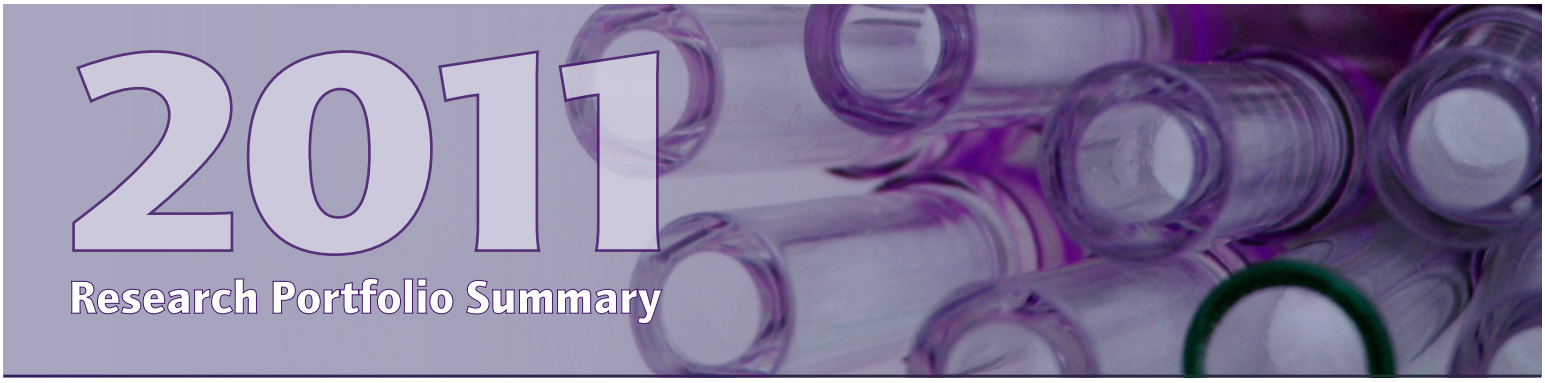
Research Portfolio Summary

2011

Alliance *for* Lupus Research

PREVENT. TREAT. CURE.



Target Identification in Lupus Grantees

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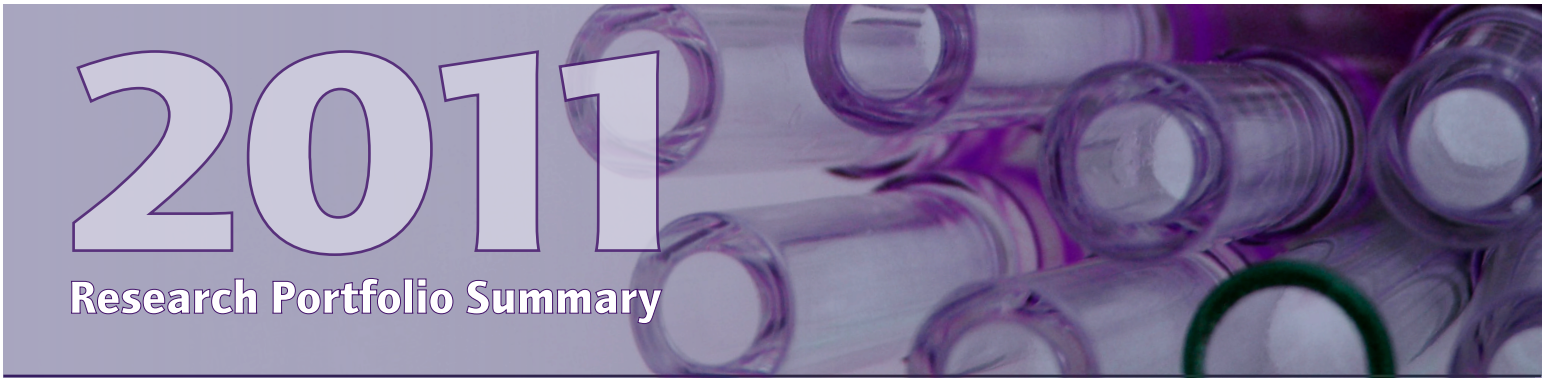

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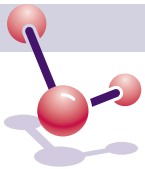


Target Identification in Lupus Grantees

Under our Target Identification in Lupus (TIL) grant program, investigators leverage a two-year, up-to-$400,000 award to remove the barriers to new treatments and a possible cure. All research funded under the TIL program is based on realizable goals for translation into therapeutic discovery programs — that is, research that can move quickly from the laboratory to the patient's bedside.

Betsy Barnes, PhD
UMDNJ-New Jersey Medical School
Targeting IRF5 activation for the treatment of lupus

While we still don't understand all the underlying causes of lupus, we do know that it tends to run in families, suggesting a strong genetic component. Indeed, researchers have already identified several genes associated with the disease. One such gene encodes for the transcription factor interferon regulatory factor 5 (*IRF5*), which helps control the expression of several inflammatory molecules that contribute to the disease.

Dr. Barnes and her team found that people with lupus have significantly higher levels of IRF5 expressed in primary immune cells than healthy people, and that this higher expression is associated with known changes in the *IRF5* gene that are linked to lupus risk. In addition, recent data suggests that IRF5 is continually activated in the blood cells of people with lupus but not in those without the disease.

With their ALR grant, the researchers plan to modify the activation status of IRF5 in the immune cells of lupus patients, thus altering the ability of IRF5 to trigger inflammatory responses that likely contribute to the disease.

What this study means for people with lupus: Results from these studies will not only contribute to a greater understanding of the actions of IRF5, but could aid in the development of new treatments that target the protein to prevent lupus and its symptoms.

Joseph Craft, MD
Yale University
Follicular Helper T Cells in SLE: Characterization and Therapeutic Targets

Patients with lupus develop autoantibodies (also called antinuclear antibodies, or ANA) that cause tissue inflammation in organs such as the kidneys. These autoantibodies are made by B lymphocytes. In order to produce the autoantibodies, however, B cells require help from other lymphocytes called T cells. The ongoing T cell/B cell interaction in the spleen, lymph nodes, and tonsils are critical for autoantibody production in lupus.

