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Abstract

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Grant Number:	5K08AR001981-04
PI Name:	JAMES, JUDITH A.
PI Email:	jamesj@omrf.ouhsc.edu
PI Title:	ASSOCIATE PROFESSOR
Project Title:	GENETIC ANALYSIS OF LUPUS AUTOIMMUNITY

Abstract: Description The applicant, and her mentor, John Harley, M.D., Ph.D., have recently demonstrated that normal rabbits immunized with specific short peptides derived from the Sm autoantigen develop high levels of both anti-Sm and anti-nRNP antibodies; the peptides used as immunogens were selected since they are also bound by human lupus autoantibodies. The peptide-immunized rabbits, in addition to anti-Sm and anti-nRNP antibodies, also develop positive ANA, antibodies to DNA, and clinical features reminiscent of human lupus including proteinuria, red cell casts, renal insufficiency, hypoalbuminemia, thrombocytopenia, alopecia, and seizures. The antibodies that develop to Sm and nRNP are apparently of high titer in that they precipitate in double immunodiffusion assays and bind RNP antigens in solution phase and by immunoblots. In essence, by immunizing rabbits with peptides, Dr. James and Dr. Harley have induced a syndrome in rabbits that resembles human lupus. In more recent, preliminary studies that form the foundation of the present proposal, Dr. James has shown that certain strains of inbred mice also respond to Sm peptide immunization with epitope spreading and genesis of autoantibodies to other parts of the Sm and nRNP autoantigens, as well as positive ANA and antibodies to DNA. In comparison, certain other strains respond to peptide immunization with antibodies against the immunogenetic peptide, but do not develop epitope spreading and other humoral features of lupus. Dr. James now proposes in the current project to identify the genes involved in this peptide-induced lupus model, using the technique of recombinant inbred (RI) mice strains. Human homologies to murine genes will then be sought. Five specific aims are proposed. First, progenitor strains of recombinant inbred mice which do and do not develop peptide induced autoimmunity will be ascertained. The essence of this aim is to carefully identify strains of mice which do and do not develop lupus autoimmunity after immunization, with lupus autoimmunity being defined as spreading of the humoral immune response beyond the peptide of immunization to other regions of the Sm and nRNP autoantigens, as well as the development of positive ANA and anti-DNA antibodies as determined by Crithidiae immunofluorescence. These experiments are already underway with eleven different RI progenitor strains. Preliminary results from these studies suggest that both responder and non-responder strains have been identified. Second, recombinant inbred substrains, produced from a cross between a progenitor responder and non-responder, will be analyzed

for evidence of peptide-induced lupus. In these experiments, the RI set derived from the progenitor strains selected in Aim 1 will be immunized with an Sm (spliceosomal) peptide and analyzed along with control mice. Animals will be assessed for development of lupus autoimmunity as defined in Aim 1. In the third specific aim, the murine locus (or loci) associated with peptide-induced lupus will be defined. In these experiments, RI sets will be analyzed for linkage of known markers of the affected progenitor strain that has evidence of peptide-induced lupus autoimmunity and/or clinical symptoms. These analyses will be accomplished using a computer program, MapManager V.2.5. Linkage will be determined by Chi square analysis using confidence intervals of $p<0.01$. Fourth, the identity of any loci found in specific aim four will be confirmed by classical genetic techniques and potential candidate genes examined. In these studies, confirmation of linkage will be obtained either using congenic lines (if one gene is involved in the production of peptide-induced autoimmunity) or via traditional back-cross analyses using the original progenitor strains of the RI set (if multiple loci are involved). In the latter case, gene mapping will be done using conventional markers as well as dispersed polymorphic DNA markers available from the mouse genome project. Finally, in aim 5, human genes homologous to those related to peptide induced autoimmunity in mice will be sought. This aim depends upon the mapping of loci involved in peptide-induced lupus autoimmunity and establishment of candidate genes, as planned in specific aims 3 and 4. If such genes are found, then the comparative human-mouse map in which 80% of the mouse genome is accounted for, will be used to establish probable location of human homologous genes. This specific aim will depend upon a large genetic linkage study in human SLE currently being performed by Dr. Harley, and which already contains 30 pedigrees with a second cohort of an additional 30 pedigrees planned.

Thesaurus Terms:
autoantibody, autoimmunity, gene expression, genetic mapping, humoral immunity, immunogenetics, systemic lupus erythematosus
clinical research, enzyme linked immunosorbent assay, gel electrophoresis, human subject, immunodiffusion, immunofluorescence technique, immunoprecipitation, laboratory mouse, polymerase chain reaction, western blotting

Institution:	OKLAHOMA MEDICAL RESEARCH FOUNDATION 825 NE 13TH ST, MS 31 OKLAHOMA CITY, OK 73104
Fiscal Year:	1999
Department:	
Project Start:	30-SEP-1996
Project End:	31-AUG-2001
ICD:	NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES
IRG:	AMS

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Grant Number:	1R01AI047575-01
PI Name:	JAMES, JUDITH A.
PI Email:	jamesj@omrf.ouhsc.edu
PI Title:	ASSOCIATE PROFESSOR
Project Title:	ROLE OF ANTIPROTEINASE 3 IN WEGENER'S VASCULOPATHY

Abstract: Wegener's granulomatosis (WG) is a multisystem disease of unknown etiology which is characterized by small to medium vessel vasculitis, pauci-immune glomerulonephritis, necrosis/granuloma formation of the respiratory tracts and autoantibodies to neutrophilic components. Although the underlying pathophysiology of this disorder remains an enigma, several lines of evidence strongly support c-ANCA, particularly anti-PR3, has a role in disease pathogenesis. Unfortunately, the specific mechanisms that initiate and perpetuate this anti-PR3 response remain to be elucidated. One approach to delineate a potential etiology for these autoantibodies, and/or to understand their role in the pathogenesis of vasculitis would be to fully characterize the antigenic determinants of the PR3 autoantigen. By defining the common autoantigenic targets of PR3 we could arrive at molecular mimicry triggers for this autoimmune response. An animal model of WG autoimmunity could show to what tissues particular epitopes are targeted. Over the past decade our lab has conducted extensive work on the immunochemistry of lupus autoantigens (1-7). These previous studies provide the technical background for this proposal. Epitope mapping experiments of the spliceosomal autoantigens have led to our peptide induced model of lupus autoimmunity (8,9). We will now apply these well-honed techniques, as well as a similar scientific strategy, to analyze the humoral fine specificity of the WG reponse to PR3. Early work in the lab of Ralph Williams suggests that sequential eptiopes are common targets of PR3. Exciting new results from our co-PI's lab uncover a potential mechanism of anti-PR3 vascular damage. He has observed that PR3 can bind to endothelial protein C receptor (EPCR), a regulatory protein in the protein C anticoagulant pathway and that this binding is inhibited by c-ANCA. He has also observed that EPCR can inhibit tight neutrophil to activated endothelium and the subsequent spreading/activation. These observations lead to the hypothesis that EPCR plays a role in regulating leukocyte adhesion and activation, in part through interactions with PR3, and that antibodies to PR3 disrupt this physiological regulation contributing to the vascular damage in WG. This RFA response seeks to integrate the strengths of two labs to build on these early observations and to identify the common humoral epitopes of PR3, to track the development of the humoral autoimmune response of WG patients to PR3 over time, to develop an animal model of vasculitis, to evaluate EPCRs influence on the c-ANCA-PR3 interaction, and to identify the

common T cell targets in WG.

Thesaurus Terms:
Wegener's granulomatosis, antigen antibody reaction, autoantigen, autoimmunity, disease /disorder etiology, endopeptidase, enzyme inhibitor, humoral immunity, pathologic process T cell receptor, T lymphocyte, disease model, histocompatibility typing, leukocyte adhesion molecule, model design /development, protein C
clinical research, enzyme linked immunosorbent assay, epitope mapping, human genetic material tag, human subject, laboratory mouse, laboratory rabbit, polymerase chain reaction, serology /serodiagnosis, transgenic animal

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Fiscal Year:	1999
Department:	
Project Start:	30-SEP-1999
Project End:	31-AUG-2004
ICD:	NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
IRG:	ZAR1

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Grant Number: 5R01AR045451-02
PI Name: JAMES, JUDITH A.
PI Email: jamesj@omrf.ouhsc.edu
PI Title: ASSOCIATE PROFESSOR
Project Title: GENETIC ANALYSIS OF EPITOPE SPREADING

