

**1. Purpose of the study-** This is a cross-sectional study that analyzes the sera of subjects in order to answer two clinical questions. First, we will assay the antibody profiles of subjects with autoimmune diseases to determine if there is cross-reactivity with the HIV epitopes 2G12, 1b12, 2F5 and 4E10, in addition to analyzing B and T cells to assess patterns that contribute to autoimmunity and autoantibody reactivity. The second goal of the study is to assess the prevalence of HIV infection in a cross-sectional cohort of subjects with autoimmune disease.

**2. Background & significance** – After twenty years of study, the HIV epidemic continues to rage world-wide. Now over 40 million people are infected and an estimated 100 million will be infected by the year 2010 (1). A safe and effective HIV vaccine is desperately needed. HIV vaccines have proven extremely difficult to develop. One of the most urgent problems to solve is to induce antibodies that broadly neutralize HIV of all subtypes. To date, 4 human monoclonal antibodies that broadly neutralize HIV, termed 2G12, 1b12, 2F5 and 4E10, have been made from HIV infected subjects (2-4). Anti-2G12 targets a carbohydrate determinate on HIV envelope protein gp120. Anti-1b12 binds to an epitope on the CD4 binding site, and anti-2F5 and anti-4E10 recognize membrane-proximal epitopes of the envelope protein gp41 near the surface of the virion.

A conundrum has developed with the observation that while multiple envelope immunogens express these epitopes on the surface of the envelope trimer, after injection into animals or man, the epitopes are not immunogenic. Moreover, most patients who are infected with HIV do not routinely make antibodies to these targets. Thus a major effort is to learn how to induce broadly reactive neutralizing antibodies against HIV by immunizing with these epitopes.

Recently we have turned our studies to examine abnormalities in the host that may prevent a robust humoral immune response to these conserved HIV epitopes. We have speculated that a critical host factor could be that the 2G12, 1b12, 2F5 and 4E10 epitopes are also self-antigens. Consequently, if host B cells generate antibodies against these HIV epitopes, they are immediately deleted because the antibodies are autoreactive. Supporting evidence for this hypothesis from our group has shown that antibodies raised against 1b12, 2F5 and 4E10 have potent cross-reactivity for autoantigens including double stranded DNA, Ro, phospholipids, centromere B, topoisomerase and histones.

The association between autoimmune disease and infection with human immunodeficiency virus (HIV) has evolved over the past two decades. Initial observations described spontaneous improvement in conditions including systemic lupus erythematosus (SLE) in patients that developed AIDS as a consequence of HIV infection. Since the advent of highly-effective anti-retroviral therapy, new-onset autoimmune diseases or relapse of previous autoimmune diagnoses have been observed after recovery of CD4+ T cell counts. Although it is consistent with the known pathophysiology of autoimmune disease that a reduction in immune function, whether by therapeutic intervention or retroviral infection, should decrease disease activity, little has been reported on the overall prevalence of HIV infection in patients with autoimmunity. In fact, relatively few cases of systemic lupus erythematosus, the prototypical systemic autoimmune syndrome, have been described in the literature. Moreover, there are no reports of HIV prevalence among cohorts of subjects with defined autoimmune disease. More recently, the sense has grown that subjects with autoimmune disease experience less HIV burden than would be expected of the general population.

## **IRB Protocol Summary –Protocol # 6804-06-2R1**

### **Title: Study of Cross Reactivity of Autoimmune Disease Autoantibodies with HIV Envelope Neutralizing Antibodies**

These clinical and laboratory observations have led to an exciting postulate that perhaps patients with autoimmune disease are protected from HIV infection due to their intrinsic loss of self-tolerance. Whatever the defect in central and/or peripheral tolerance that leads to the emergence of autoimmune disease may also permit these patients to generate neutralizing (auto-)antibodies against the HIV epitopes described above. To investigate this proposition, we plan to assess for the prevalence of HIV infection in the cohort of autoimmune disease-afflicted patients cared for in the Duke Rheumatology Clinic (particularly, the Duke Lupus Clinic and Duke Scleroderma Research Center). In addition, we will examine the reactivity of sera from subjects with autoimmune diseases including SLE, systemic sclerosis, Antiphospholipid antibody syndrome, and Sjogren's syndrome. Serum reactivity will be analyzed for a broad panel of autoantigens (centromere, topoisomerase, extractable nuclear antigens (Smith, ribonucleoprotein, Ro, and La), double-stranded DNA, cardiolipin (phospholipids), histones) and the HIV epitopes (2G12, 1b12, 2F5 and 4E10). In addition, all subjects will be tested for HIV infection with HIV PCR. These results will be compared with those obtained from a healthy control group (n=100). As part of the protocol, separated B cells will be sent to Dr. James Robinson of Tulane University for manufacture of hybridomas. This technique will generate B cells that make antibodies targeting various HIV envelope antigens. Dr. Robinson will use the hybridomas to sequence interesting B cell DNA for analysis of immunoglobulin gene structure. In addition, anonymized samples of serum will be sent to Dr. George Shaw at the University of Alabama, Birmingham for performing HIV-1 neutralization assays.