With their ALR grant, Dr. Craft and his team will begin to dissect the signals that permit such interactions and identify characteristics of these abnormally activated T cells in people with lupus They also plan to see if potential treatments now in clinical trials affect T cells that help B cells make autoantibodies.

What this study means for people with lupus: The goal of this work is to identify existing and new therapeutic targets for people with lupus.

Syamal Datta, MBBS
Northwestern University
Peptide Vaccine Suppressing Autoantigen-Specific Response in Human Lupus

The cells and molecules, also called antibodies, of the immune system fight foreign organisms. Sometimes, however, these defenses can react against the body itself. Normally, this self-reactivity is kept in check by special regulatory T cells called Treg. In autoimmune diseases like lupus, however, such regulatory cells are lacking, leading to abnormal reactivity against cell components such as DNA and histone proteins that bind to DNA (nuclear autoantigens).

Dr. Datta and his team found that replacing diseased immune system cells with stem cells that can grow healthy immune system cells creates a new source of Treg cells to repair the deficiency. It also helps maintain patients in true immunological remission. The Treg cells generated after stem cell transplantation are potent in reducing the risk of relapse and other complications from the disease and the ongoing medical treatments. They are not found in patients who achieve apparent remission via conventional drug treatments.

However, stem cell therapy is a drastic measure with significant risks that should only be tried in those in whom nothing else works. To avoid such a risky procedure, Dr. Datta and his team have developed a natural, nontoxic vaccine therapy using certain peptides or small bits of proteins that generate similar Treg cells and cause immunologic remission of the disease in lupus-prone mice.

With this grant, Dr. Datta's group will use the peptides to induce similar, autoantigen-specific regulatory T cells in peripheral blood of lupus patients in cell cultures. They also plan to identify genes and protein expression profiles of the potent Treg cells to identify unique surface markers and define the mechanisms and molecular pathways involved in their generation, maintenance and regulatory activity in humans.

What this study means for people with lupus: The ability to compare genes and proteins expressed by Treg cells before and after a stem cell transplant offers a unique opportunity to understand these remission-inducing suppressor cells and address new issues critical for developing immune-regulating therapies for people with lupus. Results from this study would ultimately be able to be used to screen the best therapeutic peptide for maintaining lupus patients in true remission by inducing autoantigen-specific Treg cells, or by infusing Treg cells created in culture.

Michael Denny, PhD
Temple University
Abnormal Neutrophil Development in SLE

We know that several parts of the immune system are involved in lupus. Dr. Denny and his team focus on the development and alteration of immune cells called neutrophils. These white blood cells are the most prevalent of all immune system cells and are crucial for responding to bacteria and fungi.

Dr. Denny and his team have identified an abnormal pool of neutrophils in people with lupus. They also developed a way to very quickly isolate these cells from blood samples for study. With this grant, they will try to understand whether these abnormal neutrophils arise from an alteration in cell development. In particular, they will look at the genetic level of the cell to identify alterations in genes that are critical for the proper development and maturation of neutrophils. Identifying the underlying mechanism responsible for the abnormalities is the first step in controlling the neutrophils involvement in disease or, possibly, eliminating them all together.

What this study means for people with lupus: Identifying the genetic causes of these neutrophil alterations could open the door to new therapies.

Betty Diamond, MD
The Feinstein Institute for Medical Research
Dendritic cell dysfunction as a path to SLE

Dendritic cells trigger reactions in the immune system. Dr. Diamond and her team previously showed that deleting a specific gene in dendritic cells leads to the development of lupus in animal models. With this grant, they will explore exactly *how* these abnormal dendritic cells change the response of immune system cells and cause lupus. This is particularly important research because the gene they're examining has already been identified as one that increases risk of lupus.

What this study means for people with lupus: These studies will provide important new insights into how lupus develops and progresses, providing valuable information that could be used to develop new treatments or even identify ways to prevent the disease in high-risk individuals.

Robert Eisenberg, MD
University of Pennsylvania
Distal Ig Receptor Revision in the Production of SLE Autoantibodies

A central feature in the underlying process of systemic lupus erythematosus is the production of autoantibodies against DNA and other self molecules. This occurs because B lymphocytes, which normally only produce antibodies to foreign substances like flu viruses, are somehow induced to produce autoantibodies, which then attack self tissue. The production of these autoantibodies signifies the failure of the process of tolerance, in which B cells learn not to view self molecules as foreign. Several studies have suggested that abnormal changes in genes that code for the autoantibodies themselves may underlie the disease process. With this grant, Dr. Eisenberg and his team will explore the hypothesis that the loss of self tolerance in lupus is due to a rearrangement of antibody genes (receptor revision) that changes the specificity of the resultant antibody. If this genetic process occurs very late in the development of the B lymphocyte, then there is no opportunity for normal tolerance mechanisms to silence newly created autoreactive antibodies.

What this study means for people with lupus: If this receptor revision mechanism is a key player in the autoimmune response of lupus, then the pathways, receptors, and regulators essential to this mechanism would provide a number of potential therapeutic targets.

Keith Elkon, MD
University of Washington
Lysis of Immunostimulatory Nucleoproteins in SLE

A characteristic feature of lupus is the presence of autoantibodies directed against self antigens, which causes overactive immune response and inflammation. Over the last several decades, investigators have characterized the antigens in lupus, finding that most are nucleoproteins (nuclear or cytoplasmic proteins attached to the nucleic acids DNA or RNA) released from one's own dead and dying cells.

There is increasing evidence that nucleoproteins accumulate in patients with lupus because the cells responsible for clearing them away don't work properly. Then autoantibodies bind to the nucleoprotein antigens to form antigen/antibody or "immune" complexes, which cause tissue inflammation. When the nucleoprotein complexes enter cells, the nucleic acid activates special receptors called toll-like receptors (TLR), triggering the release of inflammatory proteins called cytokines.

