

The Future Directions of Lupus Research

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Introduction

Systemic lupus erythematosus (SLE, or lupus) is one of more than 80 autoimmune diseases identified by scientists. An autoimmune disease is one in which the body's immune system – its normal defense against harmful invaders including viruses and bacteria – turns its attack against the body's own organs, tissues, and cells. In some diseases the target of the attack is limited to a particular part of the body – the blood vessels, moisture-producing glands of the eyes and mouth, or insulin-producing cells of the pancreas, for example – but in lupus, the attack is systemic, meaning it affects many organs and organ systems including the skin, joints, heart, lungs, kidneys, and brain. The results can be disabling or even deadly.

Disease activity often waxes and wanes. Periods of relatively mild disease may be followed by flares, or by periods of increased disease activity involving one or many organ systems, which can lead to irreversible organ damage. Some flares are evident through clinical symptoms such as a skin rash, increased fatigue, joint inflammation, oral or nasal ulcers, or seizures. In other cases, the only evidence of a flare is a laboratory test showing a low white blood cell or platelet count or protein in the urine, for example.

By the most conservative estimates, lupus affects at least 239,000 Americans. Recent independent surveys have suggested a prevalence as high as 1.5 million. Women with the disease outnumber men 9 to 1. It often strikes women in their early working and childbearing years, interfering with the ability to work, have or raise a family or in some cases even care for themselves.

The most heterogeneous of the autoimmune diseases, lupus is also one of the most difficult to understand and treat. Fifty years ago, survival was 50 percent after four years. Today, thanks to advances brought about by research on the mechanisms of diseases and aggressive therapy, it is 97 percent at five years and 90 percent at ten years. The transformation from a disease with high mortality to a chronic disease means an increased need for therapies to prevent or manage long-term manifestations of the disease. The prospect is very good for better therapies with fewer side effects.

The past two decades of research on the immune system have yielded a wealth of new information and extraordinary growth in conceptual understanding. As a result, opportunities now exist to identify potential genetic, environmental, and infectious causes of lupus and to develop novel approaches for lupus treatment and prevention. To achieve these goals the National Institutes of Health (NIH) places a high priority on cross-disciplinary research and the participation of other government agencies and private organizations in these efforts.

To facilitate collaboration among the NIH Institutes, other Federal agencies, voluntary and professional organizations, and industry with an interest in lupus, the NIH established the Lupus Federal Working Group (LFWG) in 2003. The LFWG is led by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, and includes representatives from all relevant Department of Health and Human Services (HHS) agencies and other

Federal departments having an interest in lupus. This includes among others the National Institute of Allergy and Infectious Diseases, the Office of Research on Women's Health, the National Institute of Neurological Disorders and Stroke, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Heart, Lung and Blood Institute, and other agencies of the Federal government including HHS, Food and Drug Administration, and the Centers for Disease Control and Prevention. Voluntary organizations and representatives from the private sector also attend and participate in the meetings of the LFWG.

The fiscal year 2005 House Appropriations Committee Report Language directed the NIH to develop a plan to guide the nation's investment in lupus research. To identify the opportunities, priorities and needs in lupus research that should be considered for inclusion in the research plan, a panel/workshop consisting of scientific experts in the field of lupus was brought together for two days of presentations and discussion. The highlights of their conclusions are reported here, in *The Future Directions of Lupus Research*.

This research plan highlights many unprecedented opportunities to increase our understanding of lupus at the population, individual, and molecular levels, which should ultimately allow the translation of new knowledge into more effective treatments and, eventually, prevention strategies.

The report is organized to mirror current understanding of the disease and to reflect current opportunities for advancement. The plan supplements and enhances ongoing activities of the NIH, most notably the Autoimmune Diseases Research Plan, prepared by the NIH Autoimmune Diseases Coordinating Committee and the National Institute of Arthritis and Musculoskeletal and Skin Diseases' Long Range Plan (2006-2009), the lead federal agency for research on lupus.

CHAPTER 1: Etiology

One of the first questions that come to mind when a patient receives the diagnosis of lupus is “what caused this?” While the question is understandable for someone trying to make sense of the discovery they have a chronic disease, the causes and origins (etiology) of lupus are also important to researchers, who are working to gain a better understanding of the disease. Identifying and understanding the causes of lupus will likely lead to the identification of risk factors, the design of prevention strategies and the development of targeted therapies with increased efficacy and minimal toxicity.

No one knows the precise causes of lupus (and there will likely be many that may differ by individual), but there is strong evidence supporting a role for both genetics and infectious and non-infectious environmental factors.

NIH-supported research has played an important role in the discovery of potential factors that influence susceptibility. Ongoing research should lead to the further identification and understanding of factors that play a role in the development of lupus.

Genetics

Breakthroughs

- Identification of susceptibility genes in the mouse. Results using several mouse models and varied approaches to genetic analysis suggest that some of the genetic susceptibility to lupus may be mediated by epistatic (gene-gene) interactions between polymorphic genes influencing immune responsiveness rather than purely via immunologic abnormalities caused by defective alleles.
- Development of transgenics, congenics, knockouts and knock-in murine models to resolve lupus susceptibility loci.
- Identification of susceptibility genes in the mouse – with the ability to map and clone predisposing genes and develop lupus-like disease in genetically manipulated mice (e.g., transgenics, knockouts, and N-ethyl-N-nitrosourea (ENU) mutagenesis).
- Identification of loci and genes that regulate target organ susceptibility to damage and affect disease severity.
- Identification of interferon (IFN) inducible genes in the pathogenesis of lupus.
- Development of novel induced animal models of lupus, which vary target organ involvement based on toll-like receptor (TLR) engagement.

In both human and animal models, studies suggest that multiple genes are involved in conferring susceptibility and affecting the severity of lupus. Findings suggest that the definition of genetic susceptibility in human and clinical applications of this information will require novel sophisticated approaches. For example, "reverse genetics" or “fishing" is used to find essential lupus effector genes (genes that cause a particular manifestation of disease). In general, genes define the differences between people that change the risk for lupus. There are gene differences that increase the risk of lupus (estimates vary from 10 to 1,000 genes involved), decrease the risk of lupus (there are no reliable estimates for how many genes might be protective), and do not

change the risk of lupus (the vast majority of the > 25,000 genes in human beings are expected to have no influence on the risk for lupus).

Due to the overwhelming complexity of lupus, only very few susceptibility genes have been identified so far. This area of research, however, offers some of the most promising opportunities to unravel the causes of the disease, point to new pathways of disease, and identify new targets for intervention.

Mouse Models with Manifestations of Lupus Systemic Autoimmunity

There are many mouse models of lupus, including spontaneous disease models, induced disease models, and models generated by manipulating genetic background and particular genes. Studies in these models show that lupus is not only polygenic but that the genes are different for different strains. Specific findings include the following:

- Among strains, some loci map to similar locations, but there is substantial heterogeneity.
- Some lupus loci cluster with other autoimmune loci, suggesting the same genes may be involved.
- One locus usually predisposes to several traits.
- Clinical stages and manifestations are affected by different combinations of loci.

All types of mouse models are important resources not only for studying genetics, but also for studying environmental factors and pathogenesis as well as for testing new therapies and validating findings derived from human studies. Some of these models have been available for many years and a lot has been learned about mechanisms of disease from them (e.g., MRL-*lpr/lpr* mice and NZB x NZW mice) while others have been developed more recently, primarily to explore the genetics of lupus (e.g., congenically derived NZM2410 onto a C57BL/6).

In spontaneous mouse models, candidate susceptibility genes have been proposed with the *cr2* gene, the *Slelc* locus, the cluster of SLAM family members (*cd48*, *slam*, *cd84*, and *ly108*) for the *Slelb* locus, *ifi202* for the *nba2* locus, and possibly *tlr7* for the *yaa* gene in the BSXB strain.

In general, genes involved in the development of lupus appear to affect three important steps in the development of autoimmunity and clinical disease. Some genes (e.g., *Slela*, *Slelb*, *Slelc* in the NZM2140 model) appear to influence the loss of tolerance to self and induce the production of immunoglobulin G (IgG) autoantibodies to nuclear autoantigens. Another set of genes (e.g., *sle2*, *sle3*, *fas*, *yaa*, *lyn*, *BlyS*, *112*, and *pd-1*) participate in the disruption of the normal regulation of immune responses. There appear to be complicated interactions between genes in the first and second group, but those interactions are not well understood. Some of these genes affect the mechanisms of injury in the local tissues such as immune complex deposition on basement membranes and production of inflammatory cytokines; others appear to regulate structural and physiological aspects of the target organ such as glomerular architecture or the blood-

brain barrier. Each gene is expected to make its contribution in its own special way. One of the challenges is to explain how this happens and how it generates risk for lupus.

Formal validation of the role of each gene that explains a susceptibility and severity locus awaits new transgenic models. In these systems it should be possible for many of these genes to demonstrate how the features of lupus occur.

Genetics of Human Lupus

Evidence for the importance of genes in the etiology of human lupus has been provided by studies showing that 1) there is a higher concordance of lupus between identical twins than between fraternal twins; and 2) relatives of patients with lupus are at higher risk for developing the disease. A number of genes have been found in association with lupus in human populations. Examples of genes discovered using traditional association studies include human leukocyte antigen (HLA) and complement genes. Newer approaches to gene discovery have been applied to lupus and include linkage and association studies. Using these approaches, and information derived from the Human Genome Project and the human HapMap, a number of genes related to lupus susceptibility and severity have been found (Table 1) and many more are in the “pipeline.” These should be published and become known in the next few years.

Because of the complexity and heterogeneity of lupus, however, researching the genes involved in the disease and its many features is laborious, time-consuming and expensive. To be successful, lupus gene discovery will require continued concentrated effort by many groups. In the mouse, over 50 genetic variants are known to predispose to lupus. Understanding how they work and whether they are relevant in the human situation will require years of the productive energies of the scientific community. As this occurs there is a need for enhanced collaboration and exchange between research groups.

Once the major genes are identified, then many scientists suspect that their interaction will be very important to understand. It is likely that the exploration of gene-gene interactions looking for synergies or suppression will become the major focus of some research teams.

Table 1**Confirmed Genetic Associations in Lupus**

Risk factor	Chromosome	Odds ratio	Population^a
Female risk		3–10	Global
Race		~4	African-American over European
C1*Q0	1p36	~10	European, Asian, Spanish
C2*Q0/C2*Q0 ^b	6p21	~4.5	European
C4A*Q0	6p21	1.5–5	European, Asian, African-American
C4B*Q0	6p21	1.5–6	European, Asian, Spanish, African-American
CTLA-4, +49G	2q33.2	1.3	European, Asian
DQA1*0501	6p21	3–6	European, Asian
DR2	6p21	1.5–4	European, Asian, African-American
DR3	6p21	~3	European, African-American, Hispanic
FcγRIIA, R131	1q23	~1.3	African-American, European
FcγRIIIA, F176	1q23	~1.6	European, Korean
FCRL3	1q23	1.3	Asian, Spanish
IL-10	1q32	1.4	European, Asian
IRF5	7q32	1.6	Nordic, European, Hispanic
MBL	10q22	1.4	European, Asian, African-American
Osteopontin	4q22	2.3	European
PDCD1	2q37	2.6	European, Asian
PTPN22	1p13	~1.6	European
TNFα, -308	6p21	4	European, Asian, African-American
TYK2	19p13	1.6	Nordic

^a“European” in this context means people of European extraction, wherever they now live and were studied. “African-Americans” are people of African ancestry who are now in North America.

^bHomozygous state

Source: Harley, John B., Kelly, Jennifer A., Kaufman, Kenneth M. (2006) Unraveling the genetics of systemic lupus erythematosus. *Springer Seminars in Immunopathology*. 28(2): 119-130.

Research Objectives

- Support the infrastructure needed for discovery of human and murine lupus genes and encourage the application of the newest and high throughput technologies to accelerate research on gene effects on disease.
- Identify the important gene-gene interactions in mice and humans.
- Identify additional lupus susceptibility genes in the mouse.
- Increase understanding of genetic predispositions resulting in target organ damage.
- Identify essential effector genes in lupus.
- Apply new technologies to lupus animal model.
- Better define the role of identified essential effector genes to increase the understanding of pathways that have therapeutic relevance.

Environmental Factors

Breakthroughs

- Findings that support a role for DNA methylation inhibition in patients with drug-induced and idiopathic lupus.
- The finding that the Epstein-Barr virus (EBV) is involved in the pathogenesis of lupus, apparently by antibody cross reactions, generating autoantibodies.
- The finding of evidence that supports the etiologic roles for drugs such as procainamide, hydralazine, minocycline, and some of the biologics such as TNF antagonists and interferons (IFNs) in the induction of lupus.
- Confirmation of an association of crystalline silica and lupus in human studies and descriptions of mechanisms related to autoimmunity in lupus-prone mice.

While genes play a crucial role in the development of lupus, most researchers agree that another factor – something in the environment – may contribute to disease initiation or progression in genetically susceptible individuals.

In September 2005, the Federal Interagency Working Group on Women’s Health and the Environment, led by the HHS Office on Women’s Health, was instrumental in convening a workshop which focused on three environmental exposures – solvents, silica and infectious agents – and the routes by which these exposures might lead to lupus.

