

**INVESTIGATIONS IN THE DESIGN AND CHARACTERIZATION  
OF HIV-1 NEUTRALIZING MOLECULES**

Thesis by

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This work is dedicated to the memory of my father,

RONALD ALLEN KLEIN

(1947 – 1988)

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## ABSTRACT

Human Immunodeficiency Virus (HIV) is a T-lymphotropic retrovirus that is the causative agent of Acquired Immunodeficiency Syndrome and is estimated to currently infect approximately 40 million people worldwide. Life-extending therapies are credited for the precipitous drop in HIV-related mortality in developed countries, but their high costs prevent widespread distribution in developing countries. To date, all attempts to produce a vaccine capable of preventing or controlling an HIV infection have failed, but a comprehensive explanation for these failures has yet to emerge from the available data. In this thesis the first chapter provides an overview of the pandemic, the antigenic properties of gp120 and gp41, which are the two glycoproteins that comprise the outer envelope spike of the virus, and the broadly neutralizing antibodies that have been isolated against them. The second and third chapters discuss biophysical characterizations of these monoclonal antibodies and newly designed molecules derived from them. Based on a comparison of these data with pre-existing research, a novel hypothesis called the “island effect” was developed and is presented as a possible explanation for the consistent failure of the human immune system to respond to infection or vaccination with an effective humoral response. The final chapter summarizes ongoing investigations in the capacities of broadly neutralizing monoclonal antibodies to recruit antibody-dependent cellular cytotoxicity, a mechanism by which antibodies can trigger the lysis of HIV-infected cells by the innate immune system.

## TABLE OF CONTENTS

|   |     |
|---|-----|
| ACKNOWLEDGEMENTS.....   | iv  |
| ABSTRACT .....  | v   |
| CHAPTER 1:  |     |
| Introduction.....   | 1   |
| CHAPTER 2:  |     |
| Published as Klein JS, Gnanapragasam PNP, Galimidi RP, Foglesong CP, West AP, Jr., and Björkman PJ (2009) Examination of the contributions of size and avidity to the neutralization mechanisms of the anti-HIV antibodies b12 and 4E10. <i>Proc Natl Acad Sci U S A</i> . <b>106</b> :7385-90.....   | 32  |
| CHAPTER 3:  |     |
| Assessing the impact of HIV spike arrangement on antibody avidity.....  | 49  |
| CHAPTER 4:  |     |
| Potent neutralization of HIV is not a predictor of the ability to trigger antibody-dependent cellular cytotoxicity .....  | 88  |
| APPENDIX A:   |     |
| Software: <i>VictorExtract</i> and <i>GraphExtract</i> .....  | 110 |
| APPENDIX B:   |     |
| Listing of designed anti-HIV-1 molecules .....  | 119 |
| APPENDIX C:   |     |
| Published as Halbrooks PJ, Giannetti AM, Klein JS, Björkman PJ, Larouche JR, Smith VC, MacGillivray RT, Everse SJ, Mason AB (2005) Composition of pH-sensitive triad in C-lobe of human serum transferrin. Comparison to sequences of ovotransferrin and lactoferrin provides insight into functional differences in iron release. <i>Biochemistry</i> . <b>44</b> : 15451-60. .... | 122 |

## APPENDIX D:

Published as Byrne SL, Leverence R, Klein JS, Giannetti AM, Smith VC, MacGillivray RT, Kaltashov IA, Mason AB (2006) Effect of glycosylation on the function of a soluble, recombinant form of the transferrin receptor. *Biochemistry*. **45**: 6663-73. ....133

## APPENDIX E:

Published as Win MN, Klein JS, Smolke CD (2006) Codeine-binding RNA aptamers and rapid determination of their binding constants using a direct coupling surface plasmon resonance assay. *Nucleic Acids Res.* **34**: 5670-82. ....145

## APPENDIX F:

Published as West AP, Galimidi RP, Foglesong CP, Gnanapragasam PNP, Klein JS, Suzuki M, Tiangco NE, Bjorkman PJ (2009) Design and Expression of a Dimeric Form of the Human Immunodeficiency Virus Type 1 Antibody 2G12 with Increased Neutralization Potency. *J. Virology*. **83**:98-104. ....159

## LIST OF FIGURES AND TABLES

### CHAPTER 1:

|   |    |
|---|----|
| Figure 1. X-ray crystal structures of envelope spike proteins .....                     | 12 |
| Figure 2. Schematic model of the HIV envelope spike fusion mechanism .....              | 15 |
| Figure 3. Models of the HIV envelope spike bound to CD4 and monoclonal antibodies ..... | 18 |

### CHAPTER 2:

|   |    |
|---|----|
| Figure 1. Structures of antibody constructs .....   | 33 |
| Figure 2. Biophysical characterization of the antibody constructs .....   | 34 |
| Figure 3. Bar graph of ratios of average molar IC <sub>50</sub> values (arithmetic means) for b12 constructs (blue) and 4E10 constructs (orange)..... | 35 |
| Figure S1. Surface plasmon resonance sensorgrams for binding to immobilized antigens .....  | 41 |
| Figure S2. <i>In vitro</i> pseudovirus neutralization curves .....  | 42 |
| Figure S3. Modeling of the structural requirements for IgG b12 to achieve intra-spike cross-linking .....   | 44 |
| Figure S4. Bar graph of ratios of average molar IC <sub>50</sub> values (geometric means) for b12 constructs (blue) and 4E10 constructs (orange)..... | 45 |
| Table 1. Strain-specific IC <sub>50</sub> neutralization values (nM) for each antibody construct.....   | 35 |
| Table S1. Strain-specific IC <sub>50</sub> neutralization ratios .....  | 46 |
| Table S2. IgG b12 and scFv b12 were used as internal controls to examine the reproducibility of independently determined IC <sub>50</sub> values..... | 47 |
| Table S3. Comparison of IC <sub>50</sub> values for IgG b12 and IgG 4E10 to previously published results.....   | 48 |

### CHAPTER 3:

|  |    |
|--|----|
| Figure 1. IC <sub>50</sub> values for the neutralization of various strains of HIV from clades B and C using b12 ..... | 52 |
|--|----|



|   |    |
|---|----|
| Figure 2. Modeling the dissociation to B from AB (Fab) and AB <sub>2</sub> (IgG) .....  | 56 |
| Figure 3. Comparison of IC <sub>50</sub> neutralization values for antibodies as Fabs and intact IgGs .....   | 59 |
| Figure 4. Comparison of neutralization potencies for Palivizumab and affinity-improved mutants with slower intrinsic dissociation constants (reported in (15)). .....   | 59 |
| Figure 5. Available structures of enveloped viruses showing spike densities derived by electron microscopy .....  | 61 |
| Figure 6. Protein fusion linker candidates and models .....   | 64 |
| Figure 7. Schematic of the structure of a human IgG antibody of subclass 1 with RCEs, reduced SDS PAGE results for IgG b12 with Gly <sub>4</sub> Ser RCEs, and gel filtration profile of IgG 2G12 (G <sub>4</sub> S) <sub>1</sub> ..... | 66 |
| Figure 8. SPR dissociation curves for Fab, IgG, and IgG (G <sub>4</sub> S) <sub>5</sub> constructs bound to immobilized monomeric gp120 (b12 and 2G12) or immobilized gp41 (4E10 and 2F5) .....   | 68 |
| Figure 9. Results of the <i>in vitro</i> neutralization assay comparing wild type IgG constructs with RCEs .....  | 69 |
| Figure 10. Reduced SDS PAGE analysis of IgGs that were successfully expressed with PFEs after purification by SEC .....   | 72 |
| Figure 11. Gel filtrations profiles for IgGs with protein fusion extensions .....   | 73 |
| Figure 12. Dissociation curves for PFE constructs and 2G12 dimer bound to immobilized monomeric gp120 (2G12) or immobilized gp41 (4E10 and 2F5) .....   | 74 |
| Figure 13. Comparison of neutralization activities for PFEs versus wild type IgGs (HIV strain 6535.3) .....   | 75 |
| Table 1. Half-life values (t <sub>1/2</sub> ) of antibody-antigen complexes calculated from models presented in Fig. 2 .....  | 56 |
| Table 2. Protein yields for 1 L expressions of each of the RCE constructs .....   | 66 |
| Table 3. Half-life times (t <sub>1/2</sub> ) calculated from dissociation curves in Fig. 8 .....  | 68 |
| Table 4. Half-life times (t <sub>1/2</sub> ) calculated from the dissociation curves of dimeric IgG 2G12 and PFEs in Fig. 11 .....  | 74 |

## CHAPTER 4:

|  |     |
|--|-----|
| Figure 1. Four cell lines examined for stable surface expression of gp160.....   | 93  |
| Figure 2. Summary of curves for the <i>in vitro</i> neutralization data and <i>in vitro</i> ADCC data.....   | 96  |
| Figure 3. Summary of curves from the <i>in vitro</i> ADCC data for control tests of IgG1 2G12 monomer and dimer versus untransfected CHO cells .....   | 97  |
| Figure 4. Model structure of IgG-CD16 complex .....  | 100 |
| Figure 5. Conceptual model of the distance requirements for simultaneous binding of IgG1 with CD16 and the HIV envelope spike with the assumption that the 16-residue unstructured linker connecting the extracellular domains of CD16 to the membrane is adopting a fully extended state..... | 101 |
| Table 1. Summary of calculated 50% effective doses (ED50) for specific lysis in the <i>in vitro</i> ADCC assay and 50% inhibitory concentrations (IC50) for the <i>in vitro</i> neutralization assay.....  | 97  |