One of these cytokines, interferon-alpha, has been strongly implicated in systemic lupus.

With this grant, Dr. Elkon and his team will use a mouse model to degrade the nucleic acid component of the immune complexes so they cannot be deposited in tissue or activate

TLRs. They will also investigate whether nuclease therapy is effective once the disease has already begun by administering nucleases to mice with active disease and monitoring their disease activity.

What this study means for people with lupus: This study provides information on a new avenue of exploration for compounds to treat lupus and its complications without suppressing the overall immune system.

Shu Man Fu, MD, PhD
University of Virginia
Progressions and Biomarkers of Proliferative Lupus Nephritis

The majority of people with lupus will eventually develop kidney problems as the disease attacks the kidneys and its blood supply. Called "proliferative lupus nephritis," the condition often leads to end-stage renal disease that requires either chronic dialysis or kidney transplantation, both of which have a significant impact on the quality of life.

Dr. Fu's laboratory has identified three stages of lupus nephritis, acute, transitional, and chronic glomerulonephritis (GN), in an animal model. They also found that the gene expression of affected kidney cells is different and distinct for each stage. With their ALR grant, they want to confirm their findings and use laser capture microdissection techniques and gene array analyses to biopsy kidney specimens from lupus patients with proliferative GN. Then they can determine if the genetic changes they saw in the animal model can be applied to humans. They also want to see if the resulting proteins from these genetic aberrations appear as potential biomarkers in the urine, where they could be used to more accurately and easily stage the disease.

What this study means for people with lupus: Lupus nephritis is not only difficult to treat, it is also difficult to diagnose and track, requiring a kidney biopsy to identify the stage of the disease. Finding biomarkers that could be used to track the progression of the disease would not only help with its management, but is important in designing clinical trials for new drug development.

Chaim Jacob, MD, PhD
University of Southern California
Targeted DNA Capture and Parallel Sequencing to Identify Causal Mutation

Numerous genetic mutations related to lupus have been identified. However, much work remains to better understand that specific genetic mutations that underlie the disease processes. Dr. Jacob and his team have been at the forefront of this genetic research. With this grant, they plan to identify variants in 9 genes related to lupus to better understand the specific pathways related to the disease.

What this study means for people with lupus: Discovering and characterizing genetic factors responsible for lupus is crucial in understanding the pathology of the disease and providing new novel targets for future therapies.

Caroline Jefferies, PhD
Royal College of Surgeons in Ireland
Ro52 and Siglec-E as therapeutic targets in SLE

Among the various immune system abnormalities that underlie lupus are proteins called interferons that immune system cells normally release during viral infection. However, in people with lupus, these interferons are released at other times, leading to abnormally high levels. They not only trigger inflammation, but are also involved in activating the immune system to produce autoantibodies that drive the pathology of this disease.

Thus, strategies that reduce interferon levels could also reduce lupus symptoms and slow or even halt the progression of the disease, possibly restoring the immune system to normal. Dr. Jefferies' lab focuses on identifying just such strategies. So far, they have found two molecules called Ro52 and Siglec-E, that stem the production of interferon.

With their ALR grant, they will see if activating these proteins can reduce lupus symptoms.

What this study means for people with lupus: This work could ultimately lead to the development of SLE treatments that target the Ro52 and Siglec-E molecules.

Mariana Kaplan, MD
University of Michigan
Lupus and the Inflammasome

People with lupus develop blood vessel damage that increases their risk of early heart attacks. This blood vessel damage is also thought to contribute to the severity of the kidney disease that occurs later in the disease. Dr. Kaplan's team has suggested that one mechanism leading to this accelerated blood vessel disease is related to an imbalance between cells that damage and those that repair the lining, or vasculature, of blood vessels. The researchers have previously reported that a molecule called interferon (IFN) alpha plays a crucial role in this process, promoting premature atherosclerosis and kidney damage progression.

They recently found that IFN-alpha suppresses a molecule called interleukin-1 (IL-1) beta and increases levels of another called IL-18. Both play a role in inflammatory processes. IFN-alpha also activates the machinery that processes these two cytokines, called the inflammasome.

With their grant, Dr. Kaplan and her team will use human and animal systems to better understand how IFN-alpha interacts with the inflammasome machinery, triggering blood vessel damage. They will focus on a specific inflammasome component, caspase-1, exploring how IFN-alpha alters its role to impair blood vessel function and repair.

They will also investigate how IFN-alpha reduces production of IL-1 beta and the impact this has on blood vessel function in lupus cells.

What this study means for people with lupus: Identifying the inflammasome as an important mechanism of organ damage and blood vessel abnormalities in SLE could lead to the development of new therapies to prevent the devastating complications of the disease.

Vicki Kelley, PhD
Brigham and Women's Hospital, Inc.
Colony Stimulating Factor 1: A Therapeutic: Target for Lupus Nephritis

Kidney failure is a major cause of disease and death in people with lupus. Tracking molecules that play a role in lupus-related kidney disease, or lupus nephritis, could help identify identifying therapeutic targets and biomarkers for this illness. This latter goal is particularly important because it is very difficult to detect lupus nephritis in the early stages of the disease, before kidney damage occurs. The "gold standard" for diagnosis and determining disease severity is an invasive kidney biopsy. However, such biopsies can't be repeated very often. So identifying non-invasive strategies for early detection, to determine disease severity, and to monitor therapeutic responsiveness, such as levels of certain molecules in the blood or urine, is critical for improving treatment.

Dr. Kelley and her team are targeting colony stimulating factor 1 (CSF-1) as a potential biomarker. These molecules stimulate the growth of white blood cells called macrophages, which are central to kidney disease. Studies in lupus-susceptible mice show that CSF-1 can promote lupus nephritis. In addition, the researchers have seen a dramatic rise in CSF-1 levels in the kidney, blood, and urine of patients with lupus nephritis compared to levels in healthy individuals. Thus, they hypothesize that CSF-1 is a potential therapeutic target and biomarker for human lupus nephritis.