### **3. Design & procedures –**

**I. Research Activities** – Subjects eligible for enrollment will be approached about this study in the context of their ordinary clinical care. Records reviewed by study personnel are only those of patients seen for autoimmune disease states by Drs. Joseph Shanahan, Dr. William St. Clair, Dr. Thomas Ortel or their designated clinical associates after obtaining a waiver from the IRB. No medical records or portions thereof will be copied, and data obtained and recorded from review of patient records to determine if the subjects meet study criteria will be abstracted for the study files. Data reviewed will be age, sex, diagnosis and duration of disease process, blood test results, and treatment regimen/medications. If subjects decline to participate when informed about the study, this information will be destroyed. If Subjects that provide informed consent will undergo a single phlebotomy of 120 cc. If they have not had a recent CBC (i.e. within 4-6 weeks), participation (phlebotomy) will be delayed until after measurement of a hematocrit that meets the entry criteria. It is possible that repeated phlebotomy, not to exceed removal of 400 cc over an 8-week period, may be performed if the initial specimen does not yield sufficient B cells for hybridoma generation, or if serum reactivity cannot be adequately assessed. Sera from each subject will be assayed for reactivity against a panel of autoantigens (see list above) and tested for HIV in viral load assays by PCR. Sera will also be assayed for cross reactivity of autoantibodies to HIV gp120 and gp41 proteins and the HIV epitopes 2G12, 1b12, 2F5 and 4E10. Immunocytes will be studied for multiple T and B cell subsets including T regulatory cell number and function, T and B cell subset number and function, and ability to bind to peptides reflective of gp140 neutralizing epitopes. PBL and B cells will be sent to Dr. Robinson at Tulane for production of B cell hybridomas making monoclonal antibodies that react with HIV gp140 or peptides or protein fragments of the HIV envelope. B cell DNA will be sequenced for analysis of B cell immunoglobulin gene structure.

We plan to enroll a total of 200 subjects with autoimmune disease including 125 subjects meeting the 1982 American College of Rheumatology (ACR) criteria for diagnosis of

## **IRB Protocol Summary –Protocol # 6804-06-2R1**

### **Title: Study of Cross Reactivity of Autoimmune Disease Autoantibodies with HIV Envelope Neutralizing Antibodies**

SLE (Arthritis and Rheumatism 25:1271-1277, 1982), 25 subjects meeting ACR criteria for the diagnosis of scleroderma, 25 subjects meeting ACR criteria for the diagnosis of Sjogren's syndrome, and 25 subjects meeting the Sapporo criteria for diagnosis of primary antiphospholipid antibody syndrome. Inclusion criteria include the following: 1) Ability to provide informed consent, 2) Autoimmune diagnosis meeting aforementioned criteria, 3) hematocrit >33%, 4) Age ≥18 years. Exclusion criteria will include patients/subjects with ongoing bleeding or hemorrhage. In addition, 100 healthy control subjects who do not have known autoimmune disease will be enrolled. The inclusion/exclusion criteria are unchanged for this group, with the exception of the requirement for an autoimmune disease diagnosis. We will not review medical records for controls, but will ask potential subjects to fill out a brief questionnaire (see attached) to certify that they do not have autoimmune diagnoses.

**II. Standard Care** – There will be no change in standard care (The usual and customary diagnostic and therapeutic intervention and treatment and counseling normally provided in accordance with the patient's health and disease status as determined appropriate and prudent by the attending physician) for these subjects as a result of participation in this study. The HIV testing is not routinely performed for management of autoimmune disease and as such falls under the research activity heading. The subjects will be counseled prior to consenting regarding HIV testing by Dr. Shanahan or other PIs on this study. Subjects will be asked to return to the clinic to obtain the results of the HIV testing. At this clinic visit, subjects whose HIV tests are found to be positive will be referred for immediate HIV counseling in the Duke Infectious Diseases clinic. Positive tests will also be reported to the NC Department of Public Health as required by state law. A complete blood count is performed routinely at the majority of visits for patients with autoimmune disease. Therefore, most subjects will have had a hematocrit drawn prior to enrollment. Patients with autoimmune disease are seen on an average of every 3-6 months, depending on disease status. If they have not had a recent CBC (i.e. within 4 -6 weeks), participation (phlebotomy) will be delayed until after measurement of a hematocrit that meets the entry criteria. Subjects who wish to participate in the control group will have to have a CBC drawn to determine whether their hematocrit meets inclusion criteria.

**4. Risk/benefit assessment** – The only physical risks associated with this study are those associated with phlebotomy. By excluding subjects with ongoing bleeding problems, and subjects with hematocrit less than 33%, we will minimize these risks. In addition there is a risk of anxiety associated with having an HIV test. Counseling will be provided initially by a member of the study team and if needed by referral to a counselor in the Duke Infectious Diseases Clinic. There is a confidentiality risk associated with this study since all subjects will be tested for HIV and because B cell hybridomas will be created to analyze immunoglobulin DNA. To minimize the risk of disclosing protected health information, all specimens sent to Dr. Robinson for hybridoma generation and DNA analysis and to Dr. George Shaw for HIV-1 neutralization assays will be made anonymous with a unique identifying number. Data linking the unique ID numbers with identifying information on the subject will be kept separately in a locked cabinet in Dr. Barton Haynes' research office.

**5. Subject identification, recruitment, and compensation** – Potential subjects will be identified from the Division of Rheumatology and Immunology's Duke Lupus Clinic and Duke Scleroderma Research Center, Dr. St. Clair's Sjogren's Syndrome Database, and Dr. Ortel's Antiphospholipid Syndrome Database (APSCORE) in order to ensure that subjects meet diagnostic and inclusion/exclusion criteria and have expressed relevant autoantibodies prior to being approached for enrollment in this study. Once identified, subjects will be approached in

## **IRB Protocol Summary –Protocol # 6804-06-2R1**

### **Title: Study of Cross Reactivity of Autoimmune Disease Autoantibodies with HIV Envelope Neutralizing Antibodies**

the course of an ordinary clinic visit to assess their interest in participation. Subjects who provide informed consent will undergo phlebotomy as described above. Healthy controls will be recruited from Duke Health System and Duke Clinics, with the exception of persons under direct supervision of the investigators. In addition, healthy controls that are being recruited to participate in another study with Dr. Haynes as PI, entitled Study of Lymphocyte Function (445-05-4R18ER- approved 4-28-05), will also be asked if they wish to participate in this study. Subjects will be compensated for their participation. Subjects will receive \$24.00 if they are eligible to participate and agree to phlebotomy. If subjects must return for further phlebotomy (if there were inadequate cells to create hybridomas, for example), they will receive an additional \$24.00 plus travel reimbursement at a rate of \$0.40.5/mile traveled. Some potential subjects may undergo phlebotomy and be found to be ineligible for the study due to a low hematocrit. These subjects will receive travel reimbursement at the rate described above, unless they are seen in the context of a routine clinic visit in which case they will not receive any compensation.

For those subjects who agree to participate in the study and are determined to be eligible, permission will be requested in the informed consent for the principal investigator to review his or her medical record for the purpose of recording specific information from their medical history that is relevant to this study.

**6. Subject competency** – Subjects not competent to provide informed consent will be excluded from this study.

**7. Costs to the subject** – There will be no costs to the subject for participating in this study.

**8. Data analysis & monitoring** – This is an exploratory study designed to establish whether subjects with autoimmune diseases make antibodies that cross-react with critical epitopes on the HIV virion envelope. We will make multiple comparisons between the autoimmune group and control group for frequency of cross-reactivity with various autoantigens and HIV epitopes. This will require chi-squared tests for proportions, t test for analyzing continuous variables such as strength of reactivity, and regression analysis to identify associations between reactivity to HIV and to specific autoantigens. We are also assessing the prevalence of HIV infection in an unselected cohort of subjects with established autoimmune disease. The difference in the proportion of subjects with HIV in the autoimmune group compared to the control group will be compared by a chi-squared test.

The following laboratory and/or clinical evaluations will be conducted for this study:

1. CBC, white count, and differential
2. HIV testing
3. Drawing blood for isolation of white blood cells, serum and plasma.
4. Immune cell functional assays in vitro.
5. Antibody reactivity to HIV, HIV antigens and self antigens.
6. Immune cell phenotype analysis.
7. Genetic analysis for genes that might predispose to making broadly neutralizing antibodies.

Untoward or adverse events experienced by study participants will be recorded immediately along with the response to the event and reported to the Duke University Health System

## **IRB Protocol Summary –Protocol # 6804-06-2R1**

### **Title: Study of Cross Reactivity of Autoimmune Disease Autoantibodies with HIV Envelope Neutralizing Antibodies**

Institutional Review Board for review. This report will also be made part of the subject's confidential study record.

**9. Data storage & confidentiality** – As noted above, data gathered on subjects will be stored in association with a unique subject identification number. The documentation linking the identifying data on the subject with the unique ID number will be kept separately in a locked cabinet in Dr. Haynes' research office. Identifying information will not be shared with Dr. Robinson or any other collaborators. Since we will be collecting sensitive data to identify eligible subjects during the recruitment process, we will apply for a waiver. Study files will be kept locked in the office of the principal investigator, and keys to coded samples will be kept separate from the study files also in a secured location in the office of the principal investigator. Only the key personnel listed below will have access to information obtained and stored as part of this study. No patient identifying information will be sent to Dr. James Robinson at Tulane or to Dr. George Shaw at the University of Alabama at Birmingham. Subjects will be asked if they are willing to have any cells remaining at the end of this study to be stored in the HVI Tissue Repository (IRB# 5946-0507R0ER). If they are willing to do this, they will be asked to sign an additional consent form allowing their cells to be stored in the HVI Tissue Repository.

Barton F. Haynes, MD  
Thomas Ortel, MD  
Elizabeth Petzold, PhD  
Richard Searce  
Joyce Lowery  
Michael Mugavero, MD

Joseph Shanahan, MD  
William St. Clair, MD  
M. Anthony Moody, MD  
Kelly Plonk  
Gregory Sempowski, PhD

Since we will be checking HIV status, each subject will be required to complete a separate informed consent to do HIV test and to record test results. We will use the standard Duke Form M3142 (08/01), a copy of which is attached. All subjects will be notified of their results as described above. If a subject is found to be infected with HIV, they will be asked to return to the clinic and the test results given to them by their physician. In addition they will be offered the opportunity to obtain counseling at Duke with Dr. Shanahan or in the Infectious Disease clinic (which will be arranged by Dr. Shanahan). Subjects with negative results will be notified by mail. Results of the HIV testing and screening CBC (if necessary) will become part of the permanent Duke Medical Record. In the consent form, subjects will be given the opportunity to direct communication of these results to the patient's primary care physician. This may be important particularly if the PCP is not a Duke physician.

#### Those authorized to terminate this study:

Dr. Barton F. Haynes, Dr. Joseph Shanahan, and/or the chair or representative member of the Duke University Health System Institutional Review Board are authorized to terminate this study at any point it is deemed necessary.

1. WHO-UNAIDS Statistics, 2004.
2. Ofek, G. et al. Structure and Mechanistic Analysis of the Anti-HIV Antibody 2F5 in complex with its gp41 epitope. *J. Virol.* 78: 10724-10737, 2004

**IRB Protocol Summary –Protocol # 6804-06-2R1**

**Title: Study of Cross Reactivity of Autoimmune Disease Autoantibodies with HIV Envelope Neutralizing Antibodies**

3. Calarese, D.A. et al. Antibody Domain Exchange is an immunological solution to carbohydrate cluster recognition. Science 300: 2065-2071, 2003.
4. Kunert, R, et al. Characterization of molecular features, antigen binding, and in vitro properties of IgG and IgM variants of 4E10, an anti-HIV type 1 neutralizing monoclonal antibody. AIDS Research and Human Retroviruses 20: 755-762, 2004.
5. Tan, E.M. et al. The 1982 Revised Criteria fro the Classification of Systemic Lupus Erythematosus. Arthritis and Rheumatism 25:1271-1277, 1982.