microbiology) at UKZN. I am currently completing my final year of a Masters degree in Medical Science at UKZN. I am employed at HPP as a research technician and am a part of Dr. Victoria Kasprowicz's TB Immunology group. I enjoy reading, shopping and fun activities.*

Pamla Govender: *I have an honours qualification in Medical Science (Medical Microbiology) and have been employed at HPP as a research technician since March 2009. I'm part of Dr Victoria Kasprowicz TB/Immunology group and have*

acquired skills in numerous areas throughout the course of my work. My interests include reading, shopping and I also enjoy going to the beach.

Saleha Omarjee: *I joined HPP in July 2009, after completing my Masters in Medical Science (Immunology). I am currently employed as a Research Assistant in the Immunology Core at HPP. I have a keen interest in immunology, HIV and TB research.*

Emma Siboto: *I joined HPP this year (2010). I am the Quality Control manager. My hobby is reading.*

Sharon Botha: *I joined HPP in 2009 as a finance assistant administrator. In my free time I enjoy community work.*

Morag Geach: *I am the Financial Administrator and HR Manager at HPP. My hobbies include boot camping, reading and socializing.*

Michelle Erickson: *I am the Project Co-ordinator at HPP. I enjoy wild life, gardening and reading.*

Kathy Laing: *I am the financial administrator at HPP. My hobbies include cooking, baking and reading.*

Sheryll Veerajoo: *I am currently employed as a Research Assistant at the HPP Immunology Core. I joined HPP in January this year. I have an Honours Degree and background in Medical Microbiology. My interests and hobbies include reading and music.*

Joanah Mdluli: *I am currently doing my Masters degree under the supervision of Dr. M. Gordon. I did my undergrad at the University of the Free State where I majored in biology, chemistry and biochemistry. For my honours, my research was on tuberculosis virulence genes, and currently, my focus has shifted to HIV-1 drug resistance.*

Recently I've been looking into how computer science is directly comparable to experimental work in terms of what can be achieved. This is a rapidly growing and exciting area in research, where I'm hoping to expand my knowledge.

8. CLINIC STAFF

Sr. Thathakahle Zungu: Joined HPP in 2010, she enjoys the gym and the beach.

Monika Myeza: Joined HPP in 2009. She works at McCord's Hospital and enjoys gospel music.

Sr. Thandi Sikhakhane: She joined HPP in 2001. She works at St. Mary's and enjoys watching TV, listening to music, cooking and going to social events.

Gcinile Maphanga: Joined in 2010. Works at Prince Mysheni and enjoys doing counselling.

Sizakele Myeza: Joined in 2008. Works at Prince Mysheni. She enjoys doing business training.

Sr. Nono Nkupiso: Joined HPP in 2007. She is based at CDC, which is a satellite clinic for McCords Hospital. She enjoys talking, watching cartoons, movies and listening to gospel music.

Sr. **Landiwe Nxele:** Joined HPP in 2008 and is based at Prince Mysheni.

Monica Nyawo: Joined HPP in 2008 and is based at Prince Mysheni as a counsellor. My hobbies include jogging and dancing.

Nicky Linda: Joined HPP in 2005 and is based at St. Mary's.

Lindiwe Makhathini: Joined in 2007 and is based at St. Mary's as an office assistant.

Nokuthuli Luthuli: Joined HPP in 2007 and is based at McCords Hospital.

Nokululo Noguba: Joined HPP in 2010, based at St Mary's as a counsellor.

Sr Jabu: Freelance nurse.

9. LIST OF ATTENDEES

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