They will use their grant to test this hypothesis in unique mutant lupus-susceptible mice. In one experiment, they will see if blocking the CSF-1 receptor can improve signs and symptoms of lupus nephritis in these mice; in another, they will see if levels of CSF-1 in the circulation and kidney contribute to the disease process of lupus nephritis. Finally, they will test the hypothesis that CSF-1 in the blood or urine could be used to identify and predict disease activity in people with lupus nephritis.

What this study means for people with lupus: Understanding the distinct roles of CSF-1 in the circulation and kidney is central to tailoring therapeutic approaches that target CSF-1 and treat lupus nephritis.

Kun Ping Lu, MD, PhD
Beth Israel Deaconess Medical Center
The Prolyl Isomerase PIN1: A Novel Therapeutic Target in SLE

Recent studies into the underlying mechanisms of lupus have shown that dysregulation of the immune response pathway TLR/IRAK1/IRF/IFN plays a major role in the disease. Errors in this pathway can lead to overproduction of certain hormones, particularly interferon alpha, a major culprit in lupus development. Thus, studies on how this pathway becomes dysregulated are important in identifying potential targets for new therapies.

Dr. Lu and his team previously discovered a new enzyme called PIN1 that controls protein function and plays a major role the development of numerous diseases, including cancer. Earlier work by Dr. Lu and his team suggest that PIN1 might play a major role in the development of lupus. In these studies, they found higher activity of PIN1 in peripheral blood cells of lupus patients. Importantly, Pin1 controlled the activation of the TLR/IRAK1/IRF/IFN pathway. When the researchers removed the PIN1 gene in mice and immune cells, they prevented overproduction of interferon alpha. These results led them to hypothesize that PIN1 may represent an attractive, more specific and safer novel drug target for lupus than those currently available.

With this grant, the researchers plan to test their hypothesis in three ways:

- Comparing changes related to PIN1 activity and expression in immune cells from healthy individuals and those with lupus to determine the potential role of the enzyme in human lupus
- Using animal and cell models to determine the role of PIN1 during the pathogenesis of lupus
- Delivering a highly potent and specific PIN1 peptide inhibitor specifically to certain immune cells to evaluate whether it prevents the development of lupus or suppresses lupus disease activity in cells and mouse models

What this means for people with lupus: These studies and the further development of targeted PIN1 inhibitors may represent an exciting new treatment strategy for lupus.

Timothy Niewold, MD
University of Chicago
Role of ILT Receptors in Human Lupus

Type I interferons are important immune system signaling molecules that normally function to defend the body against viruses. Increasing evidence suggests that overactivation of the type I interferon pathway increases the risk of developing lupus and is linked to increased disease activity in those who already have the disease.

The interferon 1 signaling pathway could be a prime target for lupus therapies. However, we don't know enough about how the molecules are regulated in humans. Thus, Dr. Niewold and his team will use their grant to study immune system molecules called immunoglobulin-like transcripts 3 and 7 (ILT3 and ILT7). These molecules exist on the surface of immune cells, acting to turn off inflammatory immune responses. They can also reduce the production of type I interferon, something Dr. Niewold's lab demonstrated in preliminary studies in human cells. This suggests that the ILT molecules could be useful as a target for lupus-related therapies.

The researchers plan to identify the molecule or molecules that normally activate type 1 interferon signaling via ILT3. They will also measure ILT molecules in the blood cells of people with lupus, and determine whether ILTs are related to disease activity, clinical disease features, and/or serum type I interferon levels. Finally, they will test the function of ILT molecules in human blood cells to determine their impact upon type I interferon signaling.

What this study means for people with lupus: Completing these studies will lead to improved understanding of the role of ILT receptors in lupus, essential knowledge for the development of therapies that target the interferon pathway.

Andras Perl, MD, PhD
SUNY Upstate Medical University
Mitochondrial Hyperpolarization in Lupus T Cells

Dr. Perl and his group have discovered a series of molecular defects in the mitochondria of T lymphocytes that may account for the dysfunctional actions these cells exhibit in people with lupus. Mitochondria are small cellular factories that produce fuel in the form of ATP. In lupus patients, mitochondrial defects lead to diminished production of ATP, compromising survival and appropriate functioning of cells. These cellular abnormalities also prevent the programmed destruction of self-aggressive T lymphocytes. Instead, the researchers found that this mitochondrial dysfunction causes abnormal cell death, called necrosis.

This type of cell death stimulates other cell types, initiating a relentless inflammatory response. They also discovered that nitric oxide plays a key role in the mitochondrial dysfunction of these lupus T cells. In fact, after exposure to nitric oxide, T cells of healthy donors temporarily behave like those from lupus patients.

Dr. Perl plans to use the ALR grant to precisely identify how nitric oxide works in the T cells of lupus patients and to identify other contributing factors to the mitochondrial dysfunction.

What this study means for people with lupus: Better understanding the mechanism of nitric oxide in the cells of lupus patients may provide new targets for treatments to correct malfunctioning mitochondria.

Shiv Pillai, MBBS, PhD
Massachusetts General Hospital
Targeting the SIAE pathway in lupus

We know that the risk of developing lupus is likely related to several rare genetic variants, or abnormalities. Of particular interest are rare genetic changes that affect the function of the protein the gene encodes for, rather than more "common" variants identified in the large, genome-wide association studies that have already been conducted.

Dr. Pillai and his team are employing a way to identify all such "rare" genetic variants by sequencing the gene's coding segments, or exons. The exons are like letters that form words; while the words together form the story, or, in the case of genetics, the protein the gene is responsible for making. This approach is called "complete exome sequencing," and Dr. Pillai and his team have already used it to sequence genes in people with lupus. In addition, they performed a battery of tests on each participant to examine immune cell function.