While there are potentially many environmental agents at work in lupus, those with the most evidence at present include medications, ultraviolet light (UV), silica, and EBV.

Medications

Drug-induced lupus (DIL) is a form of lupus that occurs in susceptible individuals following exposure to certain drugs. Typically, the disease resolves when the drugs are discontinued, which is the primary factor differentiating DIL from lupus.

DIL is the best and most thoroughly studied form of an environmentally-induced lupus. Strong evidence supports the etiologic roles for drugs such as procainamide, hydralazine, minocycline, and some of the biologics like TNF antagonists and IFNs in the induction of lupus. The prevalence of DIL is unknown, due in part to a lack of diagnostic criteria, and the relative risk of inducing lupus is unknown for many drugs. Established risk factors for DIL include major histocompatibility complex (MHC) alleles, slow acetylator status, and perhaps gender. The mechanisms by which hydralazine and procainamide induce lupus appear to be related to their ability to inhibit DNA methylation, a post-synthetic DNA modification that suppresses gene expression. Hydralazine, procainamide, and other DNA methylation inhibitors demethylate crucial regulatory regions and cause overexpression of T cell genes that result in autoreactivity *in vitro* and lupus-like autoimmunity *in vivo*. Importantly, the same sequences are demethylated and the same genes are overexpressed in T cells from lupus patients, providing a common mechanism for idiopathic lupus and procainamide and hydralazine DIL. How other drugs cause DIL is less certain, and whether drug exposures might play a role in triggering lupus is poorly understood.

Ultraviolet (UV) light

Many lupus patients are photosensitive and UV light is an accepted trigger of skin exacerbations in systemic and cutaneous lupus. The role of UV exposure in triggering onset of systemic lupus has not been widely studied, but some evidence suggests a potential gene-environment interaction. Although the precise mechanisms involved are unclear, research suggests UV light alters proteins and DNA in the skin. UV light is also a potent inhibitor of DNA methylation which has been shown to cause changes in gene expression that may contribute to lupus. In people with lupus who are photosensitive, UV exposure triggers the cells to release cytokines and prostaglandins, resulting in skin flares (See Skin section in Chapter 3).

Crystalline silica

Crystalline silica is a widely studied occupational risk factor for pulmonary inflammation and fibrosis (silicosis), and has well-known inflammatory effects *in vitro* and *in vivo*. A growing body of literature supports a role for silica exposure as a lupus risk factor. Studies of workers with exposure to crystalline silica have shown 10-fold higher than expected rates of lupus and other systemic autoimmune diseases compared to expected rates in the general population. Two recent studies of lupus patients have also shown higher prevalence of silica exposure in cases compared to population controls. These data are compelling given the consistent association of silica exposure with disease in different populations and using different study designs. They are also consistent with a large body of research showing an association of silica and other systemic autoimmune diseases (e.g., scleroderma and rheumatoid arthritis).

The findings of an association between silica and lupus are also supported by experimental studies that demonstrate that silica both acts as an immune adjuvant (a non-specific stimulant) and contributes to the loss of tolerance through effects on apoptosis in

accelerating lupus pathogenesis in lupus models. Although silica is unlikely to account for a majority of lupus cases in the population, it illustrates potential mechanisms by which other exposures might act to trigger or accelerate development of lupus.

Epstein-Barr virus

Among the environmental risk factors, EBV is, by far, the most closely associated with lupus. Patients have higher titers of anti-EBV antibodies than control populations, and EBV infection is more common in both children and adults with lupus than in control populations. Lupus patients have a forty-fold higher EBV viral load in peripheral white cells than control populations. This appears to be due to defective antiviral cytotoxic T cell responses and to an increase in frequency of latently infected B cells. In addition, this frequency is even higher among those undergoing a disease flare and may be modified by genetic polymorphisms influencing T-cell response.

Although EBV has long been associated with lupus, a recent study of serial blood samples drawn before lupus patients became symptomatic strongly supported a causative connection. This study also provided evidence on how the virus might trigger the disease. By studying serial samples that predated the development of lupus, scientists were able to identify the point at which anti-Ro antibodies appeared in the bloodstream of patients later diagnosed with lupus. For many, the antibodies were first produced in response to EBV infection as evidenced by the earlier or near-simultaneous appearance of anti-EBV antibodies. Other studies showed that immunization of rabbits with the critical structures of a protein from EBV also induced a lupus-like illness in these animals.

The major finding is that the progression from normal to benign disease (detectable in the blood, but causing no clinical illness) to clinically obvious lupus is preceded by findings that suggest a disease mechanism. For two of the autoantibody systems (Sm and Ro), antibodies to a structure on Epstein-Barr virus nuclear antigen-1 (EBNA-1) precede the development of lupus autoimmunity. These structures appear to be related to the first autoantibody binding sites on Sm and Ro, respectively.

While most scientists studying this area would agree that the role of any particular infectious agent remains to be unequivocally established, the possibility that EBV causes lupus is being actively pursued. Given that the vast majority of adults have EBV infection, a greater understanding is needed of the potential role of other factors in control of EBV infection and the relationship with lupus etiology.

Other Environmental Factors

Evidence from human and experimental studies suggests a possible role for exposure to heavy metals such as mercury, hydrocarbons, and solvents. In one study, occupational history of work with mercury or work in a dental office was linked to a higher risk of lupus compared to population controls. A recent study also described a cluster of lupus cases in residents living near an oil field waste site contaminated by mercury.

Development of multiple autoantibodies and acceleration of renal disease has also been seen in experimental studies in mice exposed to mercury, cadmium, and lead.

The hydrocarbon pristane has been extensively studied as a cause of lupus-like disease in animal models, including recent findings showing a female predominance in pristane-induced autoimmunity and mechanisms by which pristane may break immune tolerance through increased programmed cell death. No case-control studies have specifically addressed pristane exposure in relation to lupus risk, though pristane levels were also elevated in the oil field waste study mentioned previously. Another recent study showed increased rates of lupus in relation to specific genotypes and proximity to hazardous waste sites likely contaminated with volatile organic waste compounds, but whether pristane or related contaminants specifically played a role could not be determined. However, several experimental studies have shown immune-related effects with exposure to the solvent trichloroethylene or some of its metabolites, in drinking water or by intraperitoneal injection. Two human case-control studies showed no association with the broad category of occupational solvent exposure and lupus, though an earlier study showed increased symptoms of lupus and autoantibodies related to possible low-level exposure to trichloroethylene in water.

One human study has shown an association between high levels of occupational pesticide exposure (mixing) and lupus, while another study of a community with high level pesticide exposures and elevated lupus showed no association between lupus and pesticide levels in the blood. In recent experimental studies, however, exposure to estrogenic organochlorine pesticides was associated with accelerated features of autoimmune disease in lupus-prone mice. Effects were not clearly due to pro-estrogenic mechanisms, however, it should be noted that the role of exogenous female hormones in lupus is not well established. Nevertheless, given their abundance in the environment and effects at very low levels of exposure, xenoestrogens or other environmental contaminants with hormone disrupting or mimicking effects, remain plausible and relevant candidates for study in relation to the increasing incidence and disparities in lupus.

Smoking has been also been identified as an independent risk factor for lupus and other autoimmune diseases. A meta-analysis of several studies, however, showed that only current smoking was associated with a modest increase in risk of lupus. Given the well known role of smoking in inflammation and development of heart disease, smoking is a plausible risk factor and could be considered as a potential risk factor for lupus-related cardiovascular outcomes. More persuasive epidemiologic studies are needed to support a role for hydrazines, aromatic amines and environmental endocrine disrupters – synthetic and naturally occurring chemicals that affect the balance of normal hormone functions in animals.

The effects of ambient air pollution or passive smoking on lupus risk or severity may also be areas of interest in lupus research since findings in support of these hypotheses have been reported for other autoimmune diseases, such as type-1 diabetes, and indirectly through the association of maternal smoking and juvenile rheumatoid arthritis. Silica and

past smoking are examples of exposures best studied through the use of carefully designed and validated questionnaires. Studies that provide consistent and rigorous exposure data would enable meta-analyses and provide the large combined samples required for studies of gene-environment interactions. Biomarkers may be useful to confirm exposures to agents that accumulate in the body (e.g., pesticides and metals).

Early life exposures may “program” the immune system and thereby alter susceptibility to a later onset of lupus, for example as proposed for diseases such as asthma and allergy. These exposures may include organic dusts, infections, or exposures that modulate the response to early life infections. Modeling these effects would take careful examination of recalled data in conjunction with coded records (e.g., birth-weight). Longitudinal cohort studies are unlikely to be as effective in studying early life factors and lupus as they have been for more common conditions.

Similar to the differences between lupus genetics in animals and humans, exposures that trigger lupus in animals and people may also differ. Although broadly searching for agents related to the development of lupus in mice may help in identifying such agents for humans, experimental studies should also be concentrated on exploring the role of exposures identified in human studies (e.g., silica, solvents, or pesticides). Experimental studies may be useful when investigating mechanisms involved in both the genetic and environmental causes of lupus. Animal models may also be useful in examining *in utero* or early life environmental exposures in relation to disease risk in off-spring.

Research Objectives

- Identify environmental exposures in humans related to incidence and disease progression.
- Develop and disseminate technologies and instruments to assess environmental exposures.
- Elucidate the role of EBV in the development of lupus, including the study of genetic and environmental risk factors that might influence control of infection prior to lupus onset.
- Identify molecular or physiological targets of environmental exposures leading to either onset or progression of lupus.
- Study gene-environment interactions in both human and animal studies, including lupus-specific and non-specific genes.
- Conduct well-designed case-controlled studies to confirm the risk of developing lupus when exposed to candidate drugs.
- Establish registries of patients receiving the newer biologics to study genes and mechanisms associated with drug-induced lupus.
- Encourage collaboration and integration of investigators with expertise in environmental health research into lupus studies.
- Establish a case-controlled repository of information and biologic samples to determine the role of exogenous agents.

Conclusion

New ideas, concepts and technologies have brought renewed interest and greater insights into the factors that may predispose people to lupus and trigger its development and clinical course. As the mass of new data in genetics is analyzed, new models are tested and pathways discovered that lead to identification of new diagnostic and therapeutic targets. New technologies allow the study of how genes interact with each other and with the environment to cause disease in the predisposed individuals. The exploration of gene-environment interactions will be enhanced as more improved and harmonized methods in exposure assessment are integrated into studies with genetic data. New concepts about the environment and rapid, sophisticated and high throughput technologies give hope to scientists and patients alike that many new discoveries will be made, and that these will be translated to transform the lives of lupus patients.

CHAPTER 2: Mechanisms of Disease: Innate Immunity, Acquired Immunity and Inflammation

Lupus is characterized by a wide array of humoral and cellular abnormalities involving both up-regulation and down-regulation of critical elements of the immune system. The order in which components of immunological dysregulation occur (that is, what is a primary or secondary event) is not well understood. The defective immune responses may be genetically determined and the degree to which genes are involved in the disease is under intensive investigation (See Chapter 1).

While genetics is thought to set the susceptibility of an individual, not all people with susceptibility genes are affected, suggesting that environmental factors contribute to the disease. In addition, the majority of lupus patients have no known family history of the disease. Whether these environmental factors induce the initial onset of the disease or are responsible for subsequent flares is not known.

Research examining the role of the immune system in the pathogenesis of lupus is finding that both arms of the immune system, innate and adaptive immunity, are involved and perhaps various interplays between the two systems may result in an autoimmune response. Scientists are studying the role of both innate and adaptive immunity in the disease as well as exploring the unique role of T cells and using mouse models to better understand mechanisms of disease.

Innate Immunity

Breakthroughs

- The discovery that dendritic cells play a major role in the development of autoimmunity in lupus.
- Recognition of the importance of pattern recognition receptors *in vitro*. *In vivo* mouse experiments are underway to further understand the role(s) of these receptors in autoimmunity.
- The finding that the Yaa lupus susceptibility gene, which predisposes to an increase in male lupus, is a duplication of the TLR7 gene on the Y chromosome with increased TLR7 expression in autoimmune-prone mice.
- The finding that DNA/chromatin-containing immune complexes/DNA-binding proteins activate autoreactive B cells, by co-engagement of the B-cell receptor and TLR9.
- The finding that RNA/RNP-containing immune complexes activate autoreactive B cells by co-engagement of the B-cell receptor and TLR7. This is dramatically enhanced by type I IFN.
- The finding that DNA- and RNA-containing immune complexes induce dendritic cells to produce type I IFNs and other pro-inflammatory cytokines. The response is dependent on uptake through FcγRII and at least partially dependent on TLR9/(7).
- Discovery of the importance of type I IFN to autoimmune disease pathogenesis.

- The finding that failure to appropriately clear apoptotic debris exacerbates the lupus phenotype.
- The discovery of distinct compartmentalization of DNA response elements in dendritic cell subpopulations.

The innate immune system plays a key role in the activation of conventional immune responses against bacteria and viruses. In contrast to those of the acquired system, receptors of the innate immune system recognize unchanged structures on large groups of pathogens. Two lineages, natural killer (NK) and dendritic cells play important roles in the immune system.