Abstract: Systemic lupus erythematosus (SLE) is a serious autoimmune disease of which the etiology and mechanisms of pathogenesis are incompletely understood. It is clear that there is an important genetic component to lupus. High titers of autoantibodies, which may include anti-Sm and anti-RNP are characteristic of lupus. Recent work shows that the natural history of these autoimmune responses is to increase in complexity by involving additional structures of the autoantigen in the autoimmune response. This process is termed epitope spreading detected in this proposal as added antigenic spine specificity through time. A similar phenomenon in T cell epitopes is very important in other models of disease, such as experimental autoimmune encephalitis. The applicants suspect that this process is also very important in lupus pathogenesis. Recently a new model of lupus autoimmunity was discovered by this group induced by immunization with a short sequence from Sm B/B'. This new model of induced SLE presents opportunities to explore the genes involved in B cell epitope spreading as well as the autoimmunity of lupus. Work here with the AKXL recombinant inbred set of mouse strains has preliminarily established linkage on chromosome 4 at B cell marker 72. They propose to apply the impressive tools of mouse genetics to identify the genomic region and perhaps the specific genes associated with anti-Sm B cell epitope spreading. They will subsequently explore the syntenic regions and homologous genes in human lupus. The goals of this proposal are to analyze genetic contributions to epitope spreading and recombinant inbred strains of mice and to confirm the findings by classical genetic approaches. It will seek to confirm and narrow the region of chromosome 4 by classical back cross experiments. Simultaneously the investigators will evaluate the candidate gene Cd72 for its potential role in the observed linkage. If Cd72 does not explain linkage in this recombinant inbred set, then confirmation of this region, and a search for different candidate genes will then pursue. They will then seek linkage in other recombinant inbred strains of mice and work to identify the responsible genes. Finally, linked regions and identified genes will be tested for their potential contribution to human lupus.

Thesaurus Terms:
epitope mapping, immunogenetics, linkage mapping, systemic lupus erythematosus

autoantigen, disease /disorder etiology, lymphocyte proliferation, pathologic process
clinical research, human subject, laboratory mouse

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Fiscal Year:	1999
Department:	
Project Start:	30-SEP-1998
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ICD:	NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES
IRG:	GMA

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Grant Number: 5R03AR045084-03
PI Name: JAMES, JUDITH A.
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PI Title: ASSOCIATE PROFESSOR
Project Title: EARLY SPLICEOSOMAL PEPTIDE EPITOPES IN LUPUS

Abstract: DESCRIPTION (from the application): Autoantibodies are universally found in patients suffering from systemic lupus erythematosus. The presence of the antibodies has led investigators to conclude that SLE is an autoimmune disease. Indeed, lupus in man is probably caused by autoantibodies. In some clinical settings powerful evidence supports the conclusion that specific autoantibodies induce tissue injury and are responsible for clinical manifestations. Anti-Sm and anti-nRNP autoantibodies are commonly found at extraordinary concentrations in the sera of lupus patients. When these autoantibodies are found concomitantly they are associated with renal disease and a poor disease prognosis. Anti-nRNP autoantibodies in the absense of Sm are associated with a more limited form of lupus. Antibodies against Sm are so specific for SLE that they are considered a diagnostic criterion. This proposal sets forth to explain the development of autoimmunity in SLE. Through our previous work studying the fine specificity of autoantibodies binding to the spliceosome, we have identified over 90 peptide epitopes, 40 of which tend to be shared among patients. Using this methodology we have found that the fine specificity progresses from a small number to as many as 86 different antigenic regions from an individual patient serum. In preliminary studies, patients with anti-Sm antibodies appear to initially bind the structure defined by PPPGMRPP. This response evolves by epitope spreading to other structures of the antigen. Immunization with this peptide has led to a novel model of lupus complete with spliceosomal autoimmunity, anti-double stranded DNA antibodies, renal disease, thrombocytopenia, and seizures. Immunization with the closely related PPPGRRP sequence, which is found in a virus and which is bound by some of the autoantibodies that bind Sm, also induces anti-spliceosomal lupus autoimmunity. We suspect that there are a number of different initial epitopes that are bound by autoantibodies. We request the resources to identify the initial target epitopes of anti-Sm and anti-nRNP autoantibodies in SLE sera. We will find peptides from the environment (and, especially, from microorganisms) which are similar to the initial target epitopes and will determine if the initial target peptide epitopes from the spliceosome (and their structurally similar peptides from the environment) are cross reacting antigens. We will determine whether the peptides from the spliceosome or environment induce lupus autoimmunity after peptide immunization. This project is directly relevant to the goals of RFA: AR-97-001 and has the

potential to reveal important, previously unappreciated, mechanisms of pathogenesis and to contribute toward establishing the etiology of SLE.

Thesaurus Terms:
autoantibody, peptide structure, spliceosome, systemic lupus erythematosus
antinuclear autoantibody, autoimmunity, disease /disorder etiology, disease model, humoral immunity, immunoglobulin structure, microorganism antigen
epitope mapping, human tissue, laboratory rabbit, passive immunization, serum

Institution:	OKLAHOMA MEDICAL RESEARCH FOUNDATION 825 NE 13TH ST, MS 31 OKLAHOMA CITY, OK 73104
Fiscal Year:	1999
Department:	
Project Start:	30-SEP-1997
Project End:	31-AUG-2001
ICD:	NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES
IRG:	ZAR1