Now they will use their ALR grant to put the two together—the clinical features of the disease, including immune function, with the genetic variants. The goal is to obtain a comprehensive understanding of the genetic basis of lupus.

What this study means for people with lupus: Identifying specific defective genetic pathways can help identify new targets for novel therapies.

David Pisetsky, PhD, MD
Duke University Medical Center
Nucleic Acid Binding Polymers in the Treatment for SLE

A key underlying abnormality in the immune system in lupus is the production of antinuclear antibodies (ANA) that bind to the proteins or nucleic acids (DNA and RNA) from the cell nucleus. In fact, high ANA levels are used as a marker for diagnosis and prognosis. In lupus, these autoantibodies can form immune complexes with molecules such as DNA that are released from the cell nucleus, usually as the cell dies. These complexes may then stimulate inflammation as well as deposit in the kidney, causing damage.

The immune complexes that form in lupus have unusual properties since the bound molecules (DNA and RNA) can themselves have immunological activity. When in the form of complexes, these nucleic acids can trigger immune system abnormalities that promote autoimmunity. This activity results from the ability of DNA and RNA to stimulate nucleic acid receptors or sensors that are on the inside of the cell. Normally, DNA and RNA in the blood may not be able to bind to these receptors; but when they are available as immune complexes, DNA and RNA can get into the inside of the cell and access these receptors to promote immune disturbances.

Current therapies for lupus are based on non-specific immunosuppressive agents that, while effective, have limited benefits for many patients and significant side effects. To develop more targeted therapies, researchers are looking at blocking the stimulation of the internal nucleic acid receptors, including toll-like receptors (TLR) and non-TLR systems. Compounds that block TLR 7 and 9, which respond to RNA and DNA respectively, have demonstrated benefits in mice models; since the compounds don't block non-TLR sensors, however, they may have limited efficacy. Also, compounds that inhibit the TLRs may affect the immune response to viruses and other infections.

Dr. Pisetsky and his team are exploring an alternative strategy to block responses to nucleic acids by investigating the use of nucleic acid binding polymers (NABPs). These polymers can bind tightly to DNA and RNA and prevent the formation of immune complexes and the resulting stimulation of internal receptors, The hope is that the polymers will be able to block the ability of extracellular nucleic acids to stimulate immune system activity that can underlie autoimmunity.

Their plan is to:

- Define the activity of a series of NABPs, assessing their ability to block stimulation of various immune cells
- Assess the activity of the NAPBs in mice stimulated with DNA to determine the appropriate dosing for clinical studies
- Determine the ability of NABPs to block autoimmune disease in mouse models of lupus

What this study means for people with lupus: These experiments provide the first step in moving NABPs into clinical trials in patients to develop a new strategy to treat lupus by blocking immune responses induced by complexes.

Ian Rifkin, MD, PhD
Boston Medical Center
Adiponectin and Adiponectin Receptors in Lupus and Lupus Atherosclerosis

Two major complications related to lupus are lupus nephritis (kidney disease) and atherosclerosis (a thickening of the blood vessel wall that leads to heart attacks and strokes). Both are caused, at least in part, by excessive inflammation.

In earlier research in animal models, Dr. Rifkin and his team found that two diabetes drugs, rosiglitazone and pioglitazone, reduced inflammation and the incidence of lupus nephritis and atherosclerosis. They also found that the drugs appear to work by triggering production of a protein called adiponectin. Adiponectin itself is an anti-inflammatory molecule that is involved in regulating metabolism.

With this grant, the researchers will explore the underlying mechanism for adiponectin's anti-inflammatory effects. Specifically, they will see whether cellular receptors that adiponectin binds to, called AdipoR1 and AdipoR2, and which trigger many of the metabolic effects of adiponectin, also play a role in preventing inflammation.

What this study means for people with lupus: These studies may help identify adiponectin receptors as novel targets for new anti-inflammatory therapies that could prevent or treat lupus nephritis and atherosclerosis, either together with rosiglitazone and pioglitazone or separately.

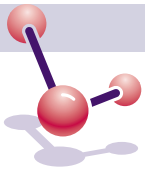
Shruti Sharma, PhD
University of Massachusetts Medical School
Innate Sensing of AT-rich DNA During Autoimmunity

Numerous immune system components contribute to disease development and progression in lupus. These include toll-like receptors (TLR), particularly TLR7 and TLR9. These proteins recognize RNA or DNA from dying cells and trigger inflammation in lupus patients by activating immune cells such as dendritic and B cells. Dendritic cells produce inflammatory molecules called type I interferons while B cells generate autoantibodies that eventually cause organ damage in the later stages of the disease.

With their ALR grant, Dr. Sharma and her colleague, Dr. Fitzgerald hope to discover what triggers autoimmune diseases like lupus and rheumatoid arthritis (RA). They suspect that the DNA released from dying cells contains clues to this event. Specifically, it appears that the location of the DNA may affect the disease differently than we initially thought. For instance, in autoimmune diseases like RA, abnormal DNA accumulates *within* cells and triggers pathways independently of TLR activation. Similarly, in lupus it appears that a different pathway operating separately from TLR9 activation may be responsible for the initial production of interferons, exacerbating subsequent symptoms and organ damage. This pathway may be related to the "nature" of the accumulated DNA. In other words, the location of the DNA within the cell and the enrichment of certain parts of the DNA could provide a clue as to its role in the disease.

The researchers also plan to use complex genetic models to investigate specific proteins involved in detecting this buildup of DNA.

What this study means for people with lupus: This research can help identify important and novel targets and pathways in the development and progression of lupus that could lead to new therapeutic targets.



Functional Genomics and Molecular Pathways in SLE

This grant mechanism was designed to help researchers move forward from the knowledge gained from the findings of the ALR-funded SLE Genetics Consortium (SLEGEN). Selected investigators receive Research Grants of up to $350,000 for two years or Pilot Grants of up to $75,000 for one year. Grantees will focus on determining how the genes identified by SLEGEN may have a role in the disease, and provide further information about the molecular pathways modulated by these genes. Ultimately, the hope is that data from these investigations will lay the groundwork for a way to "turn off" the disease at the genetic level.