Until recent years it was believed that pattern recognition receptors such as TLRs existed on the innate immune system to distinguish microbial antigens and host antigens and thus did not likely play a major role in autoimmunity; however, studies now show that some pattern recognition systems can very effectively recognize self antigens and, therefore, the innate immune system is likely to play a previously unrecognized role in autoimmune diseases such as lupus.

Investigation of innate immunity in lupus has provided clues to answer the question of how intracellular elements become targets of immune recognition and generate the production of pathogenic autoantibodies. Dendritic cells are the crucial regulators of both immune tolerance and stimulation to self and foreign antigens. Studies suggest a link between specific modifications that occur in dying cells and autoantibody recognition. Mice and humans that have defective clearance of their own dying (apoptotic) cells, have uncleared fragments of cell membranes with nucleic and intracellular proteins (apoptotic bodies and nucleosomes) bound to them. Research has shown that such mammalian self-nucleic acids (DNA or RNA) bound to autoantibodies can stimulate dendritic or B cells by binding to surface receptors on the cell, entering the intracellular compartment and binding to their respective TLR. This results in lupus-like autoimmunity in mice.

Selectively blocking aspects of the immune response has been helpful in other autoimmune diseases such as rheumatoid arthritis, although it can leave patients vulnerable to specific infections and perhaps other sequelae. Therefore, selective immune blockade should be feasible to test in lupus. If biological therapeutics (biologics) that target specific pathways in innate immunity were to be developed several elements in a pathway or related pathways may have to be targeted to address clinical disease. For example, although TLR7 and TLR9 may have similar tissue expression and signaling pathways, single blockade has shown that they have opposing inflammatory and regulatory roles. Further studies including simultaneous blockade of TLR7 and TLR9 are underway as well as studies of the influence of these receptors on B cells and dendritic cells. These intricacies reveal that the real challenge for clinician investigators is to figure out the multiple pathways to target to halt lupus progression and damage without severely compromising patients. It will be necessary to identify the role of these pathways in different phases of the disease and tailor the therapies accordingly.

Research Objectives

- Establish how mammalian DNA/RNA becomes an effective TLR ligand.
- Determine the effect of TLR3/7/9 deficiency on diverse experimental models of lupus and their applicability to human disease.
- Understand the influence of TLR3/7/8/9 expression in human lupus.
- Explore safe and effective ways to therapeutically block TLR7/9 (or TLR3/7/8/9) or to enhance the clearance of apoptotic debris.
- Evaluate whether other innate receptors contribute to the activation of autoreactive B cells or antigen-presenting cells (APCs) and their endogenous ligands (DNA, RNA, oxidized lipids).

Acquired Immunity

Breakthroughs

- Recognition of the role of peptide mimetic/tolerogens in decreasing autoantibodies/decreasing disease activity.
- Discovery of pathogenic mechanisms involved in the disease, such as the role of alpha-actinin in anti-dsDNA-related lupus nephritis and the role of anti-C1q in lupus nephritis.
- Recognition of anti-lipoprotein lipase and anti-oxidized low-density lipoprotein (LVL) in lupus and lupus-accelerated atherosclerosis.
- Discovery of defects in B-cell tolerance checkpoints in adolescent lupus; anti dsDNA binucleated (BN) cells generated by secondary heavy chain rearrangement; many others.
- Early regulation of B-cell-mediated autoimmunity.
- Recognition of the interrelationships between B cells and antibodies reactive with phospholipids, apoptotic cells, and DNA, which provides some understanding of the lupus-atherosclerosis connection (See Cardiovascular System in Chapter 3).
- The identification of new immunotherapy targets, including costimulation pathway molecules (CD28, CTLA4, PD1 ligand and ICOS/ICOSL) and other lupus-specific activation systems.

Also called specific immunity or adaptive immunity, acquired immunity lies in the capacity of immune system to distinguish self and non-self antigens. The distinction is made through T-cell receptors (TCRs) and B-cell receptors (BCRs), which are produced in thousands of different configurations. The adaptive immune system relates to the specific responding elements such as antibody and memory T cells that arise after infection and vaccination and increase protection against the particular material to which the individual was exposed.

On the other hand, researchers have also found the adaptive immune system's specific response to a non-self antigen can trigger an immune response to a self-antigen. One prime example of the adaptive immune system's response triggering an immune response to a self-antigen is a study using serial serum samples from two cohorts. By studying changes in the blood over time, researchers were able to identify the point at which 130 people, who were later diagnosed with lupus, began making self-antibodies. For many,

the researchers discovered, the antibodies were first produced in response to EBV infection. In genetically predisposed people, they found, antibodies to a portion of the EBV protein cross-reacted with self proteins, such as 60kD Ro/SSA or Sm B'. Subsequent studies showed the same autoimmune epitope spreading and features of clinical sequelae in laboratory rabbits injected with this same portion of the EBV protein.

Perhaps more important than the precise virus that apparently triggered the development of the antibodies, was the fact that a number of autoantibodies could be detected prior to the development of clinical symptoms or lupus diagnosis. Anti-Ro, anti-La and antinuclear antibodies occurred very early before diagnosis, while other antibodies, including anti-dsDNA, anti-Sm and anti-nRNP occurred closer to the time of diagnosis.

Studies have found that antiphospholipid antibodies can be found in blood before patients experience a clotting event and that patients with antiphospholipid antibodies have more severe disease, regardless of race or other risk factors. Together these findings suggest that testing high-risk groups might enable doctors to identify those who will develop lupus – specifically those who will develop more severe lupus – before they experience clotting or any disease symptoms. Doing so would allow for early treatment of disease or perhaps preventive therapy that would block components of the immune system found to initiate or drive the disease.

Extensive ongoing work also is examining the pathogenic roles of specific autoantibodies in lupus disease activity and damage, the role of historical and new autoantibodies in specific organ involvement, the use of specific autoantibodies as diagnostic tests, and the role of select autoantibodies as prognostic and biomarkers.

Already scientists are looking at a number of agents with the potential to block specific aberrant immune responses in lupus. A notable one, LJP 394, a double-stranded DNA tolerogen, was shown in clinical trials to decrease anti-dsDNA and decreased anti-dsDNA was associated with decreased renal flares.

Future research will involve better understanding of the pathogenic mechanisms particularly with some of the less-studied autoantibody specificities and for some of the lesser known disease manifestations. Additional work will focus on understanding the interactions of the innate and adaptive immune systems in the generation, propagation and damage from autoimmunity.

Research Objectives

- Better understand the pathogenic mechanisms involved in lupus.
- Better understand B cell abnormalities in lupus.
- Find ways to prevent or slow accrual of autoimmunity.
- Better understand the influence of environmental, genetic, hormonal and epigenetic interactions in lupus autoimmunity.
- Understand lupus patient humoral responses to common infections and the mechanisms by which potential environmental triggers, such as smoking and UV exposure induce damage.

- Develop better diagnostic and prognostic tests that characterize the different immunological correlates of disease activity and progression.
- Understand features of pre-clinical autoimmunity and which features forebode clinical disease – for example, what aspects of autoimmunity change with onset of clinical symptoms, what factors can downregulate autoimmune responses in individuals who do not progress to systemic autoimmune disease, etc.
- Determine why certain autoantigens are targeted in lupus and whether autoantibody specificity can predict effective therapeutic strategies.

T and B Cells

Breakthroughs

- The development of therapies to deplete B and T cells and to block T-/B-cell collaboration.
- The identification of signaling pathways and co-stimulatory molecules that activate B and T cells and that promote their collaboration.
- Recognition of the role of regulatory T cells (CD4+ CD5+ Tregs) in lupus with unique effects influencing autoimmunity.
- The identification of intrinsic (genetic) abnormalities in B (and T) cells.
- Identification and characterization of lupus-specific autoreactive T cells.
- Identification and characterization of T cell defects in lupus patients compared to controls.

Because B cells produce autoantibodies and autoantibodies induce tissue injury in lupus, lupus is often considered a disease of B cells. While research suggests that there may well be T-independent mechanisms or effects of B cells in lupus, T-/B-cell collaboration is required for the induction of antibodies and thus plays a major role in autoimmunity. Scientists are studying this collaboration, as well as how T cells are activated in lupus in mouse models and in human disease. Their findings have therapeutic implications for blocking the activation of the cells or their collaboration.

In a mouse model, estrogen treatment increased the proportion of marginal zone B cells—the B cell subset that participates in T-independent immune responses. These marginal zone B cells were spontaneously activated *in vivo* to secrete anti-DNA antibodies. Prolactin treatment in this mouse model created a similar increase in anti-DNA antibodies and immune complex deposition in the kidney.

A better understanding of T- and B-cell functions in people and the trials of B- and T-cell-directed therapies will require large numbers of patients. In recent years, the combination of increased cooperation among investigators and the development of clinical trial networks has increased the identification of available patients for such studies, but prioritizing studies with a limited patient pool is necessary.

Research Objectives

- Dissect the cellular events that lead to T- and B-cell activation and effector function and the consequences of T-and B-cell activation and effector function.
- Identify the genetic abnormalities that promote T- and B-cell activation.
- Facilitate the discovery of new approaches that allow the rapid testing of biologics targeted to autoreactive B and T cells.
- Improve understanding of how B-cell depletion ameliorates disease.
- Establish the role of regulatory T cells in lupus pathogenesis and the ability of induction of these cells to ameliorate features of clinical disease/autoimmunity.
- Utilize resources from B- and T-cell-directed therapeutic trials to further understand lupus pathogenic mechanisms.

Conclusion

Research in recent years has made great strides in understanding the immune processes involved in lupus. Both the innate and the acquired immune systems are involved in the development and perpetuation of the disease. Key inflammatory processes are triggered that lead to tissue damage. Each of these pathways of disease offers a myriad of targets for molecular and cellular intervention to halt disease progression. The hope is that a better understanding of these processes as well as sufficient numbers of patients for clinical trials of agents that block or modify those processes will lead to earlier detection of lupus and better ways to treat and perhaps prevent the disease.

CHAPTER 3: Mechanisms of Disease: Target Organ Damage

The manifestations of lupus are diverse and can affect many organs and organ systems including the joints and skin as well as vital internal organs such as the kidneys, heart, lungs, blood vessels, and brain. Although immune dysregulation plays a major role in lupus, recent evidence suggests that structural and functional properties of target organs may contribute significantly to the development of tissue damage and clinical disease. In animal models, scientists have clearly demonstrated that the threshold to renal and cardiac damage is variable. This finding is consistent with clinical observations showing that individuals with the same serologic abnormality do not necessarily share the same tissue pathology. Some of the deleterious effects of autoreactivity are likely due to interactions between antibodies and specific cellular elements in the target organ rather than the activation of the complement cascade. For example, newly identified binding properties of autoantibodies, present in sera from patients with autoimmune diseases, can trigger apoptosis or cell activation or block binding of the natural ligand.

Recent advances indicate that other target organ processes may be related to the induction and maintenance of damage, including expression of proteins related to organ function. These proteins may be expressed either spontaneously or induced by the cytokine milieu created by the inflammatory response or by events related to cell death during organ development and repair.

Vulnerability of the target organ to immune-mediated damage seems to be in part genetically determined. The genetic mapping of susceptibility genes in lupus suggests an important role for the target organ in the induction of tissue injury with contributions made by the cellular components and their interactions with both extracellular matrix and blood borne inflammatory cells.

The vasculature is often a direct target of autoimmune disease damage. Target organ damage may be due to primary damage to the vasculature in some cases. In other cases vascular damage and compromised circulation may further contribute to target organ damage. Immune complex deposition in the microcirculation appears to be particularly important in nephritis. Changes in the blood vessel walls, such as increased stiffness of the larger vessels, and /or accelerated atherosclerosis, may be contributing to the earlier incidence of strokes and myocardial infarctions seen in patients with lupus.

By better understanding mechanism of organ damage in lupus, researchers can investigate ways to modify these processes and perhaps minimize or prevent some of the most serious complications of the disease.

Kidney

Breakthroughs

- The discovery that resident renal cells contribute to kidney pathology.
- The discovery that TNF receptors on glomerular endothelial cells are critically important to that pathological process.

- The discovery of B7 expression on podocytes, which represents a new area of thinking about how the resident cells in the organism are participating in the disease process.

More than one-third of people with lupus suffer from lupus nephritis clinical signs and symptoms, such as weight gain, high blood pressure, dark urine, and swelling around the eyes, legs, ankles, or fingers. If not controlled, lupus nephritis can lead to progressive loss of kidney function.

Usually, kidney inflammation in lupus is characteristically associated with the production of nephritogenic autoantibodies, the deposition of immune complexes in the glomeruli and the local production of cytokines.

Recent discoveries point to the role of overexpression of chemokines and imbalance of dendritic cell homeostasis in the development of lupus nephritis.

Research in mice suggests that fortunately even severe proliferative lupus nephritis may be reversed and that there are critical pathological processes involved in the disease that are downstream of immune complex deposition. One study showed that mice lacking the gamma chain of Fc receptors, which is required for signaling, did not develop renal disease or kidney damage – even though they had the same amount of autoantibody deposition in the kidneys. Other research has shown that the STAT6 (STAT, signal transducers and activators of transcription) knockout mice, which have higher titers of antibodies and more deposition of antibodies in the kidneys, have less renal disease. This suggests that the widely assumed sequence of events is not correct (e.g., 1. immune complex occurs; 2. leading to complement activation; 3. leading to increased production of chemotactic factors; 4. leading to recruitment of inflammatory cells; and 5. leading to more immune complex deposition and a continuation of this cycle). Instead, current data suggest that there are clear events downstream of immune complex formation that are critical in complement activation. Recent papers suggest that Fc receptor expression on glomerular or endothelial cells may be one of those key events in activating the complement around the immune complex.

Other research points to an important contribution of resident renal cells to pathology. In recent years researchers have discovered that TNF is important to kidney disease in lupus, and there have been a few trials in Europe and some beginning in the United States using TNF blockers in the treatment of lupus nephritis. Although the thinking has been that these agents may help lupus nephritis by blocking TNF receptors on immune cells, it turns out that TNF receptors on the glomerular endothelial cells are the critical target in lupus nephritis.

Similarly, other normally immune cell-related molecules seem to be expressed by target organ cells during disease. In the kidney, B7-1 expression on podocytes is associated with lupus nephritis. It is known that bacterial lipopolysaccharide (LPS) can activate podocytes through TLR signaling. It is possible that immune complexes containing DNA or RNA may activate podocytes; however, this has not been shown. When B7-1 is

expressed on podocytes there is an alteration in the actin cytoskeleton and massive proteinuria occurs.

These recent findings represent a new area of thinking about the ways that resident cells within the kidneys – and perhaps other organs – are in fact participating in the pathology to cause damage. These findings represent interesting targets for therapy, which may be directed at the specific organ without requiring global immunosuppression.

It is important for future research to examine this collaboration and how immune cells join forces with resident cells to cause kidney damage. It is also important to better understand the reversibility of kidney disease to identify markers that will enable clinicians to predict when kidney disease can go into remission.

Immune complexes found in lupus can damage glomerular structures by attracting circulating inflammatory cells or activating resident glomerular cells to release vasoactive substances, cytokines, and activators of coagulation. The complement system, especially C5b-9, is a critical component of this inflammatory cascade. C5b-9 inserted in sublytic quantities into glomerular membranes results in cell activation, converting normal cells into resident inflammatory effector cells that cause injury. Excessive activation of the complement system is normally prevented by a series of circulating and cell-bound complement regulatory proteins. Genetic deficiencies or mutations of these proteins can lead to the spontaneous development of glomerular disease. Particularly promising future therapeutic approaches include selective immunosuppression and interference in complement activation and C5b-9-mediated cell injury.

Critical pathological processes occurring downstream of immune complex deposition and glomerular endothelial cells may be key. Recent technologies have been successfully used in humans with, and mouse models of, lupus nephritis to identify chemokines and their receptors in tissue and in the urine. For example, increased expression of interleukin (IL)-18 in glomeruli of patients with active lupus nephritis along with glomerular infiltration by plasmacytoid dendritic cells (pDCs) has been found. Since pDCs bear IL-18 receptor (IL-18R), it is conceivable that circulating pDCs may migrate toward glomeruli by IL-18/IL-18R interactions. Therefore, the relative depletion of circulating pDCs reflects the severity of inflammatory disease in lupus nephritis.

In addition, the chemokine receptors CXCR3 and CCR5 are expressed by a significant part of interstitial infiltrating T cells during glomerular diseases, and point toward a common pathophysiological pathway for tubulointerstitial injury in progressive glomerulonephritides. The correlations between clinical progression markers and CXCR3-positive cell infiltrate imply that CXCR3 might be a suitable therapeutic target for progressive human glomerular diseases.

Biopsies from patients with diffuse proliferative lupus nephritis have been shown to correlate with the severity of inflammatory cell infiltration. Sera from patients with lupus nephritis contained IgG antibodies that bound to cultured proximal renal tubular epithelial cells. This binding was accompanied by altered cell morphology and increased

cell proliferation. The binding of nonspecific antibody and specific antibody to proximal renal tubular epithelial cells resulted in differential and sequential upregulation of TNF-alpha, IL-1beta, and IL-6 secretion. These data suggest distinct immunopathogenic mechanisms during disease flare or remission. Results from these studies underscore the pivotal role of the proximal renal tubular epithelial cells in the pathogenesis of tubulointerstitial inflammation and fibrosis in lupus nephritis

Research Objectives

- Determine the genetic basis of renal pathology and the genetic basis of target organ vulnerability (predisposition to proliferative or sclerotic pathology).
- Develop a better understanding of renal cell activation and establish whether activation, injury and damage are reversible using mouse models that are translatable to human lupus nephritis.
- Evaluate renal cell rescue pathways and identify targets for intervention.

Central Nervous System

Breakthroughs

- Increased understanding of the molecular mechanisms involved in changes that occur in the brains of people with lupus, including the discovery that a subset of anti-DNA antibodies bind to glutamate receptors in the brain.
- The discovery that the same anti-DNA antibodies cause different neuropsychiatric disorders, depending on which area of the brain is exposed to them.
- The ability to obtain serial measurements of cognitive deficits with a neuropsychological testing instrument and correlate those with lesions in the brain.
- The discovery that neuropsychiatric manifestations may develop some time before the diagnosis of lupus.
- The finding that functional and structural damage to the brain may be evident early in the disease process, underscoring the need to understand the underlying brain involvement in lupus.
- The finding of predictors or risk factors for cognitive dysfunction.

A wide variety of associated neurological and psychiatric syndromes have been placed under the heading of neuropsychiatric lupus. These include stroke, transverse myelitis, cerebritis, mood disorders, inflamed cerebral blood vessels, seizures, headaches, and cognitive dysfunction (disordered thinking). Many lupus patients experience some form of neuropsychiatric involvement. Manifestations may occur as single or multiple events, with or without systemic disease activity and may precede other signs of systemic disease or a lupus diagnosis. Potential risk factors include antiphospholipid antibodies (associated with thrombosis, thrombocytopenia, and recurrent fetal loss); anti-ribosomal P antibodies (associated with psychosis); anti-glutamate receptor antibodies (associated with cognitive problems); and secondary effects of other lupus-related disease manifestations or treatment such as renal disease, atherosclerosis, infection, and medication.

In recent years, research has made significant advances in understanding the molecular mechanisms involved in changes that can occur in the brains of people with lupus. For example, researchers have discovered that a subset of anti-DNA antibodies bind to the glutamate receptor. They have shown in mice, that if these antibodies penetrate the blood-brain barrier and come in contact with neurons with glutamate receptors, the interaction of the autoantibodies with the receptors alters the neuron's metabolism and the cell dies; cognitive dysfunction or behavioral disorders can occur. Scientists suspect there may be many antibodies that function by interacting directly with cells rather than engaging Fc receptors and activating complement.

In studies of this subset of anti-DNA antibodies to the glutamate receptor, scientists have learned that agents open the blood brain-barrier in a region-specific manner. For example, when they disrupt the blood-brain barrier with lipopolysaccharide (LPS), mimicking infection, these antibodies went to the hippocampus, resulting in a memory disorder. When they opened the blood-brain barrier with epinephrine, mimicking stress, the antibodies went to the amygdala, resulting in a behavioral disorder. In other words, the same antibodies cause different neuropsychiatric disorders, depending on which area of the brain is exposed to them. This shows that non-disease-related factors such as infection, stress or smoking could lead to tissue damage and provides evidence that environmental factors can exacerbate lupus. Furthermore, scientists believe these findings may be generalizable to the entire body and that other factors – whether they be concurrent infections or toxic exposures – may sensitize the cells to lupus injury. Despite the generalizability, however, brain effects will be different from those in other organs in that they require two hits – the antibody itself and opening the blood-brain barrier to allow the antibody to enter. This two-step process makes central nervous system involvement more difficult to study than involvement of other organs.

Understanding lupus-related changes to the brain and their neuropsychiatric effects requires reliable tools to assess cognitive impairment and mood alteration. Such tools are also needed in clinical trials to provide objective proof of the effectiveness of therapies for these problems.

In recent years, there has been progress in obtaining serial measurements of cognitive deficits with a neuropsychological testing instrument and correlating those with lesions in the brain. But there are few longitudinal studies; they are all cohort studies by and large without control groups. This is an important gap in our knowledge, amplified by how common cognitive dysfunction (especially on the mild end of the severity scale) appears to be in people with lupus.

One study, the San Antonio Lupus Study of Neuropsychiatric Disease (SALUD), looked at cognitive dysfunction and 18 other neuropsychiatric symptoms in 181 primarily Hispanic subjects for up to five years with study visits every four months. Twenty-two met 1982 American College of Rheumatology (ACR) Diagnostic Criteria for Neurological Involvement (seizures/psychosis) at study entry, whereas 80 percent had one of the other 17 criteria for neuropsychiatric lupus syndromes identified by the ACR. These data compare favorably with those obtained from a Finnish group, whose lupus

cohort was entirely Caucasian. In both studies, only 20 percent were completely normal and the majority had mild cognitive dysfunction. Predictors of high-level cognitive function in SALUD included: visit number (indicating a practice effect) and higher education. Predictors of cognitive dysfunction in SALUD included: Hispanic ethnicity, higher depression scores, higher disease/damage activity, consistent prednisone use, persistently positive antiphospholipid antibodies and persistently positive anti-ribosomal P antibodies.

SALUD used computerized neuropsychological testing (ANAM) developed by the Department of Defense. This instrument is available free of charge to qualified users and is independent of language or even literacy. A study looking at the validity of ANAM and ACR battery shows there is a good comparability between the two. Healthy individuals stabilize their performance after 2 test administrations; however, there are not longitudinal studies in normal individuals and thus no long-term normative data. This poses a problem in trying to interpret longitudinal data in lupus patients.

In a study of cognitive dysfunction in a lupus inception cohort, Brain CONNECTIONS, researchers found that cognitive dysfunction is already present at the time of lupus diagnosis and the magnitude of dysfunction was similar to that seen in the SALUD cohort whose average baseline disease duration was about 10 years. Depression was found to be independently associated with cognitive dysfunction in this cohort.

Anatomic MRI studies in Brain CONNECTIONS showed a high proportion of cerebral atrophy in young patients with disease duration of less than 9 months. This suggests that some of the neuropsychiatric manifestations may be present for some time prior to the diagnosis of lupus. It may be necessary to aggressively evaluate patients with any lupus diagnostic criteria for cognitive dysfunction in order to better understand when this insult occurs in the disease course.

Research Objectives

- Identify novel mechanisms of brain disease.
- Develop assessments of brain function that allow for endpoints to be identified in less than one year to facilitate clinical trials.
- Better characterize the cognitive dysfunction in adults and children by developing standardized assessments.
- Examine the correlation between lupus and psychiatric disease, especially depression, in adults and children.
- Develop biomarkers and targets of neuropsychiatric lupus, including functional brain imaging and advanced serology.
- Develop and test novel treatment strategies including the lupus-specific treatment of depression, as well as therapies for cognitive impairment due to causes other than depression.
- Understand how regional differences in the blood-brain barrier permeability affects the neural consequences of the disease and explore therapeutic targets that modify the entry of autoantibodies into the brain. .

Skin

Breakthroughs

- The finding that certain clinical patterns of skin disease can reflect the level of activity of systemic disease.
- The development of the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI), an outcome measure specific for cutaneous lupus disease activity and damage.

Skin involvement is one of the most prevalent clinical manifestations of lupus and may present as part of the systemic disease or present as skin disease only, in the case of cutaneous lupus erythematosus. In systemic lupus erythematosus, the skin may be involved in 70-80% of the patients. In addition, the prevalence of cutaneous lupus is 2-3 times higher than systemic lupus. Skin lesions in both diseases present as inflammatory plaques predominantly in traditionally sun-exposed areas, although in cutaneous lupus large areas of the body may be covered with skin lesions. Hair loss is common in discoid and systemic lupus. The visibility of skin lesions as well as the potential for scarring and disfigurement presents both psychological stress and a lower quality of life.

Cutaneous lupus can be characterized into three broad heterogeneous clinical subtypes: acute cutaneous, subacute cutaneous, and chronic cutaneous (discoid). These characterizations are based on clinical and histopathological findings. Some patients may have more than one subtype. In addition, although cutaneous lupus occurs primarily as a skin disease, there is a potential based on subtype for cutaneous lupus to occur in the context of systemic lupus or are more likely to progress to systemic disease.

Photosensitivity is strongly associated with the manifestation of all subtypes of cutaneous lupus. Abnormal reactivity to UV light is an important factor in the pathogenesis of this disease. Deficiency in clearing apoptotic cells, changes in regulatory molecules of apoptosis and changes in the number of resident and trafficking immune cells all have been shown to be induced by UV light and also play a role in autoimmunity.

Certain cell types in the skin make attractive targets for research because their unique locale exposes them to a variety of autoantigens that may be induced by environmental factors such as microbes or UV. The Langerhans cells in the skin may get exposed to apoptotic material released from skin cells such as keratinocytes. In normal individuals, this process leads to tolerance. However, in lupus patients this response may not be properly regulated. "Plasmacytoid" dendritic cells are frequently found in skin lesions and through their secretion of type I interferons may play a pathogenic role in cutaneous lupus.

Exciting new work suggests that other cell types and inflammatory mediators may be key players in the development of cutaneous lesions in lupus. Patients with cutaneous lupus may have greater numbers of circulating T cells with skin homing molecules. Skin lesions in lupus patients also show increased quantities of chemokines or homing signals for these T cells. Once in the skin, cytotoxic lymphocytes may induce damage in specific

epidermal and skin structures such as blood vessels and hair follicles. Targeting cell surface molecules and mediators that direct T cell migration offers new opportunities to modulate and prevent skin lesions in lupus.

Better understanding the role of genetics and environmental triggers and the role of the immune system in lupus skin disease is an important goal of future research. It is also important to further understand the links between skin involvement and systemic disease as well as understand how changes in skin involvement may predict and/or reflect flare of systemic disease. Understanding skin involvement in relation to systemic disease may provide a measure for the efficacy of new therapeutics for both those with cutaneous lupus as well as systemic lupus erythematosus.

Research Objectives

- Develop a better understanding of the role of cellular and molecular mechanisms, both in the immune system and in the skin, in the initiation of lupus skin disease, via UV light, medications and other environmental triggers.
- Better understand the cellular and molecular mechanisms involved in pathogenesis of the skin manifestations of lupus both in systemic diseases as well as in cutaneous lupus.
- Use new technologies that allow the identification of patient subsets, better characterize the lesions, and identify patients with higher risk for more severe cutaneous disease.
- Further develop and validate an instrument to measure cutaneous lupus activity and response to treatment.

Cardiovascular System

Cardiovascular manifestations, including atherosclerosis and the antiphospholipid syndrome, are common among lupus patients, and are the subject of considerable interest and scientific research. While scientists are learning more about cardiovascular problems associated with lupus, a much better understanding of these processes will be required to minimize morbidity and mortality.

Atherosclerosis

Breakthroughs

- The finding that atherosclerosis is significantly increased (accelerated) in lupus, most strikingly in younger individuals.
- The finding that there is an increase in stiffness of the larger blood vessels in patients with lupus.
- The finding that risk of premature atherosclerosis in lupus patients is not attributable to traditional cardiovascular risk factors or corticosteroid therapy.
- The association of atherosclerosis with longer disease duration, higher damage score and less aggressive immunotherapy, suggesting that chronic inflammation is atherogenic in lupus.

- The institution of Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial to study the effects of statins on the development of atherosclerosis in children with lupus.

Chronic inflammatory diseases, including lupus, are associated with the premature development of atherosclerosis, or hardening of the arteries. Atherosclerosis in lupus is not solely attributable to traditional risk factors – such as diabetes, hypertension, high cholesterol, or smoking – or to the use of corticosteroid therapy. Recent research shows that atherosclerosis in lupus is associated with longer disease duration, higher organ damage scores, and the use of less aggressive immunosuppressive therapy for lupus. These findings support the premise that chronic inflammation is atherogenic.

Future research into the role of inflammation in atherosclerosis is important not only for people with lupus, but for those with rheumatoid arthritis – whose rates of preclinical atherosclerosis are identical to those for people with lupus – and other chronic inflammatory diseases. An understanding of inflammatory mediators and mechanisms will likely lead to treatments to control or prevent the problem.

To better understand the factors in atherosclerosis and to better target preventive therapies, it is important for researchers to identify lupus patients at greatest risk of cardiovascular complications. Normally, an increase in carotid intima-medial thickness (IMT, a measure of the thickness of the carotid artery wall) demonstrating plaque buildup, is a predictor of atherosclerosis. An increase in IMT, however, is not typically seen in patients with lupus, suggesting that the mechanism of atherosclerosis in lupus patients – and instruments needed to predict the development of atherosclerosis in lupus patients – is different from the general population. In lupus, more antibodies tend to be associated with more cardiovascular disease, but further research is needed to confirm and understand that connection and to stratify patients for risk.

Further research is also needed to confirm the role of statin drugs as therapy for atherosclerosis related to lupus. APPLE, a major NIH-sponsored trial is currently studying the use of statins in the prevention of atherosclerosis in pediatric lupus patients. While there is clear data on statins from *in vitro* studies and animal models, the role of statins in cardiovascular disease in lupus patients is less clear.

Research Objectives

- Identify links between chronic inflammation and the initiation and progression of atherosclerosis.
- Define inflammatory mediators and nontraditional mechanisms of atherosclerosis.
- Examine the interplay of inflammation, thrombosis and vasculopathy.
- Identify antigenic specificities on endothelial cells targeted by lupus antibodies and how these alter endothelial cell function.
- Conduct longitudinal studies to identify patients at greater risk for atherosclerosis/cardiovascular disease and validate soft endpoints.
- Develop effective treatments to prevent accelerated atherosclerosis/cardiovascular disease.

Antiphospholipid Syndrome (APS)

Breakthroughs

- Finding of evidence that inflammation is an important contributor to APS.
- The finding that activation of complement might be a causative factor in antiphospholipid antibody-mediated pregnancy complications.
- The finding that heparins may prevent obstetrical complications in women with APS by blocking activation of complement as well as excessive blood coagulation.
- The finding that antiphospholipid antibodies activate endothelial cells and alter endothelial cell phenotype.
- The finding that antiphospholipid antibodies bind to tissues subjected to ischemia-reperfusion injury and mediate tissue damage.
- The definition of antiphospholipid antibody epitope specificity by protein-based immunoassays.

APS is a disorder characterized by the presence of antiphospholipid antibodies (cardiolipin, anti-beta-2-glycoprotein 1 or lupus anticoagulant antibodies) in the blood, excessive blood clotting, and pregnancy complications, including miscarriage and fetal death. APS is a disease characterized by two closely related processes: thrombosis and inflammation. Heparin, which is the treatment of choice to prevent pregnancy complications in women with APS, blocks coagulation of blood in humans and has recently been shown to block the activation of complement in mice.

Other recent developments in APS research include the first evidence that antiphospholipid antibodies activate endothelial cells, alter endothelial cell phenotype, and affect vasculature by either inducing thrombophilia, atherosclerosis or even fibrotic changes; however, the mechanisms underlying these changes are not clear. There is a body of literature arguing that annexin II and certain low-density lipoprotein (LDL) receptors may be cross-linked by antiphospholipid antibodies and transduce signals that alter endothelial, platelet and even trophoblast function, but this has not been confirmed.

There is also evidence that antiphospholipid antibodies bind to tissue and mediate tissue damage in tissues subject to ischemia-reperfusion injury. This evidence is important not just in understanding blood vessel disease, but also in understanding more global lupus damage. Antibodies may bind to hypoxic tissues and trigger damage induced by complement.

Finally, there has been recent work describing protein antigens that are recognized by antiphospholipid antibodies, including thrombin and prothrombin. However, it is not clear whether these antigens will be useful to phenotype, understand the pathology or predict the outcome of lupus.

In the future, researchers must gain a better understanding of the link between antiphospholipid antibodies, thrombosis, and vasculopathy. It is important to find ways

to determine which patients with elevated antiphospholipid (APL) antibodies are at greatest risk of developing the syndrome. Identification of additional epitope specificity of APL antibodies may be useful. An intriguing observation that needs additional study is the presence of APL antibodies in pediatric patients with complicated thrombosis.

Research Objectives

- Examine links between inflammation, thrombosis, and vasculopathy.
- Identify patients at greatest risk for pregnancy complications and thrombosis.
- Define the role of APL antibodies in dementia.
- Study the role of APL antibodies in atherosclerotic cardiovascular disease in a younger population of lupus patients.
- Investigate gene expression profiling and proteomic studies of endothelial cells and platelets in patients with APS for the risk of thrombosis and miscarriage.
- Develop diagnostic and prognostic strategies to identify women at risk of recurrent fetal loss and thrombotic events which could result in adverse pregnancy outcomes, and the need for antithrombotic therapy in subsequent pregnancies.

Hormones/Pregnancy

Hormones

Breakthroughs

- The finding that estrogen regulates anti-DNA antibodies in mice transgenic for heavy chain of anti-DNA antibodies.
- The discovery that prolactin increases titer and renal deposition of anti-DNA in mice transgenic for heavy chain of anti-DNA antibodies.
- The finding that estrogen-induced autoimmunity proceeds through two distinct pathways, one affecting survival of autoreactive B cells, the other affecting their maturation.
- The identification of a discrete region of human chromosome 5 that carries susceptibility genes for lupus and autoimmune thyroiditis.

The role of estrogens in lupus is an area of scientific interest for a number of reasons. Lupus in women is nine times that of men, it usually presents during the child-bearing years, and estrone metabolism in lupus is skewed toward 16α -hydroxylated compounds, which are potent estrogens. Furthermore, there have been reports of lupus flares occurring in women receiving exogenous estrogens. One retrospective study of patients with preexisting kidney disease suggests that exogenous estrogens caused adverse effects. In laboratory mice, giving estrogen exacerbates lupus and, depending on genetic background, augments the survival and autoreactivity of B cells.

Concern about potential effects of exogenous estrogens on lupus has deterred many doctors from prescribing oral contraceptives or hormone therapy for women with lupus. Yet there have been no placebo-controlled trials on which to base that concern, and there are situations in which estrogen might provide benefit for women with lupus. For example, because of potential pregnancy complications and the risks of

immunosuppressive drugs on a fetus, women with lupus need reliable birth control. Hormone replacement therapy may help ease the effects of premature menopause, which can be brought on by cytotoxic drugs such as cyclophosphamide (Cytosan) or help prevent post-menopausal bone loss, which can be accelerated by corticosteroid use.

These potential benefits in light of the long-held concerns about estrogen in women with lupus prompted the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) trial, which contained two separate randomized, placebo-controlled studies examining the risks of hormone therapy and oral contraceptives. For both, the news was promising, for the most part. Neither showed an increased risk of severe flares in women taking estrogen-containing oral contraceptives or hormone replacement therapy; however, the risk of mild-to-moderate flares was increased significantly in women taking hormone replacement therapy. Patients enrolled in these studies were anti-phospholipid antibody negative and had stable disease for a period of 3 years prior to enrollment.

In mice, estrogen has been shown to regulate anti-DNA autoantibodies. In studies of mice transgenic for heavy chain of anti-DNA antibodies, estrogen breaks tolerance of the hyperreactive B cell and increases anti-DNA titers, which translates clinically into complex deposition in the glomeruli. In these same mice, estrogen rescued high affinity autoreactive B cells from negative selection, and interestingly Bcl-2, CD22 and SHP-1 are estrogen-responsive genes, which in paradoxical ways may perturb negative selection. When the role of prolactin was studied in mice, the results were similar with increased anti-DNA titers and renal deposition of anti-DNA.

Estrogen appears to induce autoimmunity through two distinct pathways – one affecting survival of autoreactive B cells and the other affecting maturation of autoreactive B cells.

Better understanding the role of estrogen in lupus is an important goal of future research. It is also important to identify lupus patients who will be able to tolerate exogenous estrogens and those for whom estrogen may cause flares. Researchers should also examine the relationship between hormones and coagulation and the risk of atherosclerosis.

Thyroid gland inflammation is common in women with lupus and it can result in over- or under-production of thyroid hormones. A study stratifying lupus patients by comorbidities found a linkage between lupus and autoimmune thyroiditis; susceptibility genes were found for both disorders in a discrete region of human chromosome 5.

Research Objectives

- Continue to explore the estrogen connection to lupus.
- Evaluate hormones and coagulation in atherosclerotic risk.
- Evaluate whether estrogen differentially regulates target organs.

Pregnancy

Breakthroughs

- The discovery of hydroxychloroquine's safety during pregnancy and that stopping hydroxychloroquine may be associated with increased flares.
- The finding that antiphospholipid antibody-associated pregnancy loss is complement-mediated.
- The finding that activation of complement is a proximal and essential causative factor in antiphospholipid antibody-mediated pregnancy complications in mice.
- The finding that heparins prevent obstetrical complications in women with APS because they block activation of complement induced by antiphospholipid as well as block excessive blood coagulation.

Pregnancy complications, such as preeclampsia, preterm birth, miscarriage, and low birth weight – are more frequent in lupus. These problems are especially seen in women with antiphospholipid antibodies. Animal studies have shown that the predominant proximal and causative factor is the activation of complement. Heparin has been the treatment of choice for these pregnancy complications. Furthermore it has been shown in studies of laboratory mice that low molecular weight heparins block the activation of complement. Understanding the role of complement in human lupus pregnancy is being followed in a multi-center study called Predictors of Pregnancy Outcome, Biomarkers in Antiphospholipid Syndrome and Systemic Lupus Erythematosus (PROMISSE) (See Chapter 5).

Another concern for some expectant mothers is congenital heart block (CHB), a condition in which the formation of fibrous tissue in the baby's heart causes interference with the electrical impulses that affect heart rhythm. CHB carries significant mortality and morbidity. In 15 percent to 30 percent of cases, it results in fetal or prenatal death; 67 percent of children who survive require cardiac pacemakers before adulthood. An important area of research is focused on understanding these problems specifically and using the knowledge gained to better understand the disease generally.

In the laboratory, CHB has been associated with autoantibodies. But the roles of autoantibodies in mediating tissue injury are not well understood; nor are antibodies likely to be the sole cause of CHB. Scientists trying to better understand these causes suspect an interplay of genetic and environmental factors.

Women in their first pregnancy with anti-Ro and anti-La antibodies have a 2 percent chance of having a baby with CHB. If a woman has had a baby with CHB, the risk of having a subsequent child with the problem is much increased. Research also shows that heart fibrosis is the most rapid of any disease in the fetus. A fetus with a normal heart rhythm as measured by echocardiogram *in utero* at week 16 or 17 of gestation can progress to third-degree block within seven to 10 days. Incomplete block can progress even after the antibody is gone.

Because fetal arrhythmias can now be evaluated prenatally using echocardiographic and Doppler ultrasound techniques, atrioventricular block is more frequently identified during the fetal period. Auscultation may be misleading because the atrial rate may be mistaken for the ventricular rate. A number of groups have reported that bradycardia, as

determined by fetal echocardiography, has been noted after the fetus had an initially normal heart rate. As previously reported in a few fetuses and confirmed in this larger study, CHB is most often detected before the 30th week of gestation. Fetal echocardiogram is essential to follow the course of disease and may suggest the presence of an associated myocarditis by finding decreased contractility and secondary changes such as an increase in cardiac size, pericardial effusions, and tricuspid regurgitation. Serial fetal echocardiography at 16, 18, 22, and 24 weeks of gestation may be reasonable for all women at risk for a child with CHB; more frequent echocardiography may be reasonable if abnormalities are detected. Dexamethasone therapy has been administered to women carrying fetuses with CHB and myocarditis in an attempt to diminish the inflammatory insult to the fetus. Resolution of pleuropericardial effusions and ascites occurred during dexamethasone administration. Dexamethasone and betamethasone have been recommended rather than prednisone because these fluorinated steroids are not metabolized by the placenta and is available to the fetus in an active form.

Research on affected hearts in some cases has shown profound apoptosis. The potential role of apoptosis in CHB has been the focus of attention in two laboratories. One group of investigators recently identified physiologic apoptosis, translocation of SSB/La, and binding of anti-SSB/La antibodies in the developing murine heart. Another group, whose work supports those findings, found that in vitro, human cardiocytes rendered apoptotic are bound by anti-SSA/Ro-SSB/La antibodies.

Physiologic apoptosis has been found to result in translocation of the intracellular SSA/Ro-SSB/La antigens to the cell surfaces where they are bound by maternal antibodies. This binding – called opsonization – triggers an inflammatory response by macrophages. In vitro studies using cardiac myocytes and fibroblasts from human fetal hearts provide evidence of a link between antibodies and injury.

By better understanding these and other factors in CHB, scientists hope that they can predict those at greatest risk and, even more importantly, prevent this serious lupus complication.

Research Objectives

- Identify fetal risk factors for cardiovascular heart block.
- Find ways to prevent cardiovascular heart block.
- Identify risk factors for antiphospholipid antibody-mediated pregnancy loss.

Conclusion

Understanding organ-specific manifestations of lupus is likely to have implications for a better understanding of disease mechanisms in general. To advance this research field, translational research and multidisciplinary approaches are critical. The rapid application of advances in kidney research and the neurosciences will be critical to improve the diagnosis of disease subsets and disease course. By understanding the cause(s) of specific manifestations, researchers can better target therapies and identify people who

are at greatest risk of complications and would benefit most from new therapies and even preventive measures.

CHAPTER 4: Pediatric Lupus, Special Populations, Epidemiology, and Health Services Research

Lupus is a disease that affects people of virtually every age, race, ethnic background and socioeconomic status. Yet some groups are affected more commonly and/or severely than others. For example, children and adolescents under the age of 18 make up only 20 percent of lupus patients, yet the disease in children is often associated with more severe renal and hematologic involvement than it is in adults. As many as 60 percent of children with lupus have renal involvement (lupus nephritis).

Lupus is believed to be three times more common in African Americans than in white women. It is also more common among women of Hispanic/ Latino, Asian and Native American descent. Black and Hispanic/ Latino women tend to develop symptoms at an earlier age than other women do. African Americans and Hispanics also have more severe organ system involvement; that is particularly the case for lupus nephritis.

Research on these high-risk populations is a major focus of lupus investigators. By studying the factors that lead to disease or more severe disease in these populations, scientists will gain a greater understanding of these health disparities and how to address them. But even more importantly, research in one group has potential implications for all groups, by providing scientists clues into the mechanisms of and eventually treatments for the disease.

Pediatric Lupus

Breakthroughs

- The recognition that lupus is similar in children and adults, yet children tend to have more severe renal and hematologic manifestations.
- Recognition of the role of type I interferon signature and abnormal myeloid cells play in active pediatric lupus.
- The establishment of the Childhood Arthritis and Rheumatology Research Alliance (CARRA), a network to study pediatric lupus and other childhood rheumatic diseases.
- The establishment of the APPLE trial in pediatric lupus. This trial has increased the understanding of institutional, regulatory, budgeting, site, coordinating center and design issues.

Because of their disease severity and young age, lupus is a particular burden for children. Thus, any research that leads to improved understanding, treatment and quality of life in children has significant, lifelong implications.

Yet research in children has the potential to do much more than help children; more generally, studying children is valuable for a number of reasons.

- Children are more likely to be referred to pediatric or adult rheumatologists earlier in the disease process than adults are, because the disease often becomes severe more quickly in children.

- Children can be followed very long-term because of their young age, offering insights into the course of the disease over time and the long-term benefits and risks of the disease and its treatment.
- Pediatric disease is similar to adult lupus. Aside from the severity of renal and hematologic manifestations, disease manifestations in children do not differ qualitatively from those of adult-onset disease. Thus, many findings about disease in children are likely to be applicable to individuals of all ages.
- Children are less likely to have comorbid conditions or to have been or be on other medications. They also have a better tolerance to medications in general and less hepatic damage from lupus treatment.
- Children with lupus, at ages prior to, and going through puberty, provide a unique view to understanding how hormones affect various aspects of lupus.
- Genetic studies may be more revealing in children, because stronger genetic contributors lead to earlier disease onset. Studying inheritance is possible because most children have at least one living parent, which allows for the study of DNA in both parent and child. Similarly, there is a wave of information emerging about autoinflammatory diseases and the genes associated with them that usually have onset in childhood or adolescence. These include Familial Mediterranean Fever (FMF), TNF Receptor-Associated Periodic Syndrome (TRAPS), and most recently, Neonatal Onset Multisystem Inflammatory Disease (NOMID). Each of these diseases is rare, but is so characteristic that it would be helpful to study them for a better understanding of other autoimmune diseases.
- Children's immune systems and microbial immunity are still developing until age 18 to 21. Lymphoid tissue grows until puberty; IgA antibodies reach adult levels only after puberty and thymic involution does not begin until puberty. Children and adolescents have somewhere in the range of 10^{14} (100 trillion) T-cell receptors, whereas adults have just 10^7 (10 million), so an enormous amount of pruning occurs at adolescence and beyond. Studying children allows scientists to compare normal immune system development to lupus etiology.
- Studying differences in microbial immunity between children in developed and developing countries may provide clues to autoimmunity as well. For example, immunity to common viruses, such as EBV and cytomegalovirus (CMV) occurs much earlier in developing countries. In Western countries, greater than 90 percent of individuals have immunity to EBV and CMV in early adulthood. In developing countries with underserved populations, however, immunity to both viruses occurs much earlier; 90 percent or greater may have immunity by age 5. Such differences could potentially affect the development of autoimmune disease.
- Studying the role of environmental triggers may in some ways be more revealing in children and adolescents since these populations tend to have a lower threshold for some environmental triggers. Studies looking for an association between lupus and microbes that commonly infect a large section of the population may be more likely to pay off in children, in whom the differences between exposed and unexposed individuals is greatest. For example, both Lyme disease and toxic shock syndrome were first recognized in children. The course of pediatric lupus varies geographically, raising questions about possible environmental exposures that may be triggering and/or exacerbating lupus in those areas.

However, challenges to studying children do exist. The primary drawback of studying children is the relatively low number of pediatric patients, which makes it difficult or impossible to find enough children in a single center to study. Multi-center studies could potentially draw together more patients; however coordinating and harmonizing several centers is challenging. Additional challenges include:

- institutional, regulatory, budgetary and design issues;
- limited or non-existent access to patient materials and technologies for translational and mechanistic studies;
- difficulties in conducting long-term studies when children transition from pediatric to adult care.

It is important to increase the number of pediatric patients in adult studies to show stratification by age. Already some clinical trials that are mainly for adult patients are accepting older children. For example, one of the trials investigating mycophenolate in adults allows enrollment of children from age 12. It is essential to find ways to access young patients who would be appropriate for those studies and to develop ways to alert pediatric rheumatologists to the availability of those studies to appropriate patients.

Research Objectives

- Examine the genomics of lupus in children.
- Examine the immune development and microbial immunity in the development of lupus.
- Elucidate the role of environmental factors in pediatric lupus.
- Improve access to clinical trials for pediatric lupus patients through clinical networks.
- Develop core services such as tissue repository, immune reagent development cores, genomics, proteomics and metabolomics capabilities, informatics, pathology, etc.

Special Populations

Breakthroughs

- The discovery that there is under-ascertainment/underreporting of deaths in death certificates of patients with lupus.
- The finding that African American ethnicity, lack of health insurance, and older age are associated with underreporting.
- The finding that African admixture is an important risk for the occurrence of lupus.
- The finding that African American children with lupus nephritis experience progression to end-stage renal disease despite aggressive treatment.
- The finding that African American children with either lupus nephritis or neuropsychiatric involvement experience high mortality rates despite aggressive treatment.

- The finding that Hispanic ethnicity is a risk factor for the occurrence of initial damage.
- The finding that poverty, not ethnicity, is a consistent predictor of mortality in lupus.

Lupus occurs disproportionately in certain ethnic populations. Until recently, studies of minorities and lupus focused on African Americans as the main minority population in the United States, but an increasing Hispanic population, now the largest minority and fastest growing – not only in the Southwest or the Northeast, but in Colorado and the Southeastern United States – has brought more attention to the unique problems of that group.

The largest study of lupus in ethnic populations to date, the Lupus in Minorities: Nature vs. Nurture (LUMINA) study, was designed to understand why minorities are affected more frequently and more severely by lupus. LUMINA is a multi-ethnic longitudinal study of outcome in three geographic areas: Alabama, Texas and Puerto Rico. Highlights of the study's findings include:

- Hispanics from Texas tend to develop damage more rapidly; however, over time, the damage is comparable to that accrued by African Americans.
- Hispanics from Texas and Hispanics from Puerto Rico have different disease manifestations, course, and outcome. Patients from Puerto Rico have much milder disease but also have much better socioeconomic status.
- Caucasians accrue less damage from lupus than do Hispanics or African Americans.
- African Americans are more likely than Caucasians and Hispanics to die from lupus, although when poverty is considered, poverty – and not ethnicity – is a consistent predictor of mortality in lupus.

Aside from the study's ethnicity-specific findings, the LUMINA study has demonstrated that hydroxychloroquine seems to prevent the occurrence of damage in lupus and increase survival by as much as 60 percent. This finding is particularly important because in the past hydroxychloroquine was used only in patients with mild to moderate, but not severe, disease.

Studies in other ethnic minority populations have offered additional insights. For example, a study by British researchers showed that African admixture was a risk factor for the occurrence of lupus among residents of the Caribbean island of Trinidad, while the incidence of lupus in Africa itself is not that common.

Research has shown that both Hispanics and African Americans have a rapid progression of kidney disease. It is not known whether this rapid progression is related to poor access to care or lack of compliance with prescribed care or whether it actually represent a less favorable response to treatment. An argument for a less favorable response to treatment is reflected in a pediatric study, looking at predominantly African American children. Children with lupus nephritis and neuropsychiatric involvement had a higher rate of

progression to end stage renal disease and a higher mortality rate than children with lupus nephritis alone despite aggressive treatment.

Yet studying lupus deaths is difficult due to significant under-ascertainment of lupus in death certificates. Underreporting has been associated with certain factors such as African American ethnicity, lack of health insurance and older age.

To fully understand the role of poverty in disease in minorities, researchers must understand what aspects of poverty are related – for example, is it that they cannot get health care or that they do not take the medication? Statistically, poverty becomes such a strong variable that it overshadows everything else.

Providing the best treatment for minorities and, in fact, all patients with lupus, requires not only aggressively treating the severe manifestations such as lupus nephritis, but also not losing sight of basic lifestyle factors. For example, smoking is a major risk factor for atherosclerosis, which is already a common problem in patients with lupus, yet many lupus patients smoke. Programs to help people with lupus stop smoking could help minimize complications of the disease.

Also, it is vital to find a systematic way to inform clinicians about proven medication benefits. For example, if a treatment like hydroxychloroquine prevents the accrual of damage, it should be introduced as a therapy for everybody with lupus as close to the diagnosis as possible.

Research Objectives

- Better define the distribution of lupus and its impact at the population level.
- Disentangle the contribution of socioeconomic vs. genetic factors to the occurrence, course, and outcome of lupus.
- Identify lupus patients at risk of poor intermediate and long-term outcomes and intervene on risk factors.
- Identify strategies to improve lifestyle habits among lupus patients, including smoking cessation.
- Study lupus in other minority groups such as Native Americans, who tend to have severe disease and antibodies that do not always correlate with that in lupus patients of other ethnicities.

Epidemiology

Accurate estimates of the number of people who have a disease – as a whole and within specific groups – are important for a number of reasons, including understanding the disease and its impact; predicting groups and individuals who are most likely to develop lupus and, thus, are candidates for screening and preventive care; and providing care and services to people with the disease. Getting accurate estimates and updating them periodically can also enable researchers to determine if disease incidence is increasing and if so, start taking steps to understand why.

But estimates about the number of individuals with lupus vary widely, depending on the study from which the estimate was derived. Different study techniques, methodologies, populations and definitions of what constitutes lupus can lead to vastly different prevalence estimates. The range is large and tells us little about the true magnitude of the problem.

What most researchers agree on is that lupus is more common in women than men and that African Americans, Afro-Caribbeans, Hispanics, Asians, and Native Americans are affected more commonly than Caucasians. But even the degree of that discrepancy is disputable. The estimates range from a five to nine or 10 times increase among those high-risk populations.

Prevalence and incidence figures for other groups and specific lupus-related problems can be even more problematic. Following are some examples:

Lupus pregnancy and neonatal lupus

As with lupus in general, there are not consistent estimates of the prevalence of lupus pregnancy or neonatal lupus. Neonatal lupus is a rare, lupus-like disease in a neonate that begins during pregnancy due to the transfer of autoantibodies from mother to child through the placenta.

In neonatal lupus there are differences in incidence estimates depending on the aspect looked at – congenital heart block vs. the presence of anti-Ro and/or anti-La antibodies vs. cutaneous involvement. Even so, there is more incidence data on the disease than prevalence data. The prevalence of neonatal lupus is difficult to determine when disease presents beyond the neonatal period (0-6 months) and long-term follow-up of children with mothers that have lupus, to a certain extent, is lacking.

Estimates of other problems in pregnancy related to lupus are also lacking. Most figures have been of spontaneous abortion, fetal death and total losses in pregnant lupus patients. Lupus-related problems in pregnancy may not necessarily result in a fetal loss, but rather fetal growth restriction, preeclampsia and preterm birth. The estimates of these vary widely. All of these problems are increased in women with lupus compared to women without the disease, but studies showing the relationship between lupus and these pregnancy complications were conducted before hydroxychloroquine came into wide use as a lupus treatment and particularly throughout pregnancy. Researchers are interested in learning how use of hydroxychloroquine in recent years may have impacted these figures.

Exogenous agent-induced lupus

Encompassing a form of lupus referred to as drug-induced lupus (DIL), exogenous agent-induced lupus is a lupus-like syndrome that occurs in response to an agent such as a drug, UV light or an infectious agent. In some cases, agents may modify the course of lupus by unmasking or accelerating disease. Examples of such agents that have been studied (but not necessarily all proven to cause or accelerate the development of lupus) include silica,

heavy metals, hair dye and smoking. The incidence and prevalence of this condition is not known.

Given the relatively low incidence rates of lupus in the general population, classical incidence and prevalence studies of environmentally-induced lupus would be difficult to conduct, especially in the absence of widespread disease registries. There is a need to develop carefully designed epidemiology (case-controlled) studies to improve the understanding of known and proposed environmental risk factors. New and improved instruments and technologies to measure environmental exposure in populations need to be developed. The current approach has relied upon recall data, and methods are needed to improve the accuracy and consistency of such data collected across different study populations. Other methods may be used to model wide-spread environmental exposures, such as residential geocoding in conjunction with data on air contaminations and traffic patterns to study the role of pollution in lung disease. These approaches may be transferable to the study of environmental exposure in a population at risk of developing lupus.

Cutaneous lupus

There are many different subtypes of cutaneous lupus, including chronic cutaneous, sub-acute cutaneous, acute, and non-specific lupus skin lesions. Defining them can have prognostic significance and therefore is important, but doing so is problematic at this point. The primary problem is that current criteria and coding methods do not allow for chart abstractors to go back and get this information and to collect the proper data in this way.

Variations among subtypes in different populations have not been captured. The thinking is that the best approach for future studies in cutaneous lupus will involve bringing together investigators who have dermatological expertise and an interest in lupus who can work with lupus epidemiologists to get the kind of information that is needed.

Antiphospholipid syndrome (APS)

Like other problems, there is a wide range in the estimates of the frequency of APS. The data are scanty in terms of how big of a problem this really is in lupus. Also not clear is whether antiphospholipid antibodies are important in and of themselves or whether it is the APS that is important.

Issues related to obtaining good prevalence figures

The bottom line is that there are not good prevalence figures for either lupus or any of these related disorders in the United States population. The reason in all cases appears to be problems with definitions, disease classification, methodological limitation and also the recruitment of special populations into studies. In addition, there are no population studies covering a wide enough range of geographic environments and groups defined by race/ethnicity and socioeconomic status to permit sensitive studies of the prevalence of and the risk factors for onset of lupus. The communities in which prevalence studies

have been done are relatively homogenous in race/ethnicity and in environmental exposures. The methods developed could be applied to larger geographic areas encompassing more heterogeneity in race/ethnicity or exposure, a strategy that would be expensive but informative.

Ideally, researchers would be able to look at lupus as well as some of these other disorders in adults and children and study the rate at which these patients go on to develop lupus. Such studies are probably not possible, yet it is possible that some of these conditions could be grouped together and examined. Alternatively, additional information about these diseases could be collected as part of a larger epidemiological study that would potentially be either hypothesis-generating or actually answer some other questions about risk factors and underlying pathophysiology.

The CDC is conducting one epidemiologic study of lupus in selected counties in Georgia and Michigan, using inpatient and outpatient records. While the CDC study should provide valuable information concerning lupus in these populations, there is no plan in that study, for example, to look at Native Americans, Asians or Hispanics, so important information is still lacking for those ethnic groups. However, once preliminary results of this study come out, it may be possible for other groups to analyze those results and study design and perhaps expand this study to areas where they can get the same information on lupus patients from other geographic locations and ethnicities not represented in the two study sites.

Research Objective

- Collect additional information as part of a larger epidemiological study, such as diagnostic classification, biomarkers of risk, environmental issues, genetics, and measures of disease activity, damage, and quality of life.

Health Services Research

Breakthroughs

- The finding that the volume of services a physician provides is inversely related to mortality, particularly lupus nephritis. Even small increases in volume made a significant difference.

In recent years, one of the most important issues in health services research has been a radical disequilibrium in and erosion of the health care system.

Research has shown that income is strongly and positively related to access to specialized care, rheumatology included. Older individuals may have more difficulty accessing health care even though they may be relatively well insured. Understanding and disentangling the impact of all these variables on access to care and health outcomes is an area of research with the potential to help some of the most vulnerable patients and would-be patients with lupus.

While most of the advances in health services research, as with that concerning the cost impact of biologics, have focused on rheumatoid arthritis, lupus-specific research has shown that the volume of services a physician provides is inversely related to mortality, particularly in lupus nephritis. While this finding is consistent with that in other areas of medicine in recent years, what was striking was that breaks occurred at very low levels. For example, physicians who saw two patients per year had significantly better outcomes than those who treated only one patient. The most dramatic effects were seen in physicians who saw three or more patients per year.

Other recent findings include:

- A study looking at the extent of social support, education and self-reported health status found these were more important than the hard measures in determining patient satisfaction. Patients in the study were reacting to the way their doctor treated them, and not so much to the objective outcome they were experiencing.
- A study showing that patient risk aversion affects subsequent treatment choices. It turns out that the proportion of the variation explained by patients' aversion to risk is about as much as the proportion explained by any of the other characteristics that one would be typically measured in the clinical environment and that radically affects the therapies that people will ultimately get. Therefore, if some therapies are more effective than others, that factor of risk aversion will affect outcome.

Research shows that participatory patients achieve better outcomes in lupus. The reason for this, however, is not known, but suggests that the personal, contextual variables have a large effect on outcomes, even when biological measures are held constant for the disease.

Better understanding of disparities among all types of groups would require comprehensive epidemiological studies, yet lack of comprehensive community-based data at present makes it impossible to do sub-group analyses. Understanding health disparities requires disentangling race/ethnicity, which have biological and cultural connotations; social class; and related individual characteristics, including health care use. Results of such studies could suggest the potential for international and perhaps interregional differences in income and outcome studies that would standardize the health care protocols and take that variable out of the system. Thus in this more controlled fashion, this would allow researchers to better understand the role of race/ethnicity and social class in determining outcomes, given they have equal access to care.

Given the emerging wisdom of the high prevalence of neurocognitive impairment in lupus, research is needed to determine how people are able to work and function, handle family and health care in the presence of this condition. Current research is looking at whether there are long-term impacts in the way health care is organized, particularly as patients experience neurocognitive deficits and are in complex systems of care. In these situations, the way patients interact with these systems of care and the points at which the health care insurance system puts obstacles in their way become critical issues. This also

helps distinguish why poverty, even in people who are relatively well-insured, comes up frequently as a predictor of health care outcome.

Research Objectives

- Delineate the role of low-income status in health care use and outcomes.
- Dissect race/ethnicity, social class, and related individual-level phenomena (including health care use) from macro-level phenomena.

Conclusion

While many of the discrepancies in health care seen in lupus apply to other rheumatic diseases, in some cases the effects may be worse due to the typically younger age of lupus patients at diagnosis. For a 40-year-old lupus patient, for example, the loss or fraying of employment-based insurance is going to have more of an impact than it would for a 65-year-old patient with rheumatoid arthritis who can get into the Medicare system. Younger workers with lupus in the United States may also be less likely to secure employment that provides insurance than an older person would. Children affected with lupus may never get insurance as they transition into adulthood. Individual factors – whether they are age, income level, race/ethnicity, or insurance status – all have a bearing on health outcomes. Better understanding how those factors influence outcomes can help in planning health services for all people.

CHAPTER 5: Diagnosis and Treatment

As with any disease, early diagnosis of lupus often leads to the most successful treatment. Yet with lupus, diagnosis is often difficult, in part because the disease evolves over time. Particularly in its early stages, lupus can resemble any number of diseases, many of which require different courses of treatment. Moreover, the manifestations can be staggered and often are not all present at once. Therefore the accumulation of enough signs and symptoms to fulfill the diagnostic criteria for lupus takes time.

Treating lupus can be difficult as well. As the features of lupus vary widely among individuals, so do optimal treatment plans. While numerous medications are used to treat lupus, none have been specifically developed for the disease and few have U.S. Food and Drug Administration (FDA) indications for lupus.

Some of the most important work in lupus in the coming years will focus on improving the ability to diagnose and treat the disease and its complications. Improvements in these areas will depend largely on further developments in three key areas: translational research, the identification and validation of biomarkers, and interventional studies. Recent advances in technology, the development of "The Guidance for Industry on Systemic Lupus Erythematosus – Developing Drugs for Treatment" draft document by the FDA (<http://www.fda.gov/CDER/guidance/6496dft.htm>), and the establishment of consortia of lupus researchers are providing inroads. However, there is still much to be done, issues to address, and obstacles to overcome as work in lupus progresses.

As with any disease, the ultimate goal of lupus research is to ensure that at-risk patients receive careful monitoring and treatment that prevent the progression of the disease and perhaps even its onset.

Translational Research

Breakthroughs

- An increased focus on translational research. The development of Centers for Research Translation (CORT) and Autoimmunity Centers of Excellence (ACE) programs at the NIH are addressing translational research in lupus.
- Development of feasible assays using real-time polymerase chain reaction (PCR) plasma/serum assays of functional activity, RBC/C', proteomics, etc.
- Discovery of the role of TLRs in immune activation.
- Emergence of evidence to suggest that RNA may mediate disease pathogenic pathways and be a therapeutic target.
- Determination of the pattern of appearance of autoantibody specificities prior to onset of clinically measurable lupus.
- Identification of cytokines and chemokines as therapeutic targets.

Translational research is that research which bridges the gap between basic science discoveries and clinical applications. More specifically, translational research is applied, and clinical, scientific research that is directed towards testing the validity and limits of

applicability of knowledge derived from basic research and engineering to the understanding of human diseases and health. Although often thought to be the route from bench to bedside, translational research is bidirectional, in that an observation at the bedside may be taken back to the lab to determine mechanism of action.

Translating basic research to clinical research is a challenge. To conduct translational research, researchers may need to both extend their own knowledge in other scientific disciplines as well as find experts in various disciplines and form collaborations to take their findings from basic to clinical research or vice versa.

The challenge with lupus is that it is an uncommon autoimmune disease; therefore, there may be only a few patients at any one research center. In addition, the disease affects many organ systems, so many disciplines may need to be brought together to study it.

An example of investigator initiated translational research taking a clinical observation to the basic research lab and returning it to the clinic is the PROMISSE study. The PROMISSE study is a prospective multi-center study recruiting from obstetrics and rheumatology practices. The primary goal of this observational prospective study is to determine whether elevations of complement split products in the serum of pregnant patients with lupus or APS predict poor fetal outcome. Investigators initiated PROMISSE after studies with laboratory mice showed that complement activation plays a critical role in antiphospholipid antibody-mediated pregnancy loss. The study is an example of translating animal findings to the clinics and asking the questions directly in a prospective way.

In recent years, translational research has become an increasingly important area of focus for NIH as researchers strive to turn discoveries about complex disease processes at the bench to new hope for patients at the bedside. Programs to promote and facilitate translational research are ongoing in several NIH Institutes, including research centers and centers of excellence. Additional efforts have been made to provide support for mechanistic studies of clinical samples from clinical trials. Larger scale efforts are being made through the NIH Roadmap initiatives.

The NIH has initiated CORT, a new program directed at elucidating the relevance of basic research to human disease in rheumatic, skin, and musculoskeletal diseases. Two major features of the CORT program are 1) the overarching aim of disease-specific research translation, and 2) the inclusion of resources and an administrative structure to facilitate research translation.

Other NIH initiatives, including the ACE program, have also focused on translational research. The goal of ACE is to enhance interactions between scientists and clinicians in order to accelerate the translation of scientific research findings into medical applications.

Collaborating with industry may be a cost-effective way to obtain data. Industry has the infrastructure to do clinical studies. Investigators involved in basic research could

identify targets for which industry could develop products. Such relationships could be beneficial for both parties and should be encouraged.

Research Objectives

- Identify pathways to be modulated by therapies – chemokines, complement, regulatory T cells (Treg), genetic and environmental triggers.
- Correlate expression of genes across patient populations.
- Develop new models for analysis of disease pathways.

Biomarkers

Breakthroughs

- The identification of potential lupus biomarkers, including type I interferon system, anti-dsDNA and complement activation.
- The establishment of the Systemic Lupus Erythematosus Biomarkers Working Group and Autoimmune Biomarkers Collaborative Network (ABCoN) to identify and validate biomarkers.
- Development of non-invasive approaches to evaluate kidney involvement and imaging to evaluate CNS involvement.
- Development of systems to collect and store patient clinical and demographic data across research and clinical centers.
- Emerging consensus on the use of biomarkers to determine biological effects of new therapies in addition to evaluation of clinical efficacy in clinical trials.

Biomarkers can be defined as physical signs or cellular, biochemical, molecular or genetic alterations by which a normal or abnormal biologic process can be recognized and/or monitored and that may have diagnostic or prognostic utility. Laboratory biomarkers have to be reliably measurable in tissues, cells or fluids. Some biomarkers have the potential to become surrogate endpoints for clinically meaningful outcomes that could predict the effect of therapeutic interventions.

The NIH-supported ABCoN is one example of NIH-funded biomarker research. ABCoN is a multi-center study to discover new clinically useful biomarkers for the diagnosis and management of lupus and rheumatoid arthritis. ABCoN researchers will examine the DNA, RNA, proteins, and cells in the blood of people with lupus using multiple blood samples taken at regular intervals. By following patients over time, the study aims to identify biomarkers in the blood that could help with diagnosis, assessment of disease activity, and treatment decisions for patients with lupus. This and other ongoing research efforts have identified several promising potential biomarkers, including the type I interferon system, anti-dsDNA and complement.

Interferons are a family of proteins produced by cells of the immune system that work in concert to direct the immune response. Studies have shown a correlation between circulating levels of interferon and the over expression of interferon-regulated genes. It is possible that distinct gene expression profiles may be associated with lupus, correlate

with specific organ involvement and change with disease activity. If research proves this true, gene expression profiles may be useful biomarkers for organ involvement and disease activity.

Anti-double-stranded DNA (anti-dsDNA), an antibody to DNA, is common in the serum of people with lupus but rare in people with other rheumatic diseases. Complement is a series of proteins that produce inflammation-causing substances when activated by antigen-antibody complexes. Scientists suspect a combination of rising anti-dsDNA levels along with declining complement levels may be useful in predicting flares of lupus nephritis and perhaps lupus flares in general.

Other potential biomarkers being studied include some subsets of lymphocytes, which are believed to correlate with lupus disease activity; B Lymphocyte Stimulator (BlyS), which drives B cells to proliferate and possibly to produce autoantibodies; and vascular endothelium adhesion molecule-1 (VCAM-1). The soluble form of VCAM-1 is higher in the serum of people with active lupus and has been used to assess kidney involvement.

Research Objectives

- Identify and validate biomarkers for disease activity and disease subsets based on mechanism and for phenotype, damage and response to treatment.
- Incorporate patient characterization based on biomarkers into clinical trials.
- Pursue pathogenic mechanisms identified in biomarker studies, RNA and RNA-binding proteins as triggers, epigenetic regulation of RNA expression and TLR role in disease.

Interventional Trials

Breakthroughs

- Increased industry interest in lupus. Numerous companies have entered into serious drug development efforts for the disease.
- Improvement in harmonization of methods to assess disease activity.
- Increasing interest in the community to engage in trials with lupus patients.

Interventional trials are those in which an intervention – a treatment – is given to patients based on a protocol. Usually such trials are conducted with controls matched for factors such as age, sex and disease severity. This allows researchers to compare the status of people who are receiving the treatment with those who are not and thereby determine the efficacy and safety of the treatment.

APPLE is one example of a major NIH-funded multi-center interventional trial. Its purpose is to study the effects of atorvastatin (Lipitor) on lipid levels, inflammation and the development of atherosclerosis in pediatric lupus patients. The trial will include 280 patients at 20 sites who will ultimately have the opportunity to serve as a collection that can be drawn upon by the research community to address questions about this all-too-common complication of lupus in both adults and children.

While designing an interventional trial, researchers must first develop an instrument to assure themselves of the outcome they are studying. The Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) trial illustrates this point. When SELENA, the first multi-center interventional trial in lupus ever done in the United States, was initiated in 1994, the investigators convened to develop precisely defined rules for mild/moderate and severe flares. As these rules are used in further studies there is perhaps a need for refinement of the mild to moderate flares so that a mild skin rash will not be given the same weight as arthritis in both knees that interferes with daily activity, for example. The British Isles Lupus Assessment Group (BILAG) definition of flare may enable investigators to further refine the mild/moderate category. Current research is focused on developing new instruments that assess disease activity and improve the definition and consistent use of “flare” as an outcome. These instruments will allow the use of innovative trial design for new therapeutics.

A lesson learned from SELENA is that one cannot only extrapolate the effects of any agent in lupus just from animal models. While hormones may be fundamental in the pathogenesis of lupus in mice or humans, the SELENA trial showed that hormone therapy and oral contraceptives can be relatively safe to some patients; however, the fact that some patients in the trial did not do well on oral contraceptives or hormone therapy prompts further questions.

The importance of optimizing treatment in patients with the disease to be treated, rather than applying the drug dosage used for another illness was well illustrated by an interventional trial to evaluate the metabolites of azathioprine (Imuran). The study showed that azathioprine, when given in doses used to treat inflammatory bowel disease, was more likely to cause toxicity in lupus patients. Interventional studies allow doctors to evaluate immunosuppressive drugs for both efficacy and toxicity for the diseases they will be used to treat. They also allow doctors to determine how medications prescribed for one disease manifestation affect others.

Investigators have shown that it is possible to conduct successful studies that evaluate efficacy based on responses of a major disease component. This was done in a recent multi-center trial supported by the FDA where the oral immunosuppressant mycophenolate mofetil (CellCept) was shown to be more effective and more tolerable than intravenous cyclophosphamide (Cytoxan) for inducing remissions in patients with lupus nephritis. CellCept induced reduction of renal symptoms in 22 percent of patients versus 5 percent treated with placebo. In addition, patients taking CellCept had fewer hospitalizations or severe infections than patients on Cytoxan.

Fortunately, there is excitement on the part of investigators and industry to bring on lupus trials based on mechanism of action. Individual sites will have to prioritize types of patients to include in these trials. Where a pathway is being inhibited will also be important in terms of design of a trial. Not every pathway will translate into a clinical change in all the diverse manifestations.

Research Objectives

- Design and conduct interventional studies to address long-term consequences of disease in vascular, cerebral, skeletal systems, etc.
- Partner with basic science to identify “biochemical coverage” of a biomarker for the drug under study.
- Establish drug safety profiles.
- Encourage novel trial designs, such as randomized withdrawal trials, in which everyone gets the drug initially, and randomized phase trials, which are started at different phases.
- Monitor tissue damage by tracking long term consequences of disease and treatment.
- Validate outcome measures of clinical response.
- Develop new instruments to classify disease and disease type, define clinical response, and characterize short- and long-term outcomes that can and should be evaluated in clinical research.
- Conduct interventional studies inhibiting interferon and chemokines.
- Incorporate quality of life and cost effectiveness studies into clinical trials.

It is important that NIH be involved in clinical studies where there is no validated, FDA-approved outcome, because industry will only look at studies where FDA accepts an outcome measurement. The NIH issued a Request for Applications to develop, apply and evaluate new clinical trial outcomes measures of safety, efficacy and effectiveness of therapies for rheumatic, skin, bone and muscle diseases. The goal is to stimulate a systematic approach to the design, evaluation and implementation of reliable outcomes to measure clinical response.

Issues in Drug Development and Testing

Breakthroughs

- Commitment to develop lupus therapeutics by multiple companies (15 to 20 are currently going to trial).
- The establishment and cooperation of clinical trials centers (consortia).
- The development of the FDA’s Lupus Draft Guidance Document, which outlines what constitutes a successful trial and shows that there will be great flexibility in trial design, etc. (<http://www.fda.gov/ohrms/dockets/98fr/2005d-0106-gdl0001.pdf>). The guidance document has accelerated the development and implementation of many trials testing new biologics in lupus.
- General agreement on trial design and the selection and consistent use of outcomes instruments.

Developing new therapeutics is a complex, costly and lengthy process. Finding a therapeutic target, such as interferon-alpha, is just a small part of the process, which also involves generating the therapeutic, formulating and manufacturing it, ensuring its coverage and, of course testing it – first in animals, then in clinical trials for safety in humans and finally clinical trials for efficacy. Each painstaking step of the process can

take years. In fact, for a successful therapeutic, the process from target identification to FDA approval ranges from the hundreds of millions to more than \$1 billion.

While timelines and costs are both major issues in drug development, of equal concern to manufacturers is finding the numbers of patients required to prove their products' benefits. As drug companies become interested in lupus, the increasing number of clinical trials brings to the forefront the vital issues of both increasing the numbers of clinical trial participants and managing the prioritization and enrollment of the current pool of participants.

In a disease that is as heterogeneous and rare as lupus, finding enough people with specific disease manifestation to test therapies is difficult. Furthermore, maintaining an adequate supply of trial participants may require rethinking some stipulations on prior therapies. For example, if a patient is enrolled in a trial for a monoclonal antibody, should that patient be excluded from subsequent trials? Reducing the pool of eligible enrollees with each trial is not desirable. If a patient has been off the monoclonal antibody or other agent for a long enough time, they should probably be able to enter another trial.

Prioritization may be a solution. Examples might include prioritization of qualified patients to specific trials or prioritization of trials, by selecting those that will allow basic or mechanistic studies that will provide the investigators understanding of the biology or the effects of the drug. Clinical trialists in lupus, as a group, might be able to assist with prioritization.

Recruiting more participants for trials may require looking beyond major tertiary care centers to smaller academic institutions, community hospitals and medical practices where many people with lupus receive their care. Efforts should be made to recruit the spectrum of patients who have lupus. Because there can be pharmacokinetic differences based on genetic factors, patients recruited should be representative of those who have the disease. For example, if lupus is over-represented in African Americans and Hispanics and other minority populations, then the majority of patients in these clinical trials should be people in those ethnic groups. However, we must be mindful of the need to enroll patients meeting American College of Rheumatology criteria for the diagnosis of lupus and the need to have trained investigators. Therefore, community outreach must include education of the referring physicians. As the number of lupus trials increases, there might be opportunities for parallel ancillary projects that are linked to industry-sponsored studies. Building on current relationships with voluntary health organizations, which are important sources of education for patient populations, could augment efforts to enhance recruitment for clinical trials.

Further resources of qualified patients may lie in the more than 120 academic medical centers in the United States and more academic hospitals, where there is likely a large cadre of academically qualified rheumatologists to establish patient databases and participate in trials. An additional opportunity exists with physicians and health care professionals who are not currently in the network but may have 50 or 100 patients that could be organized into a viable local database so that they too can participate.

Recruiting populations outside the reach of major centers has already proven useful in recruiting patients for the PROMISSE, which by its nature (patients must be pregnant and have antiphospholipid antibodies or lupus) has a narrow and limited pool of candidates. Study centers became hubs for spokes to the community. Some centers are already doing this. For example, one center has ties to community hospitals and practitioners. Researchers from another center went to a university-affiliated to find patients with more ethnic diversity. To capture those patients, investigators must think about centers that have not been part of the consortia. In some studies, the patient must be seen 10 times, so the hub approach could help with distance. Existing infrastructure does not get around barriers of distance. The key to success is placing an experienced investigator in those centers. Reliance on a busy practitioner will not provide the solution.

Research Objectives

- Make steps toward defining a path to approval of new therapeutics. This will only be clear when researchers find a therapeutic that works.
- Identify biomarkers relