Evaluation of a T-cell Assay for *Mycobacterium tuberculosis* Infection in The Gambia

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2005

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A thesis in fulfilment of the requirements for the degree of Doctor of Medicine, the University of Auckland, 2005.

Abstract

New generation T cell assays offer hope in the diagnosis of *Mycobacterium tuberculosis* infection and disease. We assessed the ELISPOT assay using cross-sectional and longitudinal studies and a natural gradient of *M. tuberculosis* exposure by sleeping proximity to a tuberculosis (TB) case in The Gambia. Two antigens, ESAT-6 and CFP-10 (EC), were compared to purified protein derivative (PPD) by ELISPOT and to the PPD skin test in 735 TB contacts. All three tests responded to the exposure gradient, the PPD skin test most dramatically. Inter-test comparison showed that the EC ELISPOT provided improved specificity in the diagnosis of *M. tuberculosis* infection, but at the cost of some sensitivity. Increasing discordance, particularly between PPD ELISPOT and PPD skin test results, down the exposure gradient to 105 community controls was identified. In 693 children, the EC ELISPOT was slightly less sensitive than the PPD skin test in the diagnosis of *M. tuberculosis* infection from recent exposure; neither test was confounded by prior BCG vaccination, even in the very young. A fusion protein of EC compared favourably with their respective peptides by ELISPOT assay in 488 TB contacts, a combined test result offered improved sensitivity. Quantitative ELISPOT and PPD-skin test responses were assessed in 1052 TB case contacts, according to an ELISPOT response to EC. Only the ELISPOT count was sensitive to the exposure gradient ($p=0.009$), revealing a positive dose-response relationship. In the longitudinal assessment, both ELISPOT and PPD skin test conversion occurred over time. PPD skin test reversion occurred in 10% of individuals after 18 months, ELISPOT reversion occurred in 39% at 3 months. In conclusion: the EC ELISPOT offers increased specificity in the diagnosis of *M. tuberculosis* infection in The Gambia, at the cost of some sensitivity; the PPD skin test appears to be down-regulated in the community; neither test is confounded by prior BCG vaccination; a fusion protein in combination with EC peptides offers optimal ELISPOT sensitivity; the quantitative ELISPOT response in specific-antigen-positive TB case contacts reflects the infectious load of *M. tuberculosis*; and significant early reversion of the ELISPOT test suggests it is unreliable in *M. tuberculosis* dormancy.

I dedicate this thesis to Marian, my wife and best friend, who established our home in The Gambia and 'hung in there' so amazingly well. I could not have done this without you.

Acknowledgments

I have been greatly privileged to work at the MRC unit, The Gambia. I thank Professor Keith McAdam, who played a key role in my personal development and inspired the TB case contact work. When I arrived in 2001, I was able to build upon plans already in place. I thank Professor Richard Adegbola for his mentorship and his friendship, which I value so highly. I thank Dr Tumani Corrah, who took over the directorship role in the unit in 2003, for his personal support and mentorship. The studies presented here required a multi-disciplinary team of people; the table on the following page acknowledges those who have played a role in these studies to the level of authorship.

I acknowledge my very able team of field workers, supervised by Abdoulie Tunkara, including Abdoulie Cham, Salieu Barry, Masaneh, Babou Faye, Fatou Jadama, Bajun Drammeh, Aziz Bangura, Rohey Jallow, Kairaba Kanyi, and Masaneh Ceesay. Roger Brookes and Annette Fox had a dedicated team of technical laboratory assistants: Momodou Darboe, Marie Gomez, Joseph Mendy, Rohey Jallow, Leticia King and Jabou Bah. Professor Richard Adegbola and Jacob Otu were assisted by Oumie Secka, Francis Mendy and Yandeh Touray.

The research in the unit would not have been possible without ongoing collaboration with the National TB control programme. I thank Dr Kebba Manneh (coordinator until 2004) and Adama Bojang (coordinator since 2004) for the very positive relationship we have had and the opportunity to take part in improving TB control in The Gambia.

The support systems in place at the MRC laboratories were crucial to the success of the research. These have been led by Mark Radford, director of operations in the time I have been working at the MRC Labs. I also acknowledge Kathy Hill (personnel), Fanta Singhateh (travel), Simon Belcher and Abdoulie Barry (finance), Seidina Joof (transport), Cherno Fye (housing), Alison Offong (administration), Arthur Roberts (facilities) and Dembo Kanteh (Bacterial diseases administrator).

I would like to thank Prof Rod Jackson at the School of Population Health, University of Auckland for hosting me in 2004 to work on this thesis. I am also grateful to Prof Charlotte Paul, Assoc Prof Rob McGee and Prof David Skegg at the Department of Preventative and Social Medicine, University of Otago, for hosting me in 2005.

