**9/14/2010**

**Statement on Teaching and Research**

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Dr. Abreu described a new variant of endemic pemphigus foliaceus (EPF) (El Bagre-EPF) that resembles pemphigus erythematosus, also known as Senear-Usher syndrome, that seem to be an overlap syndrome with features of lupus erythematosus (LE) and pemphigus foliaceus. However, further testing reveals that this disease possesses autoantibodies to multiple cell junctions between cells especially against the heart and the blood vessels, Several patients affected by El Bagre-EPF have experienced a sudden death syndrome, including persons below age fifty. El Bagre-EPF patients share several autoantigens with patients with paraneoplastic pemphigus (PNP), such as reactivity to plakins. Further, PNP patients have autoantibodies to the heart. With the cooperation of the Chair of cardiology of the University of Illinois at Chicago Dr. Samuel C. Dudley Jr. MD, PhD, we recently tested several El Bagre-EPF patients, and controls from the endemic area for autoreactivity to heart using direct and indirect immunofluorescence, by immunohistochemistry, by confocal microscopy and by immunoblotting, utilizing heart extracts as antigens. We found that 7 of 15 El Bagre-EPF patients had a polyclonal immune response to cell junctions of the heart, often colocalizing with known markers. These colocalizing markers included those of the area composita of the heart, such as anti-desmoplakins I and II; markers for gap junctions, such as connexin 43; markers for tight junctions, such as ezrin and junctional adhesion molecule A and to adherens junctions such as pan-cadherin. We also detected colocalization of the patients' antibodies with the blood vessels and with the cardiac sarcomere. The strongest patient serum autoreactivity was observed against the transverse tubule system of the heart. Reactivity to some nerves and the purkinge fibers was also noted. We conclude that El Bagre-EPF patients display autoreactivity to multiple cardiac epitopes, and, further, that the cardiac pathophysiology of this disorder warrants further evaluation.

Pemphigus is demonstrated by acantholysis and immunoglobulin deposits in the interkeratinocyte substance. The lupus component of pemphigus erythematosus is demonstrated by circulating antinuclear antibodies (ANA) and sometimes by immunoglobulin and complement deposits at the dermoepidermal junction. EPF is the only known endemic autoimmune disease occurring in some geographic rural regions of S.A, in Central America and in Africa. The occurrence of EPF has been associated with massive destruction of the rain forest and enormous and abrupt colonization. The occurrence of El Bagre-EPF has also been associated with massive mining pollution of mercuric selenides and other trace elements. The uniqueness of this autoimmune model made it a powerful tool for studying the natural course and more relevant aspect of autoimmune diseases. The geographic restriction makes EPF an invaluable natural-model for studying how the environment, genetic background, and other aspects such as life-style are capable to induce, maintain, modify, or even cure this disease.

In Brazil there are several focus of EPF where the disease is called fogo selvagem (FS). In Colombia, we have confirmed the endemic nature of El Bagre-EPF. We have performed preliminary studies in characterizing it, and in comparing it with other forms of EPF including FS. We had described a 10-year prospective, controlled epidemiologic, humanitarian, and

immunologic fieldwork case-control survey which was performed in El Bagre. Our work revealed that this disease is endemic in rural areas surrounding El Bagre. The disease appeared in 4.7% of middle-aged and older men and postmenopausal women from these rural areas. This disease differs from previously described forms of EPF. It shares some heterogeneous immunoreactivity with paraneoplastic pemphigus but seems not to be associated with malignant tumors. The disease resembles Senear-Usher syndrome (pemphigus and lupus) but occurs endemically either with a localized stable clinical course or in a possible systemic form. This systemic form may affect organs other than skin and is characterized by episodic relapses and poor prognosis in comparison with the localized form.

Some of the initial steps, to obtain more knowledge about the disease were to characterize some autoantigen profiles for several sera samples obtained from patients with this condition using case controlled molecular epidemiological studies. We used different methods, such us immunofluorescence (both direct and indirect) (DIF, IIF), various immunoblotting (IB) analyses with different antigen sources and detection methods, such as radioactivity, ECL, and immunoprecipitation (IP). By using IIF or DIF with the use of human skin sections as antigen detected IgG autoantibodies against keratinocyte cell surfaces, fibrinogen and other molecules and possible unknown molecules possibly related either to cell junction or cell-matrix junctions. The results of IB and IP analysis indicated that all sera had antibodies reactive with desmoglein 1 (Dsg1). In addition, in various IB assays, many sera reacted with several other proteins with molecular weights of 250 kd, 210 kd, and 190 kd, which later we demonstrated to correspond to desmoplakin I and II, envoplakin, and periplakin, respectively for these assays we used recombinant proteins of various Plakin domains. Molecules of the plakin family ubiquitously detected in other organs such as the heart, gastrointestinal tissue, muscle, kidney among other and preliminary studies pointed to possible affection of other internal organs and not only the skin.

In addition, we solved the identity of one of the most important antigenic moiety, a 45 kDa tryptic desmoglein 1 ectodomain, which is recognized by all sera from all the patients with active disease. We then embraced in the next project to develop a cost-effective ELISA assay capable of detecting the heterogeneous antibody population observed in these El Bagre-EPF patients to be used for epidemiological studies. We succeeded using the protein extract obtained from trypsin-digested fresh bovine cow snouts and further purified on a Con A matrix was used as an antigen. The overall sensitivity and specificity of the assay were determined to be 95% and 72%, respectively, with reproducibility of 98% (intra-assay) and 95% (inter-assay), allowing testing of multiple serum samples and correlated well with the clinical activity and extent of disease in patients with El Bagre EPF.

What needs to be done?**:** A great number of unknown antigens need to be characterized in this disease as well as the possible systemic compromise. Within this aim, we will need extensive using of mass spectrometry to discover the nature of these multiple autoantigens. In addition, we expect to uncover the mechanism that correlates the autoantigenic profiles, the immune response, (including *in situ*, and systemic inflammatory mediators), using proteomics, lipidomics, and comparing with the clinical outcome (including possible predictors factors, course, prognostic, complications and response to treatment). Further needed are studies that will ideally include mRNA and micro RNA expression profiling from archival tissue specimens.

Other techniques will ideally complement our studies including, immunohistochemistry, development and validation of molecular assays tissue microarrays, application of molecular techniques to tissue pathology, cytogenetic and FISH analyses. We also believe that people affected by El Bagre-EPF may have a common genetic ancestor. We will extend our cooperation with the Department of Genetics to pursue PCR amplification of genomic DNA and mutation detection. Other experiments will include a signal transduction via plakins, (including *in situ*, and systemic inflammatory mediators), with the clinical outcome (including possible predictive factors, course, prognostic, complications and response to treatment. We are currently cooperating with the expertise of some faculties at different universities. For studying this disease we need a group of optic nerve and a group that used angiogenesis models to investigate fundamental cellular and molecular mechanisms and pathological ocular alterations and the effect of the El Bagre-EPF autoantibodies as putative therapy for the angiogenesis. We had detected also autoantibodies to the arachnoid villi in the optic nerve which main components are desmoplakin 1 and 2 (DP 1, DP II) and the optic nerve. Incidentally we see some structures in the eyelid and palms displaying immunoreactivity to some nerves and mechanoreceptors including Pacinnian corpuscles. We also have found that the extracellular matrix seems to play an important role in this disease.

Our more recent discoveries and research focus is based on the fact that El Bagre-EPF shares several autoantigens with patients with paraneoplastic pemphigus (PNP), such as reactivity to plakins in the heart. Some known antigens for this disease include desmogleins (e.g., 1 and 3), plakins, and bullous pemphigoid antigens. Notably, patients chronically affected by EPF, including El Bagre-EPF, often present with some degree of palmoplantar keratoderma. Recently mutations in desmosomal proteins leading to inherited desmosomal cardiocutaneous syndromes have been described. Based on the genetic clustering of El Bagre-EPF, the palmoplantar compromise of their patients and on the fact that several patients affected by El Bagre-EPF have experienced a sudden death syndrome (including persons below age fifty) prompted us to test for heart autoreactivity in El Bagre-EPF serum. A pilot study, demonstrated that approximately one third of the El Bagre-EPF patients have autoantibodies to several cell junctions in the heart, its vessels and its nerves, including possibly the heart Purkinje fibers. Based on the fact that the Purkinje fibers contain several desmoplakins, we hypothesize that these autoantibodies recognize biologically important molecules in the conducting system of the heart, on the vessels and in the myocyte cell surfaces. To test this hypothesis, our specific aims are: (1) To identify selected antigens on endothelial, neural and/or in cardiomyocites that are recognized by autoantibodies found in the sera of patients with El Bagre-EPF. (2) To determine whether these antibodies to the identified antigens induce cardiovascular problems including in their conducting system using cell cultures an animal passive model. To accomplish these aims we will use direct and indirect immunofluorescence, immunoblotting, immunohistochemistry, affinity chromatography, immunoprecipitation, confocal microscopy, ELISA, cDNA libraries, frozen tissues from normal organs, multiplex antigen microarrays, and image analysis, and immune electron microscopy, surface-enhanced laser dissorption and ionization time-of-flight (SELDI-TOF) mass spectrometry. The heart samples will be compared with patient's skin samples using a Global proteomic. Our work aims to (1) profile new autoantibodies and antigens in this new variant of EPF, and to (2) establish a basis for studying mechanisms involved in the cardiovascular system in this disease vis-a-vis the role of autoantibodies to endothelium, nerves, and cardiac and cardiomyocytes. Some of our preliminary data indicated that some of the

possible antigens for El Bagre-EPF are members of the family of the armadillo protein family closely related to p120 and the plakophillins.

We believe this disease model will allow an excellent cooperation between different faculties and departments, as well as to be source for training for graduate as well as post-graduated studies. I wish to join this active multi-disciplinary group to work in research, publication, teaching and academic advancement.

**Teaching Statement**

I would love to have the ability to work with faculty and students from multiple disciplines; encourage grantsmanship; provide support to interdisciplinary programs and centers; develop existing degree programs at both the undergraduate and graduate levels; attract and retain top-tier faculty; manage the continued growth, success, and reputation of the Department; and advance the Department's and University's commitment to research in the biological sciences, teaching excellence, and community-based scholarship and outreach.

My personal definition of a great teacher is one who demonstrates passion for his/her subject and uses innovative approaches to provide the learner opportunities to apply, synthesize, and evaluate course products and processes.

- A class climate that encompasses care, compassion and creativity while also challenging learners to be critical thinkers;
- A teacher who exudes energy and enthusiasm, engaging the learners as active participants; and
- One who is organized and optimizes opportunities for learning success.
- I am aware that good teaching comes from years of trial and error;
- I am aware of the difficulties that students have at all levels in their lives, and I will be there to help each specific circumstance, as many of my professors gave me that example. I also will try to rebuild values and ethics, very evasive principles prevailing in the current society.
- In addition to faculty input, and discussions with peers, my students' responses are the best source for improving my teaching techniques which are evolving on a continuous basis.
- I will promote excellence in undergraduate and graduate arenas.

Teaching is about making some kind of impact in the world so that the world is different than it was before you practiced your craft. Knowing clearly what kind of impact you want to make in the world means that you must continually ask yourself the most fundamental evaluative questions of all–What effect am I having on students and on their learning?"

A teacher should be totally involved with the class, dedicated to his/her students and be prepared to devote time and energy for them. Love for teaching evokes passion and dedication. The enthusiasm of a motivated teacher rubs off on his/her students, who derive the inspiration and encouragement which actuate their desire to learn. This keeps the students interested and they

tend to retain the course material very well. Every module should clearly state a take-home message for the students. I know through experience that whenever my past students approach me for help for another course, I am pleased to realize during the discussions that they have retained most of the important concepts. In addition, I believe a good teacher needs to personalize the needs and problems of the students. This is observed in case of a few of the weaker, shy or some international students who need additional help but hesitate to ask for it. In my opinion, identifying the students by their full names and knowing some background information (like state, city, and country) is very beneficial. I obtain most of this information subtly through my numerous interactions with them during my office hours. Having good sense of humor is an added advantage. I believe that the best in a person comes out in a non-stressful situation. Students tend to learn more effectively from an approachable teacher who sets up a comfortable atmosphere conducive to learning. Thus, the education goes beyond the classroom and students tend to visualize the teacher as a role model from whom they seek universal advice on topics ranging from fundamental concepts to future career options, other personal problems and recommendations.

Sincerely,

Ana Maria Abreu Velez MD, PhD