Marta Alarcon-Riquelme, MD, PhD
Oklahoma Medical Research Foundation
Dissecting a Novel Molecular Genetic B-Cell Pathway in Lupus

Dr. Alarcon-Riquelme and her team have identified several lupus susceptibility genes, genes that make people more likely to develop lupus. However, they do not know how these genes lead to the development of the disease.

With this grant, the researchers will focus on two genes, BANK1 and BLK. Their own data suggests these genes may play a role in the toll-like receptor (TLR) pathway, a critical signaling pathway with which cells communicate with each other. Previous research found that this pathway is very important in the development of lupus, triggering the production of autoantibodies and highly inflammatory substances such as alpha-interferon that underlie the disease process. Up to 70 percent of all people with lupus produce too much alpha-interferon.

The researchers want to better understand the role of these two genes in the TLR9 pathway. As part of their investigation, they will work with human cell lines that do not express these genes. This will enable them to see if the lack of genetic expression changes the ability of cells in the TLR9 pathway to respond and produce inflammatory chemicals.

They will also use an animal model that does not express the genes so they can test the effects of certain stimuli on the animals' immune systems. The researchers will also see if deleting one or both genes leads to the development of autoantibodies or kidney inflammation, and how that development occurs.

What this study means for people with lupus: The results of this research will increase our knowledge of the rule of susceptibility genes in the development of lupus, enabling the construction of models and systems for testing potential therapeutic substances that could affect the underlying cause of the disease, not just its symptoms.

John Atkinson, MD
Washington University School of Medicine
Complement Mutations in End Stage Renal Disease Lupus Patients

With this grant, Dr. Atkinson and two colleagues (Drs. Jane Salmon and Robert Kimberly) are combining forces to determine how genetic variations in complement system genes contribute to kidney disease in people with lupus.

The complement cascade is an important part of the immune system. It helps the host to clear away pathogens like viruses and bacteria as well as damaged tissues and cells. Deficiencies in the complement system lead to lupus and contribute especially to kidney disease.

The researchers will identify variants or mutations in genes of the complement system to see how they contribute to the disease process in lupus.

They will focus on genetic variants that may be involved in lupus-related end-stage renal disease (ESRD) in which the kidneys fail and individuals require dialysis or a kidney transplant. To do this, they will sequence genes in people with lupus-related ESRD; in those with lupus but who do not have kidney disease; and in a group of normal controls. They have two primary goals:

- Establish that mutations in the complement system increase the risk of ESRD in people with lupus
- Determine the frequency of the genetic variants in people with lupus compared to controls

What this study means for people with lupus: Identifying genetic variants that contribute to kidney damage in people with lupus will increase our knowledge of how lupus comes about and suggest alternative means to treat the disease.

Robert Clancy, PhD
New York University School of Medicine
ITGAM R77H: Genotype/Phenotype Relationships in Dendritic Cells

Genetic discoveries have great potential to help us understand the factors that contribute to tissue injury in systemic lupus erythematous. In this study, Dr. Clancy and his team plan to build on new understandings of a variation in a gene that codes for a protein called ITGAM. This protein is found on white blood cells. Its job is to remove tagged substances, typically pathogens or dead cells or cell fragments, from circulation. It also provides a signal that prevents immune cells from activating against self tissue, a process called tolerance. Lack of tolerance is a major cause of autoimmune diseases like lupus.

What we don't know is whether variations in the gene that encodes for ITGAM results in changes in the protein and its function. That's what this study is designed to investigate.

Researchers will test the hypothesis that a genetic variation causes the protein to function poorly, contributing to the development of autoimmunity. To test this prediction, researchers will obtain DNA from people with and without lupus and test for the gene variation. They will then evaluate the ability of the protein produced by the genetic variation to increase or reduce tolerance. If successful, this study could be an important demonstration of a structural difference between common and variant forms of ITGAM with a consequence to specific functional properties of the immune system.

What this study means for people with lupus: If the variant of the ITGAM protein prevents immune tolerance, people with the genetic variant can be followed more closely to see if they develop lupus. For those who already have the disease, it could highlight a new candidate for drug therapy to "fix" the protein or make up for its weaknesses in controlling the immune system.

I-Cheng Ho, MD, PhD
Brigham and Women's Hospital
Protective roles of ETS1 in SLE

Researchers have identified numerous genes related to the lupus and other autoimmune diseases. Now they are moving onto the next step—using that knowledge to identify new targets for treatment. Dr. Ho and his team used their ALR one-year, pilot grant to explore the role of a newly identified gene called ETS1 that is associated with the risk of lupus. They theorized that the gene can counteract the pathology of the disease by inhibiting production of the inflammatory molecule interleukin 10 (IL-10). People with lupus have unusually high levels of IL-10 in their blood, which is thought to contribute to the development of the disease and its symptoms. The work they were able to do thanks to the ALR grant enabled Dr. Ho and his team to obtain a much-larger, three-year grant from the Department of Defense that will allow them to continue their research into the role of ETS1 in lupus.

What this study means for people with lupus: Results from this study may help identify new targets for lupus treatments.

Lindsay Criswell, MD, PhD
University of California, San Francisco
Functional Genomics and Pathway Analysis of the MCH Region in SLE

Dr. Criswell and her collaborators will use a large database of DNA, blood and other biospecimens, as well as genetic and clinical data, from more than 15,000 individuals to link genetic variants with key clinical aspects and biomarkers in those with lupus. One goal of the work is to transform current understanding of the major histocompatibility complex (MHC) region on genes and its relationship with non-MHC lupus genes to better understand how the MHC contributes to the disease. Major histocompatibility complex molecules are involved in immune responses as well as autoimmunity.

Specifically, the researchers will:

- Identify MHC region variants most likely to be related to lupus by sequencing DNA data from 3,800 individuals with lupus and comparing it with DNA from 12,000 individuals without the disease
- Identify complex molecular networks and cellular pathways for MHC and non-MHC variants involved in disease susceptibility and expression
- Perform DNA methylation and gene expression studies of MHC variants in lupus. To do this, they will recruit 200 additional patients who were diagnosed with the disease less than 5 years ago, and 200 individuals without lupus.

What this study means for people with lupus: A greater understanding of the genetic contributions to lupus will help identify new pathways for prevention and treatment approaches.

Yanick Crow, PhD
University of Manchester
Pathways Linking Tartrate-Resistant Acid Phosphatase, Interferon, and Lupus

Dr. Crow and his team recently demonstrated that people with changes in the *ACP5* gene have a very high risk of developing lupus. These patients also show elevated blood levels of interferon, an inflammatory molecule thought to contribute to the damaging effects of lupus on various parts of the body. High levels of interferon are a sign of lupus in many patients. *ACP5* codes for a protein called tartrate resistant acid phosphatase (TRAP). These findings suggest a role for TRAP in the development of lupus by altering control of interferon.

There is some evidence that people with mutations in *ACP5* also have higher blood levels of a protein called osteopontin, which is expressed in bone and regulated, in part, by TRAP. Osteopontin appears to play a role in the production of interferon. This finding raises the possibility that TRAP deactivates osteopontin, and that a failure of this deactivation results in an inappropriate up-regulation of interferon.

With their grant, Dr. Crow and his collaborator Keith Elkon, MD, from the University of Washington, will investigate how TRAP expression is regulated in human immune cells, concentrating particularly on the relationship between TRAP, osteopontin and type I IFN stimulation.

What this study means for people with lupus: A better understanding of the role of TRAP in interferon metabolism could help to identify new treatment targets for lupus.

Fred Perrino, PhD
Wake Forest University Health Sciences
TREX1 Mutations in SLE

One of the hallmarks of lupus is the presence of antibodies that react to DNA molecules found circulating in the blood of affected individuals. Although we don't know the source of the DNA molecules, we do know that in adults about 100 billion cells a day undergo normal cell death processes. Part of these processes involve disassembling the cells and the 2.9 billion base pairs of DNA that comprise the human genome in each cell.

Dr. Perrino and his team have identified a gene called TREX1 that encodes for a powerful DNA disassembly enzyme that appears to be responsible, in part, for the circulating DNA fragments. Mutations in this enzyme enable the persistence of DNA from dying cells, triggering an immune response. The effects of this immune response vary, but the failure of TREX1 to eliminate DNA from dying cells might be at the root cause of the aberrant immune reaction seen in some patients.

With this grant, Dr. Perrino and his tem plan to create a lupus TREX1 mutant mouse model to determine how TREX1 enzyme dysfunction leads to the development of lupus and related autoimmune disorders.

What this study means for people with lupus: These experiments will provide new insights into the origins of lupus and promising new avenues for the development of novel therapeutic strategies to treat the disease.

Derry Roopenian, PhD
The Jackson Laboratory
Novel Approach to Modeling the Functional Genomics of Human SLE in Mice

Like most autoimmune syndromes, the genetic predisposition to lupus is complex. We see this complexity in genetic association studies that have identified anomalies in more than 30 genes that provide small, but significant, contributions to the disease. However, only when several of these genetic variations occur simultaneously does the disease itself result. Thus, it is difficult to translate these complex genetic patterns into a biological explanation of the specific anomalies that cause lupus, and even more challenging to apply this information to benefit people with lupus.

With their grant, Dr. Roopenian and his team will use a mouse model to explore these issues. They will mimic the genetic changes seen in humans with lupus in the mouse and observe the results of such abnormalities. They will combine this information with gene expression studies to discover the molecular pathways that determine susceptibility and resistance to lupus in mice.

What this study means for people with lupus: Conducting studies in mice to specifically address genes we already know affect the risk of lupus in humans should provide important information for predicting, understanding, and treating lupus and related disorders in humans.

Anne Satterthwaite, PhD
University of Texas Southwestern Medical Center

Functional Relationships Between the Lupus Susceptibility Loci Lyn and Ets1

A key contributor to lupus is the production of antibodies that recognize the body's own components (autoantibodies). These antibodies, which are produced from B cell-derived plasma cells, collect in various tissues and organs, including the kidney, causing inflammation and tissue damage.

Two genes involved in the development of lupus, *Lyn* and *Ets1*, limit production of these plasma cells in mice. Without either of those genes, mice develop a lupus-like autoimmune disease. Dr. Satterthwaite, her colleague Lee Ann Garrett-Sinha, PhD, and their teams have shown that B cells that lack *Lyn* also have reduced *Ets1* levels, suggesting that the two genes operate in a common pathway to control the development of plasma cells and the production of autoantibodies.

With their grant, they will test the hypothesis that *Lyn* normally prevents autoantibody production by promoting the expression of the Ets1 protein. Among the questions they will explore:

- Does *Lyn* act in B cells or some other cell type to control *Ets1* expression?
- Which pathways regulated by Lyn are involved in altering *Ets1* expression?
- Does *Lyn* control the expression of the *Ets1* gene or regulate the stability of the Ets1 protein?

They will also explore the consequences of reduced *Ets1* levels in *Lyn*-deficient B cells, and determine whether low levels of *Lyn* and *Ets1* together contribute to the development of autoimmunity. They will also restore normal *Ets1* levels in *Lyn*-deficient B cells to see if this prevents autoantibody production.

What this study means for people with lupus: Defining and characterizing this novel pathway may reveal new therapeutic targets that could prevent the antibody-related tissue damage.

Earl Silverman, MD
The Hospital for Sick Children

How Genes Determine SLE Phenotype and Outcome

Systemic lupus erythematous is a multi-organ system disease that likely results from a complex interaction of genes and the environment. The disease can begin at almost any age, from infants as young as 1 year to individuals in their eighties and even nineties. However, the younger the patient, the more severe the disease tends to be, although its course differs in every patient. Thus, different genes may be involved based on when someone develops lupus.

So far, however, genetic studies have found one common set of genes in both children and adults with SLE. No one has looked to see whether the amount of genetic changes in children with lupus differ from those in adults with the disease.

In addition, the varying manifestations of lupus from patient to patient suggest that interactions between lupus-related genes and non-lupus-related genes my influence the course of the disease.

This grant will be used to try and answer both questions: are there differences in the number of genetic mutations between children and adults with lupus? And are there interactions between organ-specific genes and lupus-related genes that help explain the different manifestations of the disease? If so, are these interactions similar in adults and children?

To investigate these questions, Dr. Silverman and his team will assess 1,000 to 2,000 adults and a similar number of children with lupus in this international study.

What this study means for people with lupus: Identifying children who have a high genetic risk for lupus will add to our understanding of the pathogenesis of the disease and help explain why it occurs throughout the lifespan. In addition, examining the role of non-lupus genes in patients with lupus will provide important information about how other genes influence the outcome of lupus. Better understanding both of these processes will enable researchers to identify new targets for therapy and to better target existing therapies for children and adults with lupus.

Katherine Siminovitch, MD
University Health Network
Defining Functional Implications of a Human SLE Risk Allele in Mice

Among the many genes now shown to contribute to the development of lupus is *PTPN22*, a gene that codes for a tyrosine phosphatase called Lyp. Lyp is found only in blood cells and is important for suppressing T lymphocyte activity. Many people with lupus express a variant form of Lyp called Lyp620W. However, we don't know how this variant contributes to the disease process.

With this grant, Dr. Siminovitch and her team hope to identify the pathways that link the Lyp620W variant to risk for lupus. To do so, they will study mice that express this variant. Initial work with these animals found numerous immune cell defects, particularly, "hyper" activation of T and B lymphocytes. Analysis of these mice also revealed that the variant protein is unstable and is broken down too quickly ,resulting in very low levels in the mouse lymphocytes. The team then showed that these abnormalities also occur in cells from humans expressing the Lyp620 variant. These findings suggest that the *PTPN22* gene variant causes reduction in Lyp levels and in the ability of Lyp to suppress immune cell activation. The result is immune cell hyperactivity.

Dr. Siminovitch and her team will now further define the Lyp variant's effects on T and B cell functions, particularly activation, movement, and development. For instance, they will compare how cells from mutant and normal mice divide or migrate in response to select stimuli, and examine the cells' secretion of inflammatory cytokines involved in autoimmune responses.

They will also study T and B lymphocyte development in the mouse model to assess whether the variant alters early elimination of autoreactive cells, which occurs in people with normal immune systems, but may be reduced in those with lupus.

Another set of studies will define the variant protein's effects on autoimmunity and how this genetic variant interacts with other genetic variants to cause lupus.

What this study means for people with lupus: Defining the pathways related to a specific genetic variant could identify new targets for treatment.

Jun Yan, MD, PhD
University of Louisville
Regulation of Autoreactive B Cells by Integrin ITGAM/CD11b

The autoimmune responses that underlie lupus are characterized by the presence of autoantibodies (autoAbs) and T cells that turn their protective defenses against self tissue rather than invader pathogens. Genetic studies suggest that a mutation within a gene called integrin CD11b increases the risk of developing lupus, and, in those who do develop the disease, results in a more severe course.

Preliminary data from Dr. Yan's group suggests that CD11b is expressed on all stages and subsets of B-lymphocytes. These lymphocytes are responsible for producing the autoantibodies that attack self tissues and organs. Strikingly, B cells with CD11b mutations divide more rapidly and survive better than those without the mutation. Thus, it appears that the CD11b mutation may make it easier for this lymphocyte subset to become activated and drive disease processes.

This grant will be used to better understand the role of the mutation in those pathways.

What this study means for people with lupus: Better understanding the genetic mutation and its role in lupus can help identify novel targets for new drug development.

SLEGEN Consortium—ImmunoChip Study

The SLEGEN project has realized tremendous success since the ALR's initial founding of the group in 2005 and is now taking the research to a whole new level. Utilizing the most advanced technology available, called the ImmunoChip, SLEGEN scientists are more closely examining the genes that were identified in the most recent round of studies. This highly specialized and powerful tool is allowing researchers to engage in an even greater level of detail because the information contained on the ImmunoChip is based completely on findings from previous genetic studies, which means the information is extremely focused and specific. The new technology offers scientists the amazing ability to study hundreds of thousands of genetic variants – 250,000 to be exact – in more than 10,000 participants.

This landmark study will allow SLEGEN scientists to study - in unprecedented detail -ethnicity and lupus, autoimmune disease commonalities, and gene variants & biologic pathways. Understanding the genetic basis may help clinicians more closely predict when an individual might develop lupus and the complications he or she may experience and to what degree, leading to effective disease management and more precise treatments.

The realistic hope is that the ImmunoChip study will reveal, in enormously expanded detail, the critical roles that genetic variance and ethnicities play in predisposing an individual to developing lupus, age of disease onset and lupus-related complications common with the disease. Preliminary results may be available as soon as mid-2012.

ABOUT ALR

The Alliance for Lupus Research (ALR) is working tirelessly to create a world where lupus no longer exists. We believe the solution to lupus is research focused on better treatments, prevention and a cure. We support the most promising research projects from scientists at the most prestigious hospitals, universities and medical schools throughout the world. Because our Board of Directors funds all administrative and fundraising costs, one hundred percent of your donation goes directly to support research programs. Together, we will find a cure for lupus.

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Alliance *for* Lupus Research

Because ALR's Board of Directors funds all fundraising and administrative costs, 100% of all donations received goes to support lupus research programs.

To make a donation to ALR, please visit our website, www.lupusresearch.org, mail a check to ALR at 28 West 44th Street, Suite 501, New York, NY 10036, or call us toll-free at 800-867-1743.