I acknowledge the love and support of my family: my wife Marian, son Nathan and daughter Rebekah; Professor Graham and Bartha Hill (my parents), Dr Andrew and Lori Hill and Drs Douglas and Jennifer Hill.

Name and role of each person who contributed to the studies of this thesis, to the level of 'author' status. Names are listed in alphabetical order of the surnames.

Name	Role
Richard Adegbola	Unit Microbiologist, MRC Gambia. Involved in the design of the TB case contact studies. Overall coordination of microbiological work.
Ifedayo Adetifa	Paediatrician, research clinician. Involved in analysis and write up of the study of chapter 5.
Roger Brookes	Immunologist. Joint coordinator of the ELISPOT work, involved in analysis and write up of all the studies.
Tumani Corrah	Clinician-scientist – head of clinical services, MRC Gambia. Involved in the clinical care of the TB cases and contacts.
Simon Donkor	Data manager. Involved in design of the databases for all the studies. Supervised data collection and verification.
Katherine Fielding	Statistician, London School of Hygiene and Tropical Medicine. Involved in the design of the case contact work in The Gambia. Led the statistical analysis of chapter 2, and involved in the write up of that study.
Annette Fox	Immunologist. Joint coordinator of the ELISPOT work, involved in design of all the studies, analysis and write up of immunological aspects.
Kees Franken	Immunology Laboratory technician. Produced the fusion protein for the study of chapter 4. Involved in the write-up of the study of chapter 4.
Abdulrahman Hammond	Immunology Laboratory technician. Performed ELISPOT assays in all the studies of the thesis.
Dolly Jackson-Sillah	Research Clinician. Involved in recruitment and follow-up of all the study participants.
David Jeffries	Statistician, MRC Gambia. Led the statistical analysis of chapter 6 and advised the author on the statistical analysis of chapters 2, 3, 4, 5 and 7. Designed the database for all the studies and was involved in the analyses and write up of all the studies.
Michel Klein	Immunologist, Leiden University Medical Centre. Involved in the design, analysis and write-up of the study of chapter 4.
Moses Lugos	Immunology Laboratory technician. Performed the majority of the ELISPOT assays of all the studies of the thesis.
Roger Marshall	Statistician, University of Auckland. Assisted with the analysis of the studies of chapters 3 and 5, specifically the creation of rectangular Venn diagrams.
Keith McAdam	Director of MRC The Gambia and head of the TB programme until March 2003. Involved in initiation and design of the TB case contact work in The Gambia. In particular, involved in design, analysis and write up of the studies of chapters 2, 3, 5, 6 and 7.
Tom Ottenhoff	Clinical Immunologist, Leiden University Medical Centre. Involved in the analysis and write-up of the study of chapter 4.
Jacob Otu	Microbiology Laboratory technician. Performed the majority of the microbiological tests for all the studies of the thesis.
Patrick Owiafe	Immunology Laboratory technician. Performed ELISPOT assays in the study of chapter 2 of the thesis.

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Abbreviations

BCG	Bacille Calmette-Guérin
C	Centigrade
CI	Confidence Interval
E	Eastern
EC	ESAT-6/CFP-10
ELISPOT	Enzyme Linked Immunospot assay
IFN-γ	Interferon gamma
kDA	kilo-Dalton
LAL	Limulus Amebocyte Lysate
L-J	Lowenstein-Jensen
MRC	Medical Research Council
n	Number
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PHA	Phytohaemaglutinin
PPD	Purified Protein Derivative
RD	Region of Difference
rt-PCR	Reverse transcriptase Polymerase Chain Reaction
SFU	Spot Forming Units
SSI	Statins Serum 'Institut'
TB	Tuberculosis
TH	T Helper cell
TST	Tuberculin Skin Test
TU	Test Units
UK	United Kingdom
WHO	World Health Organisation
ZN	Ziehl-Neelsen

Articles arising from these studies

1. Hill P.C, Brookes RH, Fox A, Fielding K, Jeffries DJ, Jackson-Sillah D, Lugos M, Owiafe PK, Donkor SA, Hammond AS, Otu JK, Corrah T, Adegbola RA, McAdam KPWJ. Large-Scale Evaluation of Enzyme-Linked Immunospot Assay and Skin Test for Diagnosis of *Mycobacterium tuberculosis* Infection against a Gradient of Exposure in The Gambia. Clin Infect Dis 2004 ; 38 :966-73.[1]

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3. Hill PC, Fox A, Jeffries DJ, Jackson-Sillah D, Lugos MD, Owiafe PK, Donkor SA, Hammond AS, Corrah T, Adegbola RA, McAdam KPWJ, Brookes RH. Quantitative T cell assay reflects infectious load of *Mycobacterium tuberculosis* in an endemic case contact model. Clin Infect Dis 2005; 40:273-8.[3]

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