National Institute of Allergy and Infectious Diseases

Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases

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Smalls-Mantey, Sample F31 Application and Summary Statement

Introduction

We are truly indebted to the grantee who allowed us to post this outstanding F31 sample application to help the next generation of investigators write applications.

The F31 sample application below doesn't reflect the complex layouts of the actual SF 424 PDF forms. Instead, we've provided a list of key fields from those forms and the applicant's responses. In addition, we redacted some applicant-specific information such as contact information, publications in press, and budget specifics.

Questions? Contact deaweb@niaid.nih.gov. We also offer other outstanding Sample Applications.

PI	Smalls-Mantey, Adjoa R.
Title	Characterization of Antibody-Dependent Cellular Cytotoxicity in HIV Infection
Received	04/13/2011
FOA	PA11-112
Application ID	1 F31 AI098409-01

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Full Application

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  - Activities Planned Under This Award
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SF 424 R&R Face Page

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. Here is the summary that eRA Commons generated at the start of the sample app, which reflects many key fields from that form.]

PI	Smalls-Mantey, Adjoa R
Title	Characterization of Antibody-Dependent Cellular Cytotoxicity in HIV Infection
Received	04/13/2011
FOA	PA11-112
Application ID	1 F31 AI098409-01
Organization	Columbia University Health Sciences
IRG/SRG	ZRG1 AARR-J (22)L
AIDS	Yes
Expedited	No
Animals	No
Humans	Yes
Clinical Trial	No

Current HS Code	30
HESC	No
New Investigator	No
Early Stage Investigator	No

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Project/Performance Site Locations

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. Performance sites are in New York, Maryland, and the United Kingdom.]

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Research & Related Other Project Information

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. Here are the key fields from that form and the applicant's responses.]

Are Human Subjects Involved?	Yes
If YES to Human Subjects	
Is the Project Exempt from Federal regulations?	No
If yes, check appropriate exemption number.	NA
If no, is the IRB review Pending?	No
IRB Approval Date:	10/20/2010
Human Subject Assurance Number:	**[Redacted]**
Are Vertebrate Animals Used?	No
If YES to Vertebrate Animals	
Is the IACUC review Pending?	NA
IACUC Approval Date:	NA
Animal Welfare Assurance Number	NA

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Project Summary

Understanding the basis of an immune response that controls infection or provides sterilizing immunity remains a major goal in the search for effective vaccines or immunotherapies for HIV. Antibodies (Abs) induced by candidate vaccines to the surface envelope glycoprotein have not neutralized a broad array of primary virus isolates. For this reason, eliciting a cytotoxic cellular response has been the primary goal in most recent vaccine trials. However, this approach has not been successful in containing viral replication in vaccinees that have become HIV-infected. Antibody-dependant cellular cytotoxicity (ADCC) has been shown to mediate sterilizing immunity against challenge with pathogenic simian immunodeficiency virus [Hessel 2007]. In ADCC, Fc-bearing Abs bind viral epitopes coating an infected CD4+ target T cell and an Fc receptor bearing effector, most commonly natural killer cells (NKs), bind the Ab and use perforin to deliver granzymes which induce apoptosis in the target. We want to study ADCC in infected patients to

understand the magnitude and characteristics of the best responses achieved by natural infection. First, we will compare ADCC mediated by the sera of a cohort of patients using a granzyme B cytotoxicity assay developed in our lab. Based on these findings, we will select the sera of patients with the most ADCC, generate monoclonal Abs (mAbs), and characterize the mAbs based on epitope specificity, affinity, potency, breadth, IgG isotype, and Fc type. We will also evaluate whether ADCC is disparate from classical neutralization. Finally, we will use microscopy to examine the synapse between effectors, Abs, and targets. The outcome of this research will provide insight into the characteristics of Abs that mediate ADCC that are likely important goals in the design of HIV vaccines or immunotherapies.

Hypothesis: Antibody-dependent cellular cytotoxicity (ADCC) is a function that has been shown to mediate protection from lentiviral infection. We hypothesize that variations in ADCC activity of sera are dictated by the amount, specificity, and subclass of HIV-specific antibodies.

Aim 1: Characterize the potency of sera of HIV-infected individuals in ADCC.
Aim 2: Characterize the specificity and breadth of antibodies with ADCC activity.
Aim 3: Characterize the structure and function of the target-effector synapse using both fixed and live cell laser scanning confocal microscopy (LSCM), transmission electron microscopy (TEM) and cryo-electron microscopy (cryo-EM) and tomography.

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Project Narrative

Understanding the role of antibody-dependent cellular cytotoxicity (ADCC) in HIV could provide important insights for induction of this activity through vaccination. This project seeks to characterize the Abs that mediate ADCC and image the functional synapse formed by cellular components involved in ADCC with the goal of defining new goals for the development of HIV vaccines and therapeutics.

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Bibliography

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Facilities

Intellectual Support: My mentors Mark Connors and Quentin Sattentau are leading experts in their field and have mentored numerous people that have gone on to successful careers in science. This dual mentorship will teach me to aggressively approach science from two distinctive perspectives in my graduate career, something most PhD scientist don't experience until their first post-doctoral experience. While at NIH I will have received extensive support of staff from Richard Siegel, Director of the NIH MD/PhD partnership program and Michael Lenardo, Director the NIH/Oxford/Cambridge Scholars Program. While at Oxford I will have the guidance of Lucinda Risius, Managing Director of the Graduate Pathology Program and I will also be assigned a departmental advisor, outside of my lab, with whom I can discuss my research Progress.

Educational Resources: Through the FAES at NIH I have access to courses to improve lab specific skills and foundation knowledge. I have already participated a Vaccine Development course. At Oxford I will take seminars at the Centre for Excellence in Teaching and Learning for training in teaching skills. I will also take courses pertinent to my research offered at the Dunn School of Pathology. Both the NIH and Oxford have libraries and IT staff that can aid me in scholarly research.

Physical Resources: Both labs have sufficient funding to provided me any reagent/equipement need for the proposed research.

NIH

1. Lab Space – I am provided with a desk and personal desktop computer. Bench space is shared within the lab.

2. Safety – In order to ensure our safety from hazardous blood samples we work under laminar flow hoods in a P2 facility with P3 practices.

3. Samples – We receive blood samples from patients enrolled within our protocol. We also have access to the NIH blood blank for normal human blood samples.

Oxford

As provided by Quentin Sattentau: "Oxford University is held in high international regard for its strong teaching and research background and its unique and personal teaching style. Within the University the Department of Pathology has an excellent reputation for graduate teaching, and has achieved 100% success in DPhil graduation within 4 years for the past 3 years. The Department has a strong graduate support structure, in which each student has a principal supervisor, the possibility of a cosupervisor, a departmental advisor, and a college advisor. The supervisor(s) will generally meet with the student multiple times a term, and each of the non-supervisory mentors will meet with the candidate approximately once a term and will file reports on the candidate's progress. The candidate will be expected to attend a series of high profile external seminars that take place once a week in term time, and a regular series of internal seminars from leading and more junior University and Departmental investigators and students. Adjoa will attend and participate in the weekly lab journal club, in which scientific papers are taken apart critically by members of the group, and a weekly laboratory meeting in which each lab member presents their ongoing research. Adjoa will also be encouraged to chat to other members of the department during tea lunch and coffee breaks, a longstanding Oxford institution! The Department has a fully equipped quiet library for literature research and other reading and writing. Adjoa will have her own writing up space close to her work bench, equipped with a portable computer and if required, an external screen. Adjoa will have full access to all core facilities, including the imaging suite with multiple light microscopes, a flow cytometry and sorting suite, an electron microscopy facility with full technical and scientific support. We also have strong connections with the biochemistry and chemistry departments and the Weatherall Institute of Molecular Medicine at the John Radcliffe Hospital for obtaining clinical samples and expertise."

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Equipment

Mark Connors Lab, NIH

BD FACSAria IIu
BD LSRII
BD Pathways 855
VICTOR Light luminescence plate reader
AutoMACS Pro Separator

AutoMACS Classic Separator
Containment II/III Laboratory with 4 tissue culture hoods

Quentin Sattentau, Oxford

Tissue culture. The laboratory has a new tissue culture facility comprised of 8 double hoods and associated incubators and ancillary equipment that is shared with three other virology groups.

Virology. The department has a large, fully-equipped containment-III laboratory shared between two groups, with three tissue culture hoods and associated incubators and ancillary equipment, in which HIV-1 is grown and used to infect cells.

Flow cytometry. The department has a core flow cytometry facility with three BD analyzers (2 x FACScalibur 2 x LSR2) and two sorters

Molecular biology. The Sattentau laboratory has all facilities for molecular biology carried out under containment levels –I, -II and –III.

Light microscopy. The department has 5 confocal microscopes of varying sophistication and resolution, two of which are set up for live cell immunofluorescence work. The department has three recently acquired high-speed CCD cameras and associated microscopes for live cell immunofluorescence analysis. The Sattentau laboratory has a Zeiss Pascal confocal microscope and a new CCD camera set up with a Zeiss Axiovert 200 microscope for fixed and live cell work in the containment-III laboratory, allowing real time analysis of live, infected cells.

Electron microscopy. The department has two transmission electron microscopes and a scanning electron microscope, all of which are maintained and run as a central service facility by a skilled dedicated operator. Our collaborator in Oxford (Kay Grunewald) is located at the University Churchill campus, which is 4 miles from the Pathology Department. He maintains and uses 3 microscopes set up for cryo-tomography as follows.

- 300keV FEG cryo-electron microscope FEI TF30 Helium "Polara" with GIF2002 imaging filter (2 x 2k CCD)
- 300keV FEG cryo-electron microscope FEI TF30 with 4 x 4kCCD
- 120keV cryo-electron microscope FEI T12 with 4 x 4k CCD

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Research & Related Senior/Key Person Profile

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. See below for the biographical sketch attachments.]

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Biographical Sketch

NAME	Adjoa Smalls-Mantey
POSITION TITLE	Graduate Student

EDUCATION/TRAINING			
INSTITUTION AND LOCATION	**DEGREE (if applicable)**	**YEAR(s)**	**FIELD OF STUDY**
University of Maryland, Baltimore County			Biochemistry and Molecular

(UMBC)	B.S.	05/2007	Biology
Columbia University College of Physicians and Surgeons	M.D. expected 2015	2007 - 2009	Medicine (first two years)
University of Oxford	D.Phil expected 2013	2009 - Present	Immunology

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A. Personal Statement

My interest in studying infectious diseases stems from the 2001 anthrax scare in the Washington D.C. area. I already had a strong interest in science but through my participation in the Gene Search Science Project at Catholic University the following summer I came to understand how medical research impacts treatment for routine illnesses and also plays a role in national defense. Since middle school I have been preparing for a career in science. At Eleanor Roosevelt High School I was enrolled in the rigorous Science and Technology Program during which time in participated in the Gene Search Project and researched biochemical pathways that influence sickle cell anemia with Dr. William Winters at Howard University Hospital. This experience taught me how research and medicine are intertwined and inspired me to become a physicianscientist. At UMBC I was a member of the Meyerhoff Scholars Program and an HHMI Undergraduate Research Scholar where numerous lab experiences, seminars, rigorous classes, and multiple opportunities for scientific presentations prepared me for a career as a scientist. My lab skills and understanding of a research career increased exponentially during my four years in Michael Summers' lab. Not only did I learn about protein purification, gel electrophoresis, NMR, and ITC while studying MuLV RNA packaging, but more importantly of the time and discipline necessary for excellent research and the internal drive that keeps one focused. Working with Dr. Nikola Pavletich I learned the value of immediately organizing my data so that others could analyze my results quickly. In the lab of Tom Cech I had the responsibility of designing a new protocol to study telomerase assembly, a skill that has enabled me to design a novel assay to investigate AIDS specific questions in my graduate studies. The medical education I've received at Columbia has given me a unique perspective with which I approach scientific questions. Under the tutelage of Mark Connors and Quentin Sattentau, leaders in their fields on nonprogressive HIV infection and viral transmission, my understanding of infectious disease and my capacity to pursue further research in these fields will be greatly enhanced. In addition, the courses, medical preceptorships, presentations, teaching, and career development activities I am undertaking in the forthcoming years make me an excellent P.I. for this grant.

B. Positions and Honors

ACTIVITY/OCCUPATION	BEGINNING DATE	ENDING DATE	FIELD	INSTITUTION/COMPANY	SUPERVISOR EMPLOYER
Deputy Director, Counselor of Metrolite Pathfinder Club	01/95	05/07	Scouting, Volunteer	Metropolitan SDA Church	Dwight Byass, Angela Brown
Intake Counselor	09/07	12/07	Volunteer	The Door: Community based program for NYC	N/A
Mentor	09/07	05/08	Volunteer	CHAMPS High School Afterschool Program, Bronx, NY	Moya Brown
Secretary of Black and Latino Student Organization	09/07	05/09	Minority Affairs	Columbia University College of Physicians and Surgeons	Hilda Hutcherson, MD

Book Reviewer	01/08	Present	Medical Education	Wiley-Blackwell	Maddy Hurd
Art Coordinator for Vacation Bible School	07/08	07/08	Volunteer	Metropolitan SDA Church	Regina Wright
Pre-doctoral IRTA	05/08	08/08	Immunology	Lab of Immunoregulation, HIVSpecific Immunity Section	Mark Connors
Exam Proctor	09/08	05/09	Education	Office of Disability	Eileen Lograno
Tutoring Coordinator for Student Success Network	09/08	05/09	Medical Education	Columbia University College of Physicians and Surgeons	Megan Jessiman Lisa Mellman, MD

Academic and Professional Honors

- 2003 National Urban League Scholarship
- 2003 UMBC Meyerhoff Scholar
- 2003 UMBC Honors College
- 2003 HHMI Undergraduate Research Scholar
- 2004 NAD Distinguished Youth Award
- 2005 UMBC Minority Access to Research Careers Scholar
- 2005 Abby Rockefeller Mauzé Award Charitable Trust Scholar for Outstanding Achievement
- 2006 Goldwater Scholar
- 2007 UMBC Top Senior Biochemistry Major
- 2007 Graduated Sum Cum Laude from UMBC
- 2007 NIH/Oxford/Cambridge Scholar
- 2010 International AIDS Vaccine Conference Scholarship
- 2011 Keystone Symposia Underrepresented Minority Scholarship

Honor Societies

- 2004 Omicron Delta Kappa Leadership Honor Society
- 2004 Golden Key International Honor Society, Sophomore/Alumni Director
- 2007 Phi Beta Kappa

C. Publications

Research Publications

- Dey A, York D, Smalls-Mantey A, Summers MF. 2005. Composition and sequence-dependent binding of RNA to the nucleocapsid protein of Moloney murine leukemia virus. Biochemistry. 44(10), 3735-3744.

- Miyazaki Y, Irobalieva RN, Tolbert BS, Smalls-Mantey A, Iyalla K, Loeliger K, D'Souza V, Khant H, Schmid MF, Garcia EL, Telesnitsky A, Chiu W, Summers MF. 2010. Structure of a conserved retroviral RNA packaging element by NMR spectroscopy and cryo-electron tomography. J Mol Biol. 404(5), 751-772.

Poster Presentations

- Smalls-Mantey A, Winter W. 2003. Biosynthesis of 2,3-diphosphoglycerate in Erythrocytes. Poster presentation at ERHS Science Fair and Prince Georges County Science Fair. March 2003.
- Smalls-Mantey A, Dey, A, Phillip C, Summers MF. 2004. Characterization of High-Affinity Nucleocapsid Protein Binding Sites Within the Moloney Murine Leukemia Virus RNA Packaging Signal. Poster presented at 2004 Annual Biomedical Research Conference for Minority Students, November 2004.
- Smalls-Mantey A, Min J, Pavletich N. 2005. Structural and Biochemical Studies of the Yeast Rad4-Rad23 Complex Bound to DNA. Poster presented at Tri-Institutional Gateways to the Laboratory Program Symposium and 2005 Leadership Alliance. August 2005.
- Smalls-Mantey A, Miyazaki Y, Summers MF. 2007. Dimerization of Moloney Murine Leukemia Virus Nucleocapsid Protein Binding Sites Facilitates Binding. Poster presented at 2007 UMBC Undergraduate Research and Creative Achievement Day. April 2007.
- Smalls-Mantey A, Klein R, Doria-Rose N, Laub L, Rood J, Migueles S, Sattenatu. 2010. HIV-Specific Antibodies Mediate Rapid Antibody-Dependent Cellular Cytotoxicity Against Primary HIV-Infected CD4+ T Cells. Poster presented at 2010 Keystone HIV Vaccines (X5) Meeting. March 2010.
- Smalls-Mantey A, Klein R, Doria-Rose N, Laub L, Rood J, Migueles S, Sattenatu. 2010. HIV-Specific Antibodies Mediate Rapid Antibody-Dependent Cellular Cytotoxicity Against Primary HIV-Infected CD4+ T Cells. Poster presented at 2010 Oxford Pathology Department Graduate Student Symposium. June 2010.
- Smalls-Mantey A, Klein R, Doria-Rose N, Laub L, Rood J, Migueles S, Sattenatu. 2010. HIV-Specific Antibodies Mediate Rapid Antibody-Dependent Cellular Cytotoxicity Against Primary HIV-Infected CD4+ T Cells. Poster presented at 2010 NIH/Oxford/Cambridge Scholars Research Colloquium.
- Smalls-Mantey A, Klein R, Doria-Rose N, Laub L, Rood J, Migueles S, Sattenatu. 2010. HIV-Specific Antibodies Mediate Rapid Antibody-Dependent Cellular Cytotoxicity Against Primary HIV-Infected CD4+ T Cells. Poster presented at 2010 International AIDS Vaccine Conference. September 2010.

Oral Presentations

- Smalls-Mantey A. 2010. Antibody-Dependent Cellular Cytotoxicity and the Control of HIV. Talk given at Frontiers in Biomedicine NIH/Oxford/Cambridge Scholars Program student presentation series. November 2010.

D. Research Support

[Outside research support redacted from sample.]

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NAME	Mark Connors
POSITION TITLE	Chief, HIV-Specific Immunity Section Lab of Immunoregulation, NIAID, NIH

EDUCATION/TRAINING			

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Temple University, Philadelphia, PA	B.A. (Magna Cum Laude)	05/1981	Biology
Temple University, Philadelphia, PA	M.D.	05/1985	Medicine
The Boston Floating Hospital, Boston, MA	Residency	05/1989	Pediatrics

A. Personal Statement Regarding Project

Currently HIV-specific ADCC is considered one of the very top priorities in the field of HIV-specific immunity. Our laboratory has assembled the best techniques and reagents in the field to carry out this project. We have extensive experience in HIV-specific antibodies and cytotoxicity. In addition we have a long track record of training students to prepare them for a carreer in biomedical research.

B. Positions and Honors

- 1985 - 1986 Intern in Pediatrics, The Boston Floating Hospital, New England Medical Center, Boston, Massachusetts
- 1986 - 1988 Resident in Pediatrics, The Boston Floating Hospital, New England Medical Center, Boston, Massachusetts
- 1988 – 1989 Chief Resident in Pediatrics, The Boston Floating Hospital, New England Medical Center, Boston, Massachusetts
- July 1989 - October 1989 Medical Staff Fellow, Respiratory Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD
- October 1989 - June 1993 U.S. Public Health Service, Research Associate (C.O.), Respiratory Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD
- July 1993 - June 1994 U.S. Public Health Service, Clinical Associate, Clinical Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD
- January 1994 - June 1994 Clinical Fellow in Infectious Diseases, The Childrens Hospital of Philadelphia, Philadelphia, PA

Employment:

- June 1994 - December 2004 U.S. Public Health Service, Medical Officer, National Institute of Allergy and Infectious Diseases, Clinical and Molecular Retrovirology Section, Laboratory of Immunoregulation, National Institutes of Health, Bethesda, MD
- December 2007- Present Chief, HIV-Specific Immunity Section, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Recent Honors and Editorial and Review Board Activities

- 2009 - U.S. Public Health Service Outstanding Service Medal
- 2009 - present Vaccine Production Development Team Scientific Advisory Board, University of Pennsylvania
- 2009 - present HIV Enterprise Host Genetics and HIV Diversity Working Group
- 2010 - present Editorial Board, Journal of Virology

C. Peer-Reviewed Publications

- Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Schwartz D, Sullivan J, and Connors M. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV infected long term nonprogressors. PNAS 2000; 97(6): 2709-2714.

- Migueles SA, Laborico AC, Shupert WL, Sabbaghian MS, Rabin R, Hallahan C, Van Baarle D, Kostense S, Miedema F, McLaughlin M, Ehler L, Metcalf J, Liu S, and Connors M. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nature Immunol 2002; 3(11); 1061-1068.

- Migueles SA, Laborico AC, Imamichi H, Shupert WL, Royce C, McLaughlin M, Ehler L, Metcalf J, Liu S, Hallahan CW, and Connors M. The differential ability of HLA B*5701+ long-term nonprogressors and progressors to restrict HIV replication is not caused by loss of recognition of autologous viral gag sequences. J Virol 2003; 77(12); 6889-6898.

- Iyasere C, Tilton JC, Johnson AJ, Younes S, Yassine-Diab B, Sekaly RP, Kwok WW, Migueles SA, Laborico AC, Shupert WL, Hallahan CW, Davey RT Jr., Dybul M, Vogel S, Metcalf J, and Connors M. Diminished proliferation of HIV-specific CD4+ T Cells is associated with diminished IL-2 production and recovered by exogenous IL-2. J Virol 2003; 77(20); 10900-10909.

- Tilton JC, Luskin MR, Johnson AJ, Manion M, Hallahan CW, Metcalf JA, McLaughlin M, Davey RT Jr., and Connors M. Changes in paracrine IL-2 requirement, CCR7 expression, frequency, and cytokine secretion, of HIV-specific CD4+ T cells are a consequence of antigen load. J Virol 2007; 81;2713-2725.

- Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, Weeks KA, Rood JE, Berkley AM, Sacha JB, Cogliano-Shutta NA, Lloyd M, Roby G, Kwan R, McLaughlin M, Stallings S, Rehm C, O'Shea MA, Mican J, Packard B, Komoriya A, Palmer S, Wiegand AP, Maldarelli F, Coffin JM, Mellors JW, Hallahan CW, Follman DA and Connors M. Lytic Granule Loading of CD8+ T-Cells is Required for HIV-Infected Cell Elimination Associated with Immune Control. Immunity 2008; 29 (6): 1009-1021.

- Migueles SA, Weeks KA, Nou E, Berkley AM, Rood JE, Osborne CM, Hallahan CW, Cogliano-Shutta NA, Metcalf JA, McLaughlin M, Kwan R, Mican JM, Davey RT Jr, Connors M. Defective human immunodeficiency virus-specific CD8+ T-cell polyfunctionality, proliferation, and cytotoxicity are not restored by antiretroviral therapy. J Virol 2009 83(22): 11876-11889.

- Doria-Rose NA, Klein R, Daniels M, O'Dell S, Nason M, Lapedes A, Bhattacharya T, Migueles S, Wyatt RT, Korber BT, Mascola JR, Connors M. Breadth of HIV-Specific neutralizing activity in sera: clustering analysis and association with clinical variables. J Virol 2009 84(3): 1631-1636.

- Doria-Rose NA and Connors M. Antibody-secreting B cells in HIV infection. Curr Opin HIV AIDS 2009 4:426-430

- Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, Zhou T, Schmidt SD, Wu L, Xu L, Longo NS, McKee K, O'Dell S, Louder MK, Wycuff DL, Feng Y, Nason M, Doria-Rose N, Connors M, Kwong PD, Roederer M, Wyatt RT, Nabel GJ and Mascola JR. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 2010 329:856-861.

- Migueles SA and Connors M. Long-term nonprogressive disease among untreated HIV-infected individuals: clinical implications of understanding immune control of HIV. JAMA 2010 304:194-201.

- **[Redacted publication in press]**

Research Support

The research direction of the HIV-Specific Immunity Section of the Laboratory of Immunoregulation is focused upon mechanisms of immunity to HIV. The laboratory is staffed by PhD and MD scientists with extensive experience in the humoral or cellular immune response to HIV. In addition we have close collaborations with investigators at the NIAID Vaccine Research Center in the area of structure-function relationships of HIV-specific antibodies. Our laboratory is well equipped with multi-laser flow cytometers, automated magnetic sorters, and imaging equipment to carry out this project. Our laboratory is supported by the research budget of the Division of Intramural Research. Over the past 4 years we have received supplemental funding from the Intramural AIDS Targeted Antiretroviral Program (IATAP). This will continue for the next 2 years. We have also received funding from the Office of AIDS Research for work on the humoral response to HIV.

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NAME	Sattentau, Quentin James
POSITION TITLE	Professor of Immunology

EDUCATION/TRAINING			
INSTITUTION AND LOCATION	**DEGREE (if applicable)**	**YEAR(s)**	**FIELD OF STUDY**
The University of Bristol, UK	BSc (Hons)	October 1980	Microbiology
The University of London, UK	PhD	September 1985	Virology/Immunology
University College London, UK	Postdoctoral	October 1985	HIV immunology

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A. Personal Statement

N/A

B. Positions and Honors

Positions and Employment

- 1985 - 1992 Lecturer, Department of Genito-Urinary Medicine, University College London, UK
- 1989 - 1991 Visiting scientist, Howard Hughes Medical Institute, Columbia University, New York, NY
- 1992 - 1998 Staff scientist (Director of Research), Centre d'Immunologie, Marseille, France
- 1999 - 2003 Reader, Section of Infectious Disease, Imperial College London, London UK
- 2003 - Professor of immunology, Department of Pathology, University of Oxford, Oxford UK

Other Experience and Professional Memberships

- 1995 - Member, American Association for the Advancement of Science
- 1998 - Member, American Society for Microbiology
- 1998 - Member, British Society for Immunology, UK
- 1998 - Member, Society for General Microbiology, UK
- 2005 - Member of the Pasteur Institute Paris France Scientific Evaluation Committee.
- 2005 - Member of the Vaccine Research Center (VRC) NIH Board of Scientific Councilors
- 2007 - Member of BMGF Vaccine Monitoring Centre SAB
- 2008 - Member of NIH HIVRAD/Novartis Neutralizing Antibody Vaccine Consortium SAB
- 2009 - Member of study section for Swedish Program for International Development
- 2010 - Member of NIH study section for the B cell Immunology Partnership Program for HIV-1 Vaccine Discovery (U19)

C. Selected Peer-Reviewed Publications

Most relevant to the current application

1. Martin N, Welsch S, Jolly C, Briggs JA, Vaux D and Sattentau QJ. (2010) Virological synapse-mediated spread of human immunodeficiency virus type-1 between T cells is sensitive to entry inhibition. J. Virol. 84: 3516-3527.

2. González N, Bermejo M, Calonge E, Jolly C, Arenzana-Seisdedos F, Pablos JL, Sattentau QJ and Alcamí J. (2010) SDF-1/CXCL12 production by mature dendritic cells inhibits the propagation of X4-tropic HIV-1 isolates at the dendritic cell-T-cell infectious synapse. J Virol. 84:4341-51

3. Groot F, Welsch S and Sattentau QJ. (2008). Efficient HIV-1 transmission from macrophages to T cells across transient virological synapses. Blood 111: 4660-4663.

4. Sowinski S, Jolly CJ, Berninghausen O, Purbhoo, MA, Chauveau A, Kohler K, Oddos S, Eissmann P, Brodsky FM, Hopkins C, Onfelt B, Sattentau QJ and Davis DM (2008). Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. Nature Cell. Biol. 10: 211-219.

5. Jolly C and Sattentau QJ (2007). HIV-1 assembly, budding and cell-cell spread in T cells takes place in tetraspanin-enriched plasma membrane. J. Virol. 81:7873-7884

6. Jolly C and Sattentau QJ (2007). Adhesion molecule interactions facilitate human immunodeficiency virus type-1-induced virological synapse formation between T cells. J. Virol. 81: 13916-13921.

7. Jolly C, Mitar I and Sattentau QJ (2007). Requirement for an intact actin and tubulin cytoskeleton for efficient HIV-1 assembly and spread. J. Virol. 81: 5547-6

8. Jolly C and Sattentau QJ. Human Immunodeficiency virus type-1 virological synapse formation in T cells requires lipid raft integrity. J. Virol. 2005 79: 12088-12094.

9. Jolly C, Kashefi K, Hollinshead M and Sattentau QJ (2004) HIV-1 cell-to-cell transfer across an Env-induced, actin-dependent synapse. J. Exp Med. 199: 283-193.

Additional recent publications of important to the Field (in chronological order)

1. Wegmann F, Krashias G, Luhn K, Laamanen K, Vieira S, Jeffs SA, Shattock RJ and Sattentau QJ (2011) A novel strategy for inducing enhanced mucosal HIV-1 antibody responses in an anti-inflammatory environment. PlosOne 6: e15861.

2. Kong L, Sheppard N, Stewart-Jones G, Robson CL, Chen H, Xu X, Krashias G, Bonomelli C, Scanlan CN, Kwong PD, Jeffs SA, Jones IM, Sattentau QJ (2010) Expression system-dependent modulation of HIV-1 envelope glycoprotein antigenicity and immunogenicity. J. Mol. Biol. Aug 25th epub.

3. Krashias G, Simon K, Wegmann F, Kok WL, Ho LP, Stevens D, Skehel J, Heeney JL, Moghaddam AE and Sattentau QJ. (2010). Potent adaptive immune responses induced against HIV-1 gp140 and influenza virus HA by a polyanionic carbomer. Vaccine 28: 2482-2489.

4. Zanetti G, Briggs JAG, Grunewald K, Sattentau QJ and Fuller SD. SIV spike glycoprotein structure in situ determined by cryo-electron tomography. Plos Pathogens August 2 (8): e83

5. Sheppard NC, Bates AC and Sattentau QJ (2007). A functional human IgM response to HIV-1 Env after immunization with NYVAC-HIV C. AIDS 19: 524-527.

D. Research Support

[Outside research support redacted from sample.]

PHS Fellowship Supplemental Form, Research Plan

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. The attachments are included below. In the original version, these attachments met the page limits.]

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Specific Aims

Understanding the basis of an immune response that controls infection or provides sterilizing immunity remains a major goal in the search for effective vaccines or immunotherapies for HIV. Antibodies (Abs) induced by candidate vaccines to the surface envelope glycoprotein have not neutralized a broad array of primary virus isolates. For this reason, eliciting a cytotoxic cellular response has been the primary goal in most recent vaccine trials. However, this approach has not been successful in containing viral replication in vaccinees that have become HIV-infected. Antibody-dependant cellular cytotoxicity (ADCC) has been shown to mediate sterilizing immunity against challenge with pathogenic simian immunodeficiency virus [Hessel 2007]. In ADCC, Fc-bearing Abs bind viral epitopes coating an infected CD4+ target T cell and an Fc receptor bearing effector, most commonly natural killer cells (NKs), bind the Ab and use perforin to deliver granzymes which induce apoptosis in the target. We want to study ADCC in infected patients to understand the magnitude and characteristics of the best responses achieved by natural infection. First, we will compare ADCC mediated by the sera of a cohort of patients using a granzyme B cytotoxicity assay developed in our lab. Based on these findings, we will select the sera of patients with the most ADCC, generate monoclonal Abs (mAbs), and characterize the mAbs based on epitope specificity, affinity, potency, breadth, IgG isotype, and Fc type. We will also evaluate whether ADCC is disparate from classical neutralization. Finally we will use microscopy to examine the synapse between effectors, Abs, and targets. The outcome of this research will provide insight into the characteristics of Abs that mediate ADCC that are likely important goals in the design of HIV vaccines or immunotherapies.

Hypothesis: Antibody-dependent cellular cytotoxicity (ADCC) is a function that has been shown to mediate protection from lentiviral infection. We hypothesize that variations in ADCC activity of sera are dictated by the amount, specificity, and subclass of HIV-specific antibodies.

Aim 1: Characterize the potency of sera of HIV-infected individuals in ADCC.

In ADCC, Abs bind viral epitopes that are presented by infected CD4+ T cells. NKs expressing an Fc receptor bind the Fc domain of the Ab and use perforin to deliver granzymes to the HIV-infected cell. Subsequently, granzymes induce apoptosis within the cell. Our lab has developed a flow cytometric assay that measures granzyme B delivered to an HIV-infected CD4+ target T cell. We will classify ADCC by the percent of target cells receiving granzyme and the elimination of targets as defined by residual percent of targets expressing p24, HIV capsid.

a. Compare the serum of HIV+ individuals with various rates of progression and viral loads to determine which contain Abs capable of mediating the highest levels of ADCC.

b. Compare the ADCC and neutralizing activity of patient sera.

Aim 2: Characterize the specificity and breadth of antibodies with ADCC activity. Our laboratory has panels of NAbs derived from patients with known serum neutralizing or ADCC-mediating activity.

a. Determine whether recognition of specific epitopes is required for ADCC.

b. Define the breadth of the polyclonal sera by its ability to mediate ADCC in CD4+ T cells infected by different clades of HIV.

c. Titer serum total IgG, IgG1, and IgG3 binding infected CD4+ T cells.

Aim 3: Characterize the structure and function of the target-effector synapse.

Using both fixed and live cell laser scanning confocal microscopy (LSCM), transmission electron microscopy (TEM) and cryo-electron microscopy (cryo-EM) and tomography, we will examine the synapse formed between NK and other cells with potential ADCC activity (macrophages and neutrophils) and infected target cells. We will specifically investigate:

a. The structure of a functional ADCC synapse.

b. The kinetics of ADCC function in real time and its relation to antibody type and specificity.

c. A role for antibody-dependent cell-mediated phagocytosis (ADCP) in elimination of HIV-infected cells. d. Receptors and effector molecules central to ADCC activity against HIV infected cells.

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Research Strategy

SIGNIFICANCE

Introduction

Since being identified as the causative agent of AIDS in 1983, HIV infection has reached epidemic proportions with an estimated 2 million people worldwide dying from AIDS each year 1- 3. There is more public awareness about how to prevent HIV infection but with the growing incidence of HIV infection and the limitations of current antiretroviral therapy, there is an imperative need for a vaccine.

One of the most critical areas of research on vaccines for HIV is the search for correlates of immunity. At present the only method for determining the potential benefits of a vaccine with regard to protection from infection or lowering of viral load upon infection remains very large clinical efficacy trials. Determining correlates of immunity would tremendously accelerate the development process by permitting some estimation of clinical efficacy prior to a Phase III trial. Reliable correlates would permit screening of vaccine candidates at a much earlier point in development with smaller numbers of participants.

Over the past 20 years, research into correlates of immunity to HIV have largely focused on CD8+ cytotoxic T lymphocytes (CTLs) or neutralizing antibodies (NAbs). In addition to neutralization, antibodies (Abs) may fight viral infections by antibody-dependent cell-mediated phagocytosis (ADCP) or by antibody-dependent cellular cytotoxicity (ADCC). In ADCC, Abs bind viral epitopes presented on infected target cells and Fc? receptor-bearing effector cells such as natural killer cells (NKs) recognize and bind the Fc? region of these Abs. Upon cross-linking of the Fc? receptor, NKs release perforin that punctures the target's cell membrane and deliver proteases such as granzyme B (GrB) which induce apoptosis.

Humoral Response to HIV

ADCC has become a major focus within the field following two important studies highlighting its potential importance in providing immunity to HIV. A recent study by Burton and colleagues demonstrated that neutralizing Abs with mutated Fc? receptor binding regions were less effective in protecting macaques from HIV whereas Abs with impaired complement binding conferred protection to macaques, suggesting that ADCC may be more important in preventing infection than neutralization or complement activation alone 4. Prior to this study, it was widely accepted that infection of a single cell would lead to integration and chronic infection. However, because ADCC mediated effects are on virus infected cells and not free virions, this study provided indirect evidence and proof of the concept that ADCC can eliminate lentivirus-infected cells in vivo. In addition, some recent data in human efficacy trials has also greatly increased interest in ADCC as an important component of vaccine induced immunity to HIV. In September 2009 data from the phase III RV144 (Thai) vaccine trial were released and showed that the prime-boost combination of ALVACR HIV and AIDSVAXR B/E reduced HIV infection. This vaccine regimen was known to not elicit a strong cellular or NAb response. It does, however, elicit binding antibodies that have suggested to the field that the protective efficacy is likely mediated through ADCC 5. Our laboratory is part of a large international effort to characterize ADCC antibodies from this trial in order to determine whether there were higher ADCC-mediating antibodies in uninfected vaccinees compared to those that were infected.

Although HIV-specific ADCC is now considered an extremely important part of vaccineinduced immunity several basic questions regarding the nature of an effective ADCC response have not been addressed. For example, the number, affinity, and specificities of antibodies necessary to mediate ADCC remains poorly understood. Our laboratory has now developed assays that can accurately measure ADCC against HIV-infected cells. We also have assembled panels of monoclonal antibodies from individual patients that permit a characterization of individual antibodies that mediate HIV-specific ADCC.

PRELIMINARY STUDIES

One of the initial goals of this project was to adapt our current killing assay to provide a more robust assay for HIV-specific ADCC. Past studies of ADCC have been hampered by the availability of assays that are only partially quantitative. There are four primary methods that people use to measure ADCC 6. The most prevalent is the chromium release assays (CRA), which is highly variable, does not specifically account for

the mechanism of killing, and is insufficiently quantitative 7. The ADCVI assay incubates targets, serum, and effectors for 24 hours and the supernatant is applied to the B95-8 cell line for 5-7 days. The endpoint is reduction of viral infection of the B95-8 cells 8. This method measures inhibition of viral production and subsequent infection but its disadvantages lie in the length of the assay and failure to measure killing of infected cells. Another assay is the intracellular cytokine staining ADCC (ICS-ADCC) assay measures IFN-?, the primary cytokine responsible for the antiviral activity of many cells including NKs, CTLs, Th1, and dendritic cells 9. The advantages of this assay are that multiple effector functions can be measured and all cells and Abs are from the same patient, but it is still a proxy that does not measure HIV-infected cell killing. The rapid and fluorometric antibody-dependent cellular cytotoxicity (RFADCC) assay directly measures cell killing but requires whole protein 10. While use of protein pulsed targets is a sensitive method, it likely places artificially high amounts of protein on the surface of infected cells, potentially overestimating the efficacy of ADCC.

Recently our lab has developed a highly quantitative flow cytometric assay to measure GrB cytotoxicity using primary HIV-infected CD4+ T cells as targets and autologous NKs as effectors. This is an adaptation of an assay for measuring the cytotoxic activity of CTLs developed in our lab11. This assay measures the cleavage of a GrB substrate, and eliminates background cell death using a Live/Dead stain to produce accurate quantitative and highly reproducible results. In brief, CD4+ T cells are stimulated for 3 days and HIV infected. Autologous, negatively selected NKs are used as effectors. Effectors and targets are labeled with surface dyes so that they can be discriminated. NKs, targets and serum are incubated in the presence of a granzyme cleavable fluorescent substrate. Granzyme delivery to the target cell and elimination of HIV-infected targets can then be measured by flow cytometry (Figure 1).

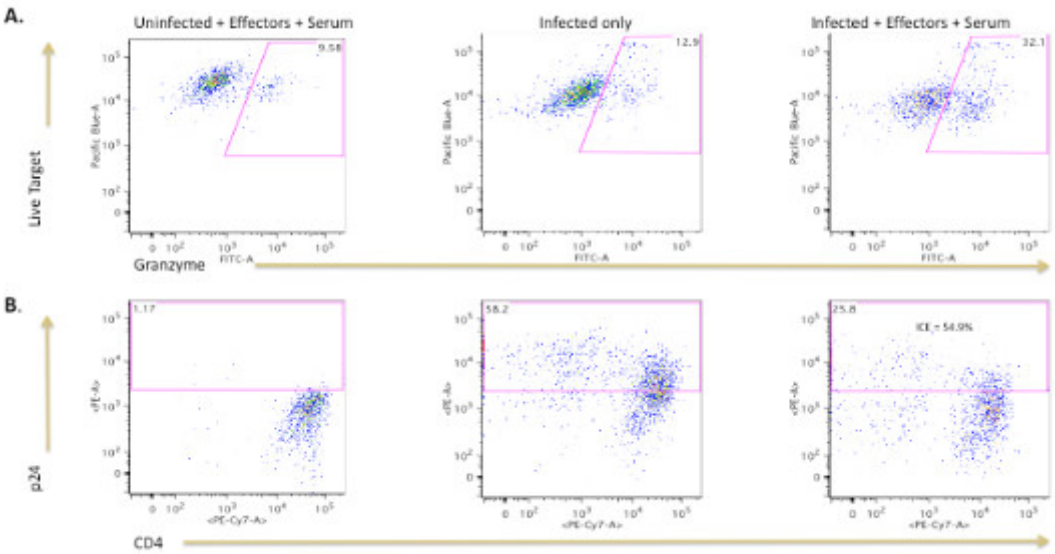

Figure 1. Fluorometric granzyme cytotoxicity assay effectively measures serum-mediated
cytotoxicity against CD4+ T cells.

A. Cellular GrB content. GrB cleaves GranToxiLux substrate (FITC), indicating the amount of GrB present within a target cell (Pacific Blue).

B. Elimination of HIVSF162-infected cells. Live targets are stained for intracellular HIV capsid, p24 (PE). Some infected cells show downregulation of CD4 (PE-Cy7). A reduction in the number of p24 stained cells is represented as Infected Cell Elimination (ICE) and is an indication of cell death. In the example above, ICEInfected+Effectors+Serum = [(58.2-25.8)/58.2] x 100 = 54.9%.

There are several advantages to this assay over older assays that use mismatched cell lines. Because NKs lyse target cells in the context of MHC mismatch and cause nonspecific killing, autologous cells are used in our assay to reduce background. 12. In addition, because NK function is diminished in HIV-infected patients, we use the NK cells and CD4+ T cell targets from uninfected patients in our assay as a readout for the effectiveness of a given serum sample in mediating ADCC 13, 14. We observed variations in NKs

from normal individuals in their ability to mediate ADCC and choose donors that provide optimal signal to noise ratio that we will use to compare the serum of HIV+ individuals (Figure 2).

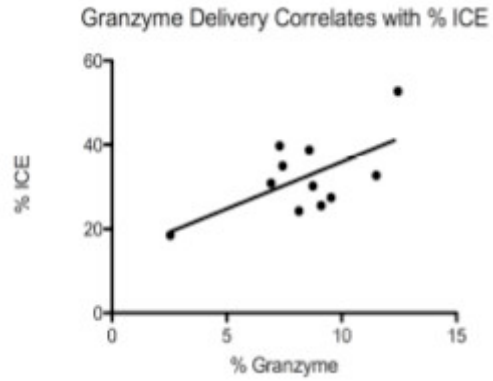

Figure 2: GrB delivery correlates with infected cell elimination (ICE). There is an inherent difference in the degree of ADCC mediated by an individual's cells. In the presence of pooled HIV+ sera, NKs with autologous CD4+ T cells mediate a variable degree of ADCC.

The targets used in this assay are likely closer to the in vivo situation given autologous HIV-infected targets are used in the place of antigen pulsed cell lines. Other advantages include our assay's ability to measure the mechanism of ADCC (granzyme delivery) and the outcome (death of infected cells) (Figure 2). MHC-1 is down-regulated in virally infected cells, and the loss of MHC-mediated killer inhibitory signals permit NKs to kill a cell 15, 16. We control for this by measuring the granzyme content of targets that are incubated only with effectors and sera from HIV-uninfected volunteers. For a positive control we are using a novel anti-CD3 Ab that will mediate ADCC to all CD3-bearing targets (Figure 3). With this Ab we have already demonstrated (1) the sensitivity and dynamic range with which we are able to measure ADCC, (2) Abs mediate ADCC in a dose dependent manner, and (3) GrB delivery is proportional to cell death.

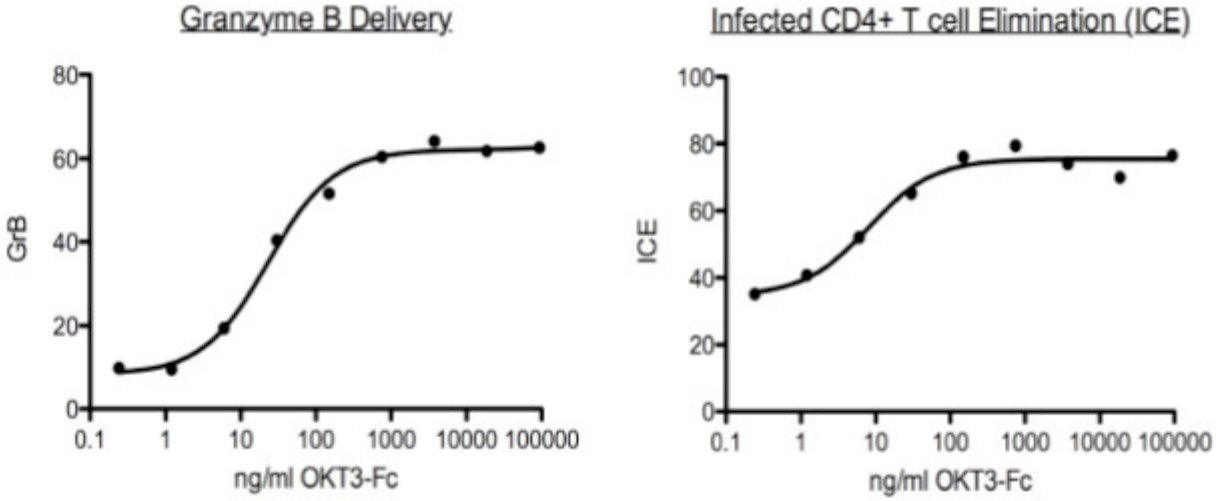

Figure 3. Antibodies mediate ADCC in a dose-dependent manner. GrB correlates with ICE. A novel humanized anti- CD3 antibody (OKT3-huIgG1) was constructed and bound to CD4+T cell targets in the ADCC assay. Left: Granzyme B delivery. Right: ICE.

APPROACH

Aim 1: Characterize the magnitude of ADCC in HIV infected individuals.

Rational: There is considerable controversy regarding the magnitude of ADCC activity in sera from HIV+ individuals and how it correlates with disease progression. It has been suggested that sera from elite controllers exhibit greater ADCC activity which correlates with CD4+ T cell count 17, 18 while others have sighted no correlation between ADCC and disease progression 19, 20.We will compare the serum of HIV+ individuals with various rates of progression and viral loads to determine which contain Abs capable of mediating the highest levels of ADCC. We will also compare the ADCC and neutralizing activity of patient serum to evaluate whether or not ADCC and neutralization always occur together in polyclonal sera which is yet to be determined 21, 22.

Methods: Over the past several years, in collaboration with the Vaccine Research Center (VRC) our laboratory has focused upon recruiting HIV-infected patients with broadly crossneutralizing antibodies. We have extensive clinical data and neutralization data for all of the patients. With the data we generate from our ADCC assay we are able to compare multiple indicators of immunity with ADCC.

Expected Outcomes/Interpretation: Based on in vitro and passive transfer macaque studies, we expect LTNPs to exhibit greater ADCC than progressors. Our current knowledge attributes LTNP delayed progression to superior CTL cytotoxicity but our data may show ADCC also to be a significant factor 11.

Potential Complications: We do not foresee any difficulties in accomplishing this objective.

Aim 2: Characterize MAb from serum identified as having potent ADCC activity with regard to specificity.

Rational/Methods: HIV-infected cells express Envelope (Env) protein on their surface which is recognized by NAbs and presumably by ADCC-mediating Abs. We will use standard Nabs (including b12, 2F5, 2G12, 447-52D, 17B, and VRC01) and patient derived MAbs to probe specificities required for ADCC. An ideal HIV vaccine would be able to protect against diverse virus strains so it is important to determine whether Abs raised against one clade of virus would be able to mediate ADCC in CD4+ T cells infected by different clades. We will use multiple strains of virus from clade A/E, B, and C to infect CD+ T cells and compare killing of target cells in the presence of serum pooled from clade B HIV+ patients. IgG1 and IgG3 are the predominant IgG isotypes with roles in ADCC. It is possible that variations in ADCC activity of sera are mediated by variations in IgG subclass. We will use infected CD4+ T cells, apply sera with various levels ADCC at the same dilutions used in our killing assay, and measure total bound IgG, IgG1, and IgG3 by flow cytometry. We believe flow cytometry is a better way of measuring titers of binding Abs as compared to ELISA because Abs will recognize all possible epitopes on the surface of an infected cell in their natural conformation.

Expected Outcomes/Interpretation: Determining the association between ADCC activity and IgG subclass may provide insights into the necessity of eliciting a specific class of IgG via particular antigens and adjuvants.

Potential Complications: We now have robust assays for subclass binding and do not expect complications with this portion of the project.

Aim 3: Evaluation of the target-effector synapse.

Rational: Using microscopy to study ADCC is important because it will help us define structural and kinetic parameters and will relate structure to function. Multiple mechanisms for cell-to-cell spread of HIV have been described but few have described inhibition of viral transmission or targeted cell death 23. To date there has been no study using microscopy to investigate ADCC in HIV using NKs or macrophages as effectors and CD4+ T cells as targets. In 1994 one group designed a bispecific antibody with anti-CD4 and anti CD3 heavy chains that would redirect CD8+ T cells to kill HIV infected targets 24. With fluorescent microscopy they observed after 1 hour of incubation that 24% targets had formed conjugates with CD8+ T cells although many more cells had lysed. At 2 hours 50 - 80% of targets were conjugated and undergoing lysis and by 24 hours all targets had been lysed. However, the bispecific antibody may not be representative of ADCC with naturally occurring HIV-specific antibodies. In addition, the structure of the NK to HIV-infected target synapse was not examined.

Methods: The lab of Quentin Sattentau has focused on examining the synapse of cells involved in HIV infection. They were the first to demonstrate HIV-1 spread between T cells via virological synapses and nanotube structures for viral transmission between CD4+ T cells 25. They have recently demonstrated that

macrophages can infect CD4+ T cells every 6 hours and that cell-tocell transfer of virus is 10-fold more efficient than free virus spread 26. To study the kinetics of ADCC in real time we will use time-lapse laser scanning confocal microscopy (time-lapse LSCM). Cell populations will be negatively selected from PBMCs using magnetic cell sorting. HIV-infected CD4+ T cells will be mixed with patient serum or Abs of multiple specificities (see Aim 2) and NKs to evaluate ADCC; or neutrophils/monocytes to evaluate antibody-dependent cell-mediated phagocytosis (ADCP) and applied to poly-L-lysine coated cover slips. Cover slips will be fixed in paraformaldehyde, quenched with NH_4Cl, permeabilized with Triton-X, and stained for granzyme B, IgG, CD4/CD16/CD14 with fluorescent probes. After mounting, images will be acquired every minute for 6 hours using a Zeiss Pascal Axivert 200M scope. We will use transmission electron microscopy and tomography (TEM) to generate higher resolution static images of the ADCC functional synapse. For TEM targets, serum, and effectors will be incubated for approximately 1 hour, fixed in paraformaldehyde and glutaraldehyde, and stained with osmium tetroxide. Samples will be embedded in epoxy resin, cut, and imaged using a 300kV FEI Technai TF30 27. If necessary cryo-EM will also be used for structural studies with the aid of Oxford collaborator Kay Grunewald. These experiments will be completed using the pathology department's core bioimaging facility and I will take department's Advanced Light Microscopy and Electron Microscopy course in preparation for this research.

Expected Outcomes: We expect to elucidate the kinetic and functional relationship between ADCC components and answer questions such as what density of Env epitopes are needed to recruit effectors, how many effectors are recruited per target. We do not foresee any difficulties in accomplishing this objective.

CONCLUSION

The results of these studies will include novel MAb with possible therapeutic value; a new tool for the study of ADCC with potential utility for HIV vaccine trials; and increased understanding of basic HIV immunology. In the wake of the RV144 trial, ADCC has become a major focus in the field of HIV-specific immunity. We are very well positioned to carry out these important studies because of our large collection of cells, sera, and detailed clinical data from patients that are part of the NIAID HIV clinic, and both labs' extensive experience in this area.

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Protection of Human Subjects

Samples are collected under the NIAID IRB-approved protocol 02-I-0086. Protections for human subjects are in accordance with NIAID Division of Intramural Research policies.

Hazards/Risks/Discomforts

Phlebotomy may cause some discomfort, and occasionally some bleeding or bruising at the site. On rare occasions, people faint while having blood drawn. Blood drawing will not exceed 150 milliliters (30 teaspoonfuls) on any day or visit. In the course of any time frame during this study, the total amount of blood will not exceed 10.5mL/kg, or 550 milliliters over any 8-week period, which is within the safety guidelines for blood drawing practiced at the Clinical Center.

Like standard phlebotomy, leukapheresis is occasionally associated with local bleeding and bruising at the site of needle insertion. Lightheadedness can also occur, and people faint on rare occasions. Occasional tingling in the arms and legs can occur with the procedure and can usually be relieved by chewing a calcium-rich tablet that the staff will provide. In addition, on very rare occasions (less than 1 in every 1,000 times), malfunction of the leukapheresis machine can result in blood loss of up to 450 milliliters. Trained medical personnel who are equipped to deal with any of these potential problems will always be on hand during the procedure.

Accrual Target: n = 300

Inclusion of Women and Minorities

Gender, Ethnicity and Race Consideration

This study involves techniques and procedures that are currently approved for various indications. Male and female adult patients (18 years of age or older) will be enrolled without preference to gender, race or

ethnicity from the pool of patients that receive care in Clinics 8 at the NIH Clinical Center. Patients will be recruited from outside the NIH community by advertisements posted in the Clinical Center, the NIAID web site, and advertised in other media. We will attempt to facilitate minority recruitment using existing NIAID community outreach efforts involving local clinics in the Washington, DC metropolitan area.

In adult HIV-infected patients planning to interrupt HAART, patients will be fully counseled prior to entry into the study as to the potential risks of discontinuing HAART therapy and the potential benefits of continuing HAART therapy, in the setting of a documented virologic and/or immunologic response. Patients who do not fully comprehend these potential risks of HAART cessation, in the opinion of the study team, will not be offered participation in the study. Patients stopping HAART therapy will be monitored closely for virologic and immunologic relapse and will be offered therapy, based upon the algorithm for safety outlined above.

Targeted/Planned Enrollment

Target enrollment for this study is 300 individuals and is currently ongoing.

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Inclusion of Children (and Pregnant Women)

Inclusion of Children and Pregnant Women

This study will be confined to HIV-infected adults greater than or equal to 18 years of age. As an observational study there is no therapeutic benefit or clinical intervention resulting from participation in this trial, thereby children are not being denied the potential benefits of a research intervention. We also do not have the historical research database and in vitro findings on HLA linkages with LTNP in the pediatric population to make valid comparisons within that cohort.

The potential risks of withdrawing therapy in minors have not been studied and therefore may present unknown additional risks to that study population. Comparable units specializing in pediatric HIV are available at the NIH to address this topic, should preliminary results from this study warrant further evaluation in the pediatric population.

The safety of discontinuing antiretroviral therapy has not been established during pregnancy and nursing, and current guidelines continue to advise HIV-positive women to take antiretroviral therapy to prevent viral transmission to the child during that time period. Therefore, pregnant or nursing women will be excluded from this study. Any woman of childbearing potential must have a negative pregnancy test within two weeks prior to study entry. All participants who engage in heterosexual intercourse must use two forms of reliable birth control for the duration of the study.

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Respective Contributions

When I entered the NIH/Oxford/Cambridge Scholars program I identified Mark Connors and Quentin Sattentau as two HIV researchers with whom I would like to train with because of their focus on HIV immunology, vaccine design, and their expertise in basic immunology techniques, flow cytometry, and microscopy. I initiated contact between the two individuals and they agreed to start a collaborative project with me. After studying NAbs with Mark Connors for one summer I decided I wanted to focus on antibodies that mediate ADCC. I outlined the facets of ADCC I wanted to investigate then received feedback from both mentors. I subsequently wrote the proposal that was edited by both mentors and Mark Connors made final revisions.

I meet multiple times during the week with Dr. Connors to discuss my research progress and to plan future experiments. We also conduct quarterly phone conferences with Dr. Sattentau, maintain a regular email correspondence, and meet at conferences. Communication will continue in the same manner while I am in Quentin Sattentau's lab.

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Selection of Sponsors and Institution

Selection of Institution

I decided to join Columbia's MSTP program because of its tradition of exemplary medical education, medical research, and unparalleled support offered to students from program administrators. Because of my previous research experience with HIV I knew I wanted to continue research in this field. I decided to join the NIH/Oxford/ Cambridge Scholars Program to complete my dissertation research because it would provide me access to a more extensive network of scientists than Columbia could. Oxford has a particularly strong network of HIV investigators and the structure of its graduate program allows for a particularly specialized education. As a graduate student in the pathology department I am allowed to select seminars and accelerated 1-day to 1-week courses that allow me to attain skills quickly and immediately implement them into my research. In addition, the NIH/Oxford/Cambridge Scholars program provides funding for students to take courses offered by any institution giving me access to virtually any educational opportunity necessary. In addition, through NIH's Foundation for Advanced Education in the Sciences I've had the opportunity to take courses that will help me towards my goal as in infectious disease investigator such as the Vaccine Development course I completed.

Selection of Mentors

The NIH/Oxford/Cambridge Scholars Program is designed so that students work with a mentor at NIH and one at a university. When I joined the program I searched for HIV investigators throughout NIH and Oxford and identified Mark Connors and Quentin Sattentau as potential mentors because I wanted to conduct research that had direct implications in vaccine development. Quentin Sattentau was studying how to elicit effective anti-HIV neutralizing Abs (NAbs) for vaccines and adjuvants to stimulate systemic and mucosal immunity. Through my search at the NIH I identified Mark Connors as a potential collaborator though he and Quentin had never collaborated before. I believed Dr. Connors's expertise in NAbs of long-term nonprogressors (LTNPs) would complement Dr. Sattentau's vaccine development goals. In addition, Dr. Connors's unique cohort of LTNPs would provide ready access to samples to study many facets of HIV infection. Finally, I believe Dr. Sattentau's approach to research as a PhD and Dr. Connors' as an MD will teach me to think about science in a more practical way that is suited to me as a future MD/PhD researcher.

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Responsible Conduct of Research

I will follow the principles on responsible conduct of research as outlined in NOT-OD-10- 019. As an undergraduate at UMBC I took a semester-long course entitled "Ethics/Integrity in Scientific Research" which first help me to understand the responsibility scientists have to one another and medical community honestly share information. As a Columbia medical student, I have taken the Hippocratic Oath to guide my patient care and I am expected to adhere to the code of ethics as outlined in student handbook. Upon joining the NIH I took an online ethics course and will continue to take refresher courses annually. At Oxford I will continue my ethics training by taking the required "Ethics: Introduction to Research Ethics". Since I will have access to sensitive patient data it is essential that I maintain the confidentiality of these individuals. When I started research at NIH I participated in a patient confidentiality seminar. Oxford offers an "Ethics: Confidentiality" course in which I will enroll to better my further my understanding and ability to maintain ethical practices with regards to research.

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Goals

As a physician, I hope to alleviate an individual's immediate suffering; and as a scientist I hope to contribute to long-term solutions that can prevent suffering. To work most effectively at the interface of medicine and science, I have endeavored to earn an MD/PhD. I believe that the combined degree's greatest value lies in the perspective that it affords: to approach patient care with a researcher's eye, and to approach research with a physician's focus on improving the quality of life.

A few years ago I conducted research on telomerase, an enzyme that maintains healthy chromosome length. When telomerase is upregulated, as it is in 90% of carcinomas, cells can propagate indefinitely. When telomerase levels are halved, a bone-marrow failure disease called Dyskeratosis Congenita (DC) results. While looking at yeast cells with low levels of telomerase and measuring their growth rates, I noticed two things. The first was that cells with low levels of telomerase grew slowly. Secondly, these cells had abnormal morphologies, as do tumor cells. These observations led me to wonder whether the

progression of cancer in individuals with DC was slower than in patients without it because DC patients have less active telomerase. Upon discussing this with my P.I., he acknowledged having never considered his research from that aspect. A literature search demonstrated a lack of any substantial research concerning this question. Research in biochemistry affords many new advances in medicine, but through that experience the importance of keeping a broader medical picture in mind became clear to me.

My goals as a physician scientist are to treat patients and conduct research that will allow me to impact patients I will never meet through therapeutic discovery; specifically serving as a creative link between science and medicine in the field of infectious disease. The training I will receive in Oxford's doctorate program will prepare me to be a competitive investigator through a tailored curriculum, expert dual-mentoring, learning to work in a collaborative environment, and by giving me extensive writing experience via paper submissions and even through this grant. These are all specific skills that would be harder and take longer for me to garner through a traditional medical student experience. The advantage I have in understanding that I want to be a physician-scientist has given me the opportunity to carefully cultivate these skills early in my career.

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Activities Planned Under This Award

Year 1: Mark Connors Lab (NIH)

Research 80%, Seminars/Courses 10%, Presentation/Career Development/Clinical 10%

Research Skills: While working in Mark Connors's lab I have acquired specific skills such to creatively study immunology such as learning to use flow cytometry. I discuss my research progress frequently during the week with Dr. Connors. We also have phone conferences and meet with Dr. Sattentau at numerous conferences throughout the year.

Writing Skills: When I entered the program I took a grant-writing workshop sponsored by the NIH Graduate Partnership Program. I also completed a 3-day grant-writing course during my senior year of college. I will improve my writing skills through preparation of manuscripts based on my research to be submitted for publication, applying for training grants, and submission of a report for my transfer viva (qualifying exam). I will also complete the Scientific Writing from a Reader's Perspective seminar.

Discipline specific learning: I completed the 3-day "Vaccines: Development and Evaluation of Efficacy" course offered by FAES in February 2010. I also attended "Psychiatry and HIV," a 2-day workshop offered by the American Psychiatric Association so I could learn to effectively interact with HIV+ patients I encounter. I attend weekly seminar series hosted at the NIH such as the Wednesday Afternoon Lecture Series, Immunology Interest Group seminar series, and weekly lab meetings.

Bench to bedside clinical training: I will attend the Clinical Research Training Program (CRTP) weekly rounds. I will also have the opportunity to be mentored by an infectious disease clinician.

Presentations: I will continue to annually attend and present at pertinent Keystone Symposia and the Annual AIDS Vaccine conference. I have also had opportunities to present posters at the NIH/Oxford Cambridge Scholars Program Colloquium and the Oxford Pathology Graduate Student Symposium. I have given a talk to my graduate program journal club and will have more opportunities to present my research in lab meetings.

Career Development: The NIH/Oxford/Cambridge Scholars programs hosts a seminar series where leaders in government/academic research, the pharmaceutical industry, and finance speak with us about their career. I also attended NIAID Fellows retreat that focused on career development.

Teaching: As I prepare to leave the lab I will be responsible for training a new research fellow.

Year 1 (continued) and Year 2: Quentin Sattentau Lab (Oxford)

Research 75%, Seminars/Courses 10%, Presentation/Career Development/Clinical 10%, Teaching 5%

Research Skills: While working in Quentin Sattentau's lab I will learn to apply microscopic imaging to studying HIV infection. I will have weekly meetings to discuss my research progress. We will have phone

conferences and meet with Dr. Connors at various conferences throughout the year. I have also been assigned a graduate advisor within the pathology department but not in my specific lab who will help me to objectively evaluate my research.

Writing Skills: I will improve my writing skills through preparation of manuscripts based on my research to be submitted for publication and writing reviews. I will complete the following 1-day courses offered at Oxford: Writing Skills – Paper and Thesis; Doctoral Thesis Writing,

Discipline specific learning: I will participate in courses and Skills Workshops offered by Oxford including "Advanced Light Microscopy," "Electron Microscopy," and "Introduction to Statistics."

Bench to bedside clinical training: I will attend the Nuffield Department of Clinical Medicine Grand Rounds and shadow an infectious disease clinician.

Presentation: See above.

Career Development: See above. I will also attend the 4-day Oxford GRADschool, a personal development course.

Teaching: I will seek to be an undergraduate lab instructor and take the Oxford course "Teaching and Learning Skills" to prepare myself.

Year 3 and Year 4: Medical School (Columbia)

100%

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Research Experience

In the summer after 11th grade I participated in the intensive 6-week Gene Search Program at the Catholic University of America that introduced me to molecular biology and the exciting race to sequence the human genome. That summer, I learned much more than how to run a gel; I learned to read and comprehend primary research articles and how to design experiments. Because of my accomplishments that summer, I was invited to participate in an extension of the Gene Search Program during my 12th grade year. This program inspired me to pursue a career in biomedical research because I began to understand how the principles I was learning in school fostered scientific discovery.

As a high school senior, I worked with Dr. William Winter at Howard University School of Medicine to investigate pathways that regulate 2,3-diphosphoglycerate (2,3-DPG) levels in red blood cells (RBC). 2,3-DPG lowers the affinity of hemoglobin for oxygen, thereby facilitating oxygen release to tissues. Patients suffering with sickle cell anemia have high levels of 2,3- DPG. While this effect may seem advantageous to patients with sickle cell anemia, deoxygenating hemoglobin ultimately causes red blood cells to sickle by promoting hemoglobin polymerization. Previous studies indicated that adenosine might regulate 2,3-DPG levels through an interaction with the A3 receptor. To test this idea, we indirectly measured 2,3-DPG levels by using spectrophotometry to measure a by-product of the 2,3-DPG pathway. An exciting therapeutic prospect of our research is that an antagonist of the A3 receptor may prevent the accumulation of 2,3-DPG and RBC sickling in patients with sickle cell anemia. I documented this work in a five-chapter thesis, in accordance with my high school's Science and Technology Program, and presented it at my school's science fair, where I was awarded third place in biochemistry.

During college I spent four years working with Dr. Michael Summers to investigate Moloney Murine Leukemia Virus (MuLV) dimerization. Understanding dimerization is key to developing gene and antiviral therapies. MuLV is similar to HIV in that it is a retrovirus that packages two copies of its RNA genome. Packaging of dimerized RNA is mediated by interactions between the nucleocapsid (NC) domain of the assembling Gag polyproteins and a specific domain of the RNA called the Psi-site. Previous work in the Summers lab suggested a riboswitch mechanism for MLV genome packaging, where, in monomeric RNA, Psi-site UCUG segments capable of binding NC are sequestered by base pairing, but upon dimerization, become exposed to bind NC. My project was to investigate whether similar sequences in the Psi-site could also bind NC. Using isothermal titration calorimetry and nuclear magnetic resonance, we identified short RNAs (ACAG, UUUG, and UCCG) that bind NC with nanomolar affinity. Our findings suggest that: 1) binding depends on an unpaired guanosine, 2) binding is enhanced in short RNAs containing terminal

phosphates, and 3) binding affinity varies by more than one order of magnitude depending on which three nucleotides lie upstream of the guanosine. The paper I co-authored (Dey, A., et. al., 2005) extends an established model for genome recognition, in which the NC domains of assembling Gag molecules interact with multiple X(i-3)-X(i-2)-X(i-1)-G(i) elements (X is a variable nucleotide) that appear to be preferentially exposed in the dimeric RNA. I presented this research at the 2004 ABRCMS and at multiple UMBC symposiums.

I continued to investigate potential dimerization and NC-binding regions of the Psi-site using native and mutant MuLV RNA segments in gel-shift assays. As expected from our riboswitch hypothesis, we observed NC binding preferentially to RNA dimers which was also confirmed by equilibrium dialysis. Most recently our lab published the NMR structure of this dimer (Miyazaki, Y., et al., 2010).

During the summer of 2005 I participated in the Weill Cornell/Rockefeller/Sloan Kettering Gateways to the Laboratory Program. I worked with Dr. Nikola Pavletich toward solving the crystal structure of the Saccharomyces cerevisiae Rad4-Rad23 complex bound to a DNA substrate. Rad4-Rad23 is a homolog of human XPC-HR23B, a DNA damage-repair protein. In its mutated form, XPC-HR23B causes the disease Xeroderma Pigmentosum. Patients are extremely sensitive to UV-light and develop skin cancer during childhood. I identified DNA substrates to be used in crystallization trials by HPLC-purifying DNA, performing gel-shift assays, and setting up crystal trays. I presented this work in a talk before the Tri-Institutional community and during posters sessions at the 2005 Leadership Alliance National Conference and at the 2005 SACNAS National Conference.

In 2006 I studied telomerase assembly in the lab of Dr. Thomas Cech at the University of Colorado at Boulder. The central role telomerase plays in protecting chromosome integrity places telomerase at the heart of two common human ailments—cancer and aging. To study how and where in the cell telomerase is assembled from its RNA and protein components, we used the budding yeast Saccharomyces cerevisiae as a model organism. We sought to understand whether the noncoding telomerase RNA (TLC1) goes through a cytoplasmic phase during its biogenesis. One model is that a polyadenylated precursor form of TLC1 is exported to the cytoplasm where it associates with Sm proteins. Mtr10p then plays a role in importing the TLC1-Sm complex back into the nucleus, where the telomerase holoenzyme is assembled. Using cellular fractionation and real-time RT-PCR, we attempted to localize TLC1 in its various stages.

During the summer between my first and second year of medical school I joined the lab of Mark Connors where I screened patient sera for breadth and potency of neutralizing Abs against HIV using a TZMBL neutralization assay. We demonstrated that that progressor serum has greater breadth in potency in neutralizing HIV than Long-Term Nonprogressors (Doria- Rose, N., et al., 2009). Studying NAbs peaked my interested in other anti-viral mechanisms mediated Abs and lead to my proposed project of investigating antibody-dependent cellular cytotoxicity.

Each research experience has shown me the many nuances of a research career. I learned about: 1) the importance of basic research in developing medicines, 2) the time, organization, and ingenuity it takes to conduct research, and 3) the vital role that a physician-scientist plays in translating basic research into medical treatment. Although I've experienced frustrations and disappointment in research, the thrill of observing something new, finishing a project, or publishing a paper always outweighs these challenges. Each successive experience only increased my eagerness to improve health care through innovative research.

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MARK CONNORS MENTOR STATEMENT

[Redacted from sample.]

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QUENTIN SATTENTAU MENTOR STATEMENT

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Summary Statement

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[Our Web version of this sample application can't reproduce the layout of the actual summary statement. See below for the data fields and critiques.]

SUMMARY STATEMENT **(Privileged Communication)**	
PROGRAM CONTACT:	Diane Adger-Johnson
Release Date:	07/22/2011
Application Number:	1 F31 AI098409-01
Principal Investigator	Smalls-Mantey, Adjoa R
Applicant Organization:	Columbia University Medical Center (CUMC)
Review Group:	ZRG1 AARR-J (22) Center for Scientific Review Special Emphasis Panel Fellowships: AIDS Predoctoral and Postdoctoral
Meeting Date:	07/15/2011
Council:	OCT 2011
Requested Start:	NA
RFA/PA:	1 F31 AI098409-01
PCC:	X79A
Project Title:	Characterization of Antibody-Dependent Cellular Cytotoxicity in HIV Infection
SRG Action:	Impact/Priority Score: 10
Human Subjects:	30-Human subjects involved - Certified, no SRG concerns
Animal Subjects:	10-No live vertebrate animals involved for competing appl.

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RESUME AND SUMMARY OF DISCUSSION:

In this application, the fellowship candidate proposes to investigate antibody-dependent cellular cytotoxicity (ADCC) in HIV-infected patients do determine the extent of protection that ADCC provides. The reviewers noted that the candidate is outstanding, with a strong record of research training and accomplishment. The candidate appears to be highly motivated. Her sponsors, at both institutions, are well qualified to provide the necessary training and the letters of recommendation were uniformly laudatory. Minor weaknesses of the application included the ambitious research plan to be accomplished in two years, the risk of conducting the research at two sites, and the failure to address pitfalls and alternatives of the research project. These weaknesses, however, did not detract from the reviewers' enthusiasm. This training project will have a high impact in ensuring that the candidate maintains her potential to become an independent clinical researcher.

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DESCRIPTION (provided by applicant):

Understanding the basis of an immune response that controls infection or provides sterilizing immunity remains a major goal in the search for effective vaccines or immunotherapies for HIV. Antibodies (Abs) induced by candidate vaccines to the surface envelope glycoprotein have not neutralized a broad array of primary virus isolates. For this reason, eliciting a cytotoxic cellular response has been the primary goal in most recent vaccine trials. However, this approach has not been successful in containing viral replication in vaccinees that have become HIV-infected. Antibody-dependant cellular cytotoxicity (ADCC) has been shown to mediate sterilizing immunity against challenge with pathogenic simian immunodeficiency virus [Hessel 2007]. In ADCC, Fc-bearing Abs bind viral epitopes coating an infected CD4+ target T cell and an Fc receptor bearing effector, most commonly natural killer cells (NKs), bind the Ab and use perforin to deliver granzymes which induce apoptosis in the target. We want to study ADCC in infected patients to understand the magnitude and characteristics of the best responses achieved by natural infection. First, we will compare ADCC mediated by the sera of a cohort of patients using a granzyme B cytotoxicity assay developed in our lab. Based on these findings, we will select the sera of patients with the most ADCC, generate monoclonal Abs (mAbs), and characterize the mAbs based on epitope specificity, affinity, potency, breadth, IgG isotype, and Fc type. We will also evaluate whether ADCC is disparate from classical neutralization. Finally, we will use microscopy to examine the synapse between effectors, Abs, and targets. The outcome of this research will provide insight into the characteristics of Abs that mediate ADCC that are likely important goals in the design of HIV vaccines or immunotherapies. Hypothesis: Antibody-dependent cellular cytotoxicity (ADCC) is a function that has been shown to mediate protection from lentiviral infection. We hypothesize that variations in ADCC activity of sera are dictated by the amount, specificity, and subclass of HIV-specific antibodies. Aim 1: Characterize the potency of sera of HIV-infected individuals in ADCC. Aim 2: Characterize the specificity and breadth of antibodies with ADCC activity. Aim 3: Characterize the structure and function of the target-effector synapse using both fixed and live cell laser scanning confocal microscopy (LSCM), transmission electron microscopy (TEM) and cryo-electron microscopy (cryo-EM) and tomography.

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PUBLIC HEALTH RELEVANCE:

Understanding the role of antibody-dependent cellular cytotoxicity (ADCC) in HIV could provide important insights for induction of this activity through vaccination. This project seeks to characterize the Abs that mediate ADCC and image the functional synapse formed by cellular components involved in ADCC with the goal of defining new goals for the development of HIV vaccines and therapeutics.

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CRITIQUE 1:

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 1
Research Training Plan: 2
Training Potential: 1
Institutional Environment & Commitment to Training: 2

Overall Impact/Merit: This proposal is from an applicant who is currently enrolled in the NIH M.D./Ph.D. Partnership Training Program and is a participant in the NIH/Oxford/Cambridge Scholars Program, which

promotes joint research in an NIH laboratory and in a university research lab. Ultimately, she will receive her Ph.D. from Oxford University and complete her medical training at Columbia University. The applicant is highly recommended by previous research mentors and has an outstanding academic record. Her research experience, which includes four years of undergraduate research, is matched by her productivity; she is a contributing author on two published papers and has presented her work at numerous scientific venues. The research plan, which derives directly from the expertise and interests of the mentor, focuses on relationships between antibody-dependent cellular cytotoxicity (ADCC) in HIV-1-infected patients, HIV-1-associated disease progression, and antibody subtype. Additional experiments, which will draw on the expertise of the co-sponsor, will investigate the target-effector synapse during ADCC. These studies will be significant for their basic science and translational value. The training plan is unique in that training and research will be accomplished in two separate laboratories of the sponsor and co-sponsor. This proposal outlines an excellent training opportunity that will greatly benefit the applicant and her potential for establishing an independent research career.

1. Fellowship Applicant:

Strengths

- The applicant, who is currently enrolled in an M.D./Ph.D. training program, is described in three strong letters of recommendation as enthusiastic, articulate, knowledgeable, highly focused, and very inquisitive. Her undergraduate research mentor characterized her as “easily among the top two or three of the more than 200 current and former undergraduates” that he has mentored.
- The applicant has four years of undergraduate research experience, which involved work with the Moloney Murine Leukemia Virus, and research performed during a summer program at the Howard Hughes Medical Institute focused on studies of telomerase.
- Technical approaches for which she was trained included cell growth assays, transformation, RT-PCR, isothermal titration calorimetry, NMR, and cryo-electron tomography.
- Through her prior research experience, the applicant has been included as a contributing author on two published papers and has presented eight posters and one oral presentation at various local, national, and international meetings.
- The applicant has an outstanding academic record (3.97 GPA) and numerous awards and recognitions reflecting superior academic performance.
- The collaborative project outlined in the proposal was an outgrowth of a dialog initiated by the applicant between the sponsor and co-sponsor.
- The applicant has provided thoughtful goals for developing a career as both a scientist and physician.

Weaknesses

- No weaknesses were noted.

2. Sponsors, Collaborators, and Consultants:

Strengths

- The applicant’s mentor and sponsor, who is currently Chief of the HIV-Specific Immunity Section in the Laboratory of Immunoregulation, NIAID/NIH, is an established and productive senior investigator with research support provided by the NIH.
- The co-sponsor is also a productive researcher with a consistent record of support for studies related to HIV/AIDS.
- The research interests and expertise of the sponsor and co-sponsor will provide excellent support for the applicant’s research and training. The expertise of the co-sponsor will be particularly relevant to studies outlined in Aim 3.

- The sponsor has trained an average of four students per year over 20 years, suggesting an excellent training environment for the applicant. Similarly, the co-sponsor has trained nine Ph.D. trainees since 2003.
- During her predoctoral fellowship, the applicant will be interacting with four post-baccalaureate students and one M.D. in the sponsor's lab, and four Ph.D. trainees in the co-sponsor's laboratory. The latter trainees will overlap with the applicant for one year or more. These interactions will enrich the applicant's training.

Weaknesses

- No weaknesses were noted.

3. Research Training Plan:

Strengths

- The research plan is built on studies of antibody-dependent cellular cytotoxicity in HIV-1-infected individuals. Experiments will examine the potency of sera of HIV-1-infected individuals in ADCC (Aim 1), the specificity and breadth of antibodies with ADCC activity (Aim 2), and the structure/function of the target-effector synapse that mediates ADCC.
- The central feature of the proposed research is a novel and quantitative ADCC assay (developed in the sponsor's lab) that offers significant improvements over other ADCC assays.
- Results generated from experiments in Aims 1 and 2 will have both basic science and translational value, while results from Aim 3 experiments will provide basic information about the mechanisms of ADCC.
- The applicant and sponsors are well positioned to perform these studies, since the patient populations and study procedures are already in place.
- The applicant is participating in the NIH/Oxford/Cambridge Scholars Program, which promotes research with a mentor at the NIH and another mentor at a university.
- Consistent with the Scholars Program, the research training plan includes studies and training in year 1 in the sponsor's lab at the NIH, continued studies and training in year 2 in the co-sponsor's lab at Oxford, and years 3 and 4 in medical school.
- Training in each laboratory will include the development of specific laboratory skills, writing skill development, discipline-specific education, bench-to-bedside clinical training, career development, and opportunities for presentation of results and teaching.
- Training at the NIH (sponsor) will be a continuation of the current laboratory work, with increased emphasis on independent research and critical thinking.
- Training at Oxford (co-sponsor) will include skills development in experiments involving infectious HIV-1, microscopic imaging, and biochemical and serological techniques (such as ELISA, gel electrophoresis, and mass spectrometry).

Weaknesses

- Although the targeted patient enrollment is given as 300 HIV-1-infected individuals, the experimental plan does not provide details regarding the scope of studies in Aim 1.
- Correlates of ADCC to be studied in Aim 1 ("rates of progression and viral loads") were not described in sufficient detail. How will rates of progression be measured? Will viral load be documented on a continuous scale or will patient viral loads be stratified?
- An explanation of how the results of Aim 1 experiments will be used to guide the selection of pooled patient sera in Aim 2 would have been informative.

4. Training Potential:

Strengths

- A research project spanning the laboratories of two different principal investigators will offer diversity in training and acquired expertise, and enhance the capability of the applicant to develop an independent and productive research career.

Weaknesses

- No weaknesses were noted.

5. Institutional Environment & Commitment to Training:

Strengths

- The resources and facilities at both institutions will provide outstanding environments for training and research.

Weaknesses

- A more detailed plan regarding the coordination of training plans between the sponsor and co-sponsor would be valuable, considering the distance and time difference between the two performance sites.

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- The description of RCR training is brief and does not provide a detailed plan for formal training. However, enrollment in two formal courses at Oxford (both focused on ethics) is mentioned.

Comments on Subject Matter (Required):

- Training courses and certifications at the NIH and Oxford should provide information on relevant subjects.

Comments on Faculty Participation (Required):

- Faculty participation could not be evaluated from the description of RCR training.

Comments on Duration (Required):

- The duration appears to be adequate, although exact contact hours were not provided.

Comments on Frequency (Required):

- The frequency of training could not be discerned from the description of RCR training.

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CRITIQUE 2:

Fellowship Applicant: 2
Sponsors, Collaborators, and Consultants: 1
Research Training Plan: 2
Training Potential: 2
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit: This is an outstanding proposal from a highly qualified applicant. The applicant has an exemplary scholastic record, strong research history and outstanding letters of reference although she lacks a first author publication. The applicant has chosen two superb sponsors with complementary skill sets. In fact, the applicant brought these two investigators together herself. Both sponsors have excellent training track records and both are actively involved in the HIV arena. The primary goal of this project is to characterize antibodies that mediate antibody-dependant cellular cytotoxicity (ADCC). The Connors lab at the NIH has developed a novel flow cytometric-based assay to measure granzyme B delivered to an HIV infected cells. Using this novel assay the applicant plans to compare ADCC in HIV+ subjects with various rates of disease progression and to define the specificity and breadth of antibodies with ADCC activity (Aim 1 and 2). The third Aim to characterize the structure and function of the target-effector synapse will be carried out at Oxford under the guidance of Dr. Sattentau. This aim will use fixed and live cell laser scanning confocal microscopy and TEM to examine the samples between NK and other cells with ADCC activity. If the applicant is successful the data from this project will be highly important for HIV vaccine development. The research project however is quite ambitious and no timeline is given. It appears that the applicant plan to spend 1 more year in the Dr. Connors lab and 1 year in Dr. Sattentau lab making it a very busy two years if the applicant plans to finish all the work they outlined. Despite the highly ambitious nature of the proposed research project the overall project goals warrant further study and the applicant herself is of the highest caliber making this application worthy of funding.

1. Fellowship Applicant :

Strengths

- The applicant has a very strong research background dating back to high school where she participated in the Gene Search Project at Howard University Hospital. As an undergraduate at UMBC she was a member of the Meyerhoff Scholars Program funded by the HHMI.
- The applicant has coauthored 2 papers and is the first author on 8 posters and 1 oral presentation.
- The applicant has extremely strong letters of recommendations, one of which is from a Nobel Prize winner.
- Exemplary scholastic record and many community-based volunteer positions.

Weaknesses

- Lack of a first author papers.

2. Sponsors, Collaborators, and Consultants:

Strengths

- Two suburb sponsors are listed. Dr. Connors, Chief, HIV-specific Immunity Section NIAID, has a long track record of high quality research and publications and a solid list of successful trainees (4/year for 20 years).
- The other sponsor, Dr. Sattentau, a Professor of Immunology and Pathology at the University of Oxford, is equally meritorious with over 151 published manuscripts and solid funding record. Dr. Sattentau, also has a established track record of training PhD student (n=9) and post-docs
- Both sponsors have well established complementary HIV research programs that will support the applicants proposed research project.

Weaknesses

- none

3. Research Training Plan:

Strengths

- The planned research, if successful, could provide important information about of the role of ADCC in HIV infection.
- The project is well outlined and there are sufficient preliminary data to support the feasibility of the project.
- The plan is to utilize a novel recently developed (Connors lab) quantitative flow cytometric assay to measure GrB cytotoxicity and determine which Abs are capable of mediating the highest level of ADCC. Using this technique the applicant plans to characterize the potency of sera from subjects with different rates of disease progression, characterize the specificity and breadth of antibodies with ADCC activity.
- Using advance microcopy techniques (fixed and live cell laser scanning confocal microcopy and electron microscopy) the applicant plans to examine the structure and function of the target-effector synapse. This work will be done under the guidance of Dr. Sattentau who is an expert in studying the virologic synapses.

Weaknesses

- This is a highly ambitious grant. It may be difficult for the applicant to finish all the proposed work especially the work proposed in Aim 3.
- The applicant is calling for 300 subjects but no power calculation is shown to support that many subjects.
- A timeline for the proposed work would be helpful.
- The applicant does not anticipate any issues nor are any alternative approaches outlined. This maybe a little naïve.

4. Training Potential:

Strengths

- The research training plan is well laid out by year. The plan includes research, writing skill (grant writing course), presentation, career development, teaching (both in class room training of new personnel) and discipline specific learning (i.e. in house courses and seminar series, lab meetings). Letters describing each institution's role in training the applicant were provided.
- Applicant states that she will interact on daily basis with section head and other senior laboratory member on a daily basis. Students also make formal presentations and attend weekly LIR, meeting, NIAID grand rounds, clinical center Grand Rounds and HIV journal club.

Weaknesses

- The training plan indicates that the applicant will be at the NIH for one year and Oxford for one year and then back to medical school for the last 2 years. This raises some concerns regarding the duration of the funding (3 vs. 2 years).

5. Institutional Environment & Commitment to Training:

Strengths

- The work will be conducted at the NIH/NIAID and University of Oxford. Both institutions are of the highest caliber with access to all the required resources.
- Oxford has a program in place for graduate students to take accelerated courses and the NIH Foundation for Advanced Education in the Sciences also has specialized courses that will facility the applicants goal to become an infectious disease investigator.

Weaknesses

- No concerns

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- On line ethics course at the NIH and formal classroom course at Oxford.

Comments on Subject Matter (Required):

- Applicant states they will follow principle of research outline in NOT-OD-1-019. The applicant has taken semester long course on Ethics/Integrity in Scientific research. The applicant took another Ethic course upon entering the NIH. Oxford offers a similar course.

Comments on Faculty Participation (Required):

- No comment on faculty participation.

Comments on Duration (Required):

- Formal classroom course at Oxford.

Comments on Frequency (Required):

- Online course and annual refresher at the NIH.

Additional Comments to Applicant (Optional):

- Nicely written application.

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CRITIQUE 3:

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 1
Research Training Plan: 2
Training Potential: 1
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit: This proposal comes from an excellent candidate who is part of the NIH/Oxford/Cambridge Scholars program, carrying out her PhD research under the guidance of two excellent researchers and mentors, Drs. Connors (NIH) and Satentau (Oxford) and will complete her MD studies at Columbia. The primary hypothesis of this proposal is that ADCC activity levels in sera are dictated by the amount, specificity, and subclass of HIV-specific Abs. This represents a highly relevant area

of research that will be tackled through 3 straightforward and well-planned aims – (i) characterize the potency of sera from individuals at different stages of HIV infection in ADCC (comparing to clinical status and Ab neutralization), (ii) characterize the specificity and breadth of Abs mediating ADCC (comparing the sera to panels of known neutralizing Abs), and (iii) characterize the ADCC synapse. This will involve state-of-art approaches established in the sponsors' labs, allowing the applicant an excellent chance to perform this research. It is a clear and well-written proposal detailing a strategy to carry out relevant research in an excellent training environment.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

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