Specificity and Properties of Anti-HLA Antibodies Associated with Renal Allograft Rejection

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iv. Summary

Identification of the complement C4d fragment in peritubular capillaries as a specific marker for antibody mediated rejection in renal transplantation revealed the critical role of antibodies in graft survival. In this thesis, I document the design and findings of studies performed to investigate the clinical impact of anti-HLA antibodies present before and/or after transplantation.

Over time, the detection techniques for anti-HLA antibodies has evolved from the less sensitive complement-dependent lymphocytotoxicity (CDC) crossmatching (XM) to more sensitive solid phase assays such as Luminex®. Studies have been conducted to compare the predictive value of different antibody detection techniques.

The first result chapter presents antibody specificity in positive CDC B-cell crossmatch (BXM), analysed with highly specific Luminex® assays. The study also investigates the predictive value of BXM in the general transplant population. I found that donor-specific anti-HLA antibodies (DSA) are only present in one third of positive BXM and are associated with poor outcomes. The novel finding is that >80% of the DSA detected by BXM are complement-fixing IgG_1 and IgG_3 subclasses.

Transplant glomerulopathy (TG) is type of chronic renal graft rejection. The pathogenesis of TG is unclear. In the second result chapter, I report risk factors and involvement of anti-HLA antibodies in the development of TG. This study shows that glomerular rejection, delayed graft function, HLA presensitization and DSA have a univariate effect on TG development. Multivariate analysis revealed that DSA are an independent predictor of TG, after adjustment for other risk factors.

I have further investigated the role of BXM in a unique, well-matched, highly sensitized patient group transplanted under the national renal exchange programme. I compared Luminex® antibody analysis with BXM in predicting transplant outcomes. In highly sensitized patients, DSA are found in two thirds of positive BXM. In univariate analyses, BXM is associated with humoral rejection whereas DSA defined by Luminex® are associated with total and all rejection types. The major finding is that, by multivariate analysis, DSA defined by Luminex® are an independent predictor of total and humoral rejection, but BXM are not. These interesting findings are reported in the third result chapter.

Studies reported in this thesis define the clinical significance of anti-HLA antibodies in renal transplant outcomes. Method comparison studies provide useful information on antibody specificity and their impact on graft survival. Collectively, a better understanding of alloantibodies associated with graft rejection and limitation of antibody detection methods may facilitate donor selection and choice of immunosuppressants, and consequently improve transplant outcomes.

v. Dedication

For my father Kheng Choo, mother Sew Kee, siblings Hooi Ling, Inn Soo, Hooi Ping, Soo Tchuen, Chuen Sin, Sin Zheng and husband Lee Khuan.

vi. Acknowledgements

A thesis, with multiple research studies and high quality collaboration, such as this can only be completed with a brilliant and exceptional supportive team of supervisors, collaborators, and team assistance. I have had the privilege to be supervised by Professor Graeme Russ, Dr. Toby Coates and, and Dr. Peter Bardy, without whom, my study would not be possible. I am so honoured and most grateful to have the opportunity to work in such an amazing team environment. My most heartfelt thanks for all your professional advice, your guidance, and unwavering support.

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My thanks to all the participating hospitals, and tissue-typing laboratories, who have collected serum and data for contribution to this research. Your professional accurate and timely support has made this research possible.

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vii. Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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viii. Thesis Structure

Chapter 1 is a review of Human Leukocyte Antigen (HLA) system consists of the genetics, structures and functions of HLA molecules. It also describes role of HLA antigen in renal graft rejection, and current tissue typing and antibody detection techniques.

Chapter 2 contains the materials and methods used to perform the studies described in Chapter 3 to 5. The steps of tissue typing and Luminex® assays and reagent preparation are documented. Statistical tests for patient demographics and transplant outcomes comparisons are recorded in this chapter.

This thesis consists of three studies performed on patients with different transplant characteristics. The first study investigated the clinical significance of positive B-cell crossmatch in an Australian renal transplant population using Luminex® assays. This study also examined the specificity of antibody causing positive B-cell crossmatch and correlation with renal transplant outcomes. The design and results of the study are described in Chapter 3.

The pathogenesis of transplant glomerulopathy remains unknown. The second study described in Chapter 4 reported the involvement of anti-HLA antibodies in development of transplant glomerulopathy. This study also indentified the influence of anti-HLA antibodies on the progression of transplant glomerulopathy.

The Australia National Renal Exchange Programme commenced in October 2004 to improve the HLA antigen matching and transplant rate for highly sensitized patients. Chapter 5 reported the overall performance of patients transplanted under this programme in comparison to general transplant population. Clinical predictive value of B-cell crossmatch and Luminex® antibody analysis in highly sensitized patients were also compared.

Chapter 6 brings together the findings of the studies reported in Chapter 3 to 5, and examined the uses of BXM and Luminex® assays in current tissue typing protocol. The results are compared to previous studies and appropriate comments are included.

A list of references and then an appendix are attached after Chapter 6. The appendix consists of the published journal article that has arisen from the findings presented in this thesis.

ix. Abbreviations

AAT	amini acid triplets
ADCC	antibody–mediated cell cytotoxicity
ELISA	enzyme-linked immunoabsorbent assay
AHR	acure humoral rejection
APC	antigen- presenting cells
BXM	B-Cell crossmatch
CAN	chronic allograft nephropathy
CDC	complement-dependent lymphocytotoxicity
CN/HP	current serum negative/ historic serum positive
CNX	calnexin
CREGs	cross-reactive groups
CRT	chaperone calreticulin
CTLRs	C-type lectin receptors
cTLs	cytotoxic T-lymphocytes
DISC	death-inducing signal complex
DS/SAPE	dilution Solution/PE Streptavidin
DSA	donor specific antibodies
DTH	delayed hypersensitivity reaction
DTT	dithiothreitol
EPO	eosinophil persoxide
ER	endoplasmic reticulum
ERAAP or ERAP 1	ER associated aminopeptidase
ERp57	thiol oxidoreductase
FasL	Fas-ligand
Fc-receptors	fragment , crystallisable
H	histocompatibility
HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
IMGT	ImMunoGeneTics
IR	Immune Response
ITIMs	immunonoreceptor tyrosine-based inhibitory motifs
KIR	killer like receptor
LD	linkage disequilibrium
Li	Invariant chain
MESF	Molecules of Equivalent Soluble Fluorochrome
MFI	Median Fluorescence Intensity
MHC	Major Histocompatalibity complex
MIC	MHC class 1 chain-related genes
MMF	mycophenolate mofetil
Mya	million years ago
NK	natural killer

NO	nitric oxide
PBS	phosphate buffered saline
PBS/citrate	trisodium citrate
PFD	probability of finding a donor
RSA	recurrent spontaneous abortions
TAP	transporter associated with antigen processing
TCR	T-cell receptor
TG	transplant glomerulopathy
TH1	T helper
TN/BP	negative T-cells/positive B-cells crossmatches
TNF	tumour necrosis factor
TPSN	Tapasin
TXM	T-cell crossmatch
UNOS	United Network for Organ Sharing Registry
XM	crossmatching

x. Publications

1. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Luminex® Investigation: Low titer HLA donor-specific antibodies in positive B-cell crossmatches predict late graft loss. Nephrology 2007:Supp 1;A19.

2. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Luminex® Investigation of Positive B-cell Crossmatches: low titler HLA donor-specific antibodies associated with inferior outcomes. Human Immunology 2007:68 Supp 1:S31.

3. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex® predict late graft loss. American Journal of Transplantation 2008 8(11):2335-42.

4. Hooi Sian Eng, Scott Campbell, Graeme Russ, P. Toby H. Coates, Peter Bardy, Greg Bennett, Sean Chang, Brian Tait, On behalf of the Renal Transplant Advisory Committee (RTAC). A retrospective study of B-cell crossmatch with Luminex® technology in well-matched highly sensitized patients from the AUSTRALIAN interstate exchange. Nephrology 2008:13;A112.

5. Hooi Sian Eng, Greg Bennett, Eleni Tsiopelas, Sean Chang, Peter Bardy, Graeme Russ, P. Toby H. Coates. Alloantibody involvement and risk factors in transplant glomerulopathy. Nephrology 2008:13;A99.

6. Hooi Sian Eng, Scott Campbell, Graeme Russ, P. Toby H. Coates, Peter Bardy, Greg Bennett, Sean Chang, Brian Tait, On behalf of the Renal Transplant Advisory Committee (RTAC). Predictive value of B-cell crossmatch and Luminex® antibody analysis in well-matched highly sensitized patients from the Australian National Interstate Exchange (ANIE). Human Immunology 2008:69;S13.

7. Hooi Sian Eng, Greg Bennett, Eleni Tsiopelas, Sean Chang, Peter Bardy, Graeme Russ, P. Toby H. Coates. Donor specific anti-HLA antibodies in transplant glomerulopathy. Human Immunology 2008:69;S109.

8. Hooi Sian Eng, Greg Bennett, Peter Bardy, Patrick Coghlan, Graeme R. Russ, P. Toby H. Coates. Clinical significance of anti-HLA antibodies detected by Luminex®: Enhancing the interpretation of CDC-BXM and important post-transplant monitoring tools. Human Immunology 2009 *In press*.

xi. Presentations

1. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Clinical relevance of a positive B-cell crossmatch on renal transplantation: A single centre study. The Queen Elizabeth Hospital Research Day 20 October 2006, Adelaide, Australia.

2. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Analysis of the specificity and properties of alloantibodies associated with rejection of renal transplant. The 3rd International Summer School on Immunogenetics 17-21 November 2006, Bangkok, Thailand.

3. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. B-cell crossmatching and kidney allograft outcomes in cadaveric transplant recipients. The 30th Annual Scientific Meeting of The Australasia and South East Asia Tissue Typing Association 22-25 November 2006, Chiang Mai, Thailand.

4. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Luminex® investigation of positive B-cell crossmatches in renal transplantation. The 31st Annual Scientific Meeting of The Transplantation Society of Australia and New Zealand 28-30 March 2007, Canberra, Australia.
   TSANZ Young Investigator Award 2007

5. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Luminex® Investigation: Low Titer HLA Donor-specific Antibodies in Positive B-cell Crossmatches Predict Late Graft Loss. The 43rd Annual Scientific Meeting of The Australia and New Zealand Society of Nephrology (ANZSN) 8-12 September 2007, Queensland, Australia.
   Kidney Health Australia (KHA) Award for The Best Clinical Science Presentation

6. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Luminex® Investigation of Positive B-cell Crossmatches: Low Titer HLA Donor-specific Antibodies Associated with Inferior Outcomes. The 33rd Annual Meeting of American Society For Histocompatibility and Immunogenetics (ASHI) 8-12 October 2007, Minnesota, USA.

7. HS Eng, G Bennett, I Humphreys, E. Tsiopelas, M Lake, SH Chang, P Bardy, PTH Coates and G. Russ. Low Titer Donor-specific Antibodies in Positive B-cell Crossmatches Predict Late Graft Loss. The 31st Annual Scientific Meeting of Australasia and South East Asia Tissue Typing Association 15-18 November 2008, Perth, Australia.

8. Hooi Sian Eng, Scott Campbell, Graeme Russ, P. Toby H. Coates, Peter Bardy, Greg Bennett, Sean Chang, Brian Tait, On behalf of the Renal Transplant Advisory Committee (RTAC). Clinical significance of B-cell positive crossmatches and Luminex® defined donor-specific antibodies in well-matched highly sensitized patients from the AUSTRALIAN interstate exchange.
   The XXII International Congress of The Transplantation Society 10-14 August 2008, Sydney, Australia.

- *Top 10 Abstracts Young Investigation Award of The XXII International Congress of The Transplantation Society 10-14 August 2008, Sydney, Australia.*

- *Nominee for The President's Award of The Transplantation Society of Australia and New Zealand (TSANZ).*

- *TSANZ Young Investigator Award*

- *Amgen Young Investigator Award*

9. Hooi Sian Eng, Greg Bennett, Eleni Tsiopelas, Sean Chang, Peter Bardy, Graeme Russ, P. Toby H. Coates. Risk factors and associations with alloantibodies in transplant glomerulopathy. The XXII International Congress of The Transplantation Society 10-14 August 2008, Sydney, Australia.

10. Hooi Sian Eng, Scott Campbell, Graeme Russ, P. Toby H. Coates, Peter Bardy, Greg Bennett, Sean Chang, Brian Tait, On behalf of the Renal Transplant Advisory Committee (RTAC). A retrospective study of B-cell crossmatch with Luminex® technology in well-matched highly sensitized patients from the AUSTRALIAN interstate exchange. The 44th Annual Scientific Meeting of Australia and New Zealand Society of Nephrology (ANZSN) 6-10 September 2008, Newcastle, Australia.
Finalist for The Clinical Science Young Investigator Award

11. Hooi Sian Eng, Greg Bennett, Eleni Tsiopelas, Sean Chang, Peter Bardy, Graeme Russ, P. Toby H. Coates. Alloantibody involvement and risk factors in transplant glomerulopathy. The 44th Annual Scientific Meeting of Australia and New Zealand Society of Nephrology (ANZSN) 6-10 September 2008, Newcastle, Australia.

12. Hooi Sian Eng, Scott Campbell, Graeme Russ, P. Toby H. Coates, Peter Bardy, Greg Bennett, Sean Chang, Brian Tait, On behalf of the Renal Transplant Advisory Committee (RTAC). Predictive value of B-cell crossmatch and Luminex® antibody analysis in well-matched highly sensitized patients from the Australian National Interstate Exchange (ANIE). The 34th Annual Meeting of American Society For Histocompatibility and Immunogentics (ASHI) 27-31 October 2008, Toronto, Canada.

13. Hooi Sian Eng, Greg Bennett, Eleni Tsiopelas, Sean Chang, Peter Bardy, Graeme Russ, P. Toby H. Coates. Donor specific anti-HLA antibodies in transplant glomerulopathy. The 34th Annual Meeting of American Society For Histocompatibility and Immunogentics (ASHI) 27-31 October 2008, Toronto, Canada.

xii. Awards

1. Young Investigator Award of Amgen-The Transplantation Society of Australia and New Zealand. The 31st Annual Scientific Meeting, 28-30 March 2007, Canberra, Australia.
2. Kidney Health Australia For The Best Clinical Science Presentation Award. The 43rd Annual Scientific Meeting of The Australia and New Zealand Society of Nephrology (ANZSN) 8-12 September 2007, Queensland, Australia.

3. Top 10 Abstracts Young Investigation Award of The XXII International Congress of The Transplantation Society 10-14 August 2008, Sydney, Australia.

4. Young Investigator Award of Amgen-The Transplantation Society of Australia and New Zealand. The XXII International Congress of The Transplantation Society 10-14 August 2008, Sydney, Australia.

5. Young Investigator Award of The Transplantation Society of Australia and New Zealand. The XXII International Congress of The Transplantation Society 10-14 August 2008, Sydney, Australia.

6. Nominee for The President's Award of The Transplantation Society of Australia and New Zealand. The XXII International Congress of The Transplantation Society 10-14 August 2008, Sydney, Australia.

7. Finalist for The Clinical Science Young Investigator Award The 44th Annual Scientific Meeting of Australia and New Zealand Society of Nephrology (ANZSN) 6-10 September 2008, Newcastle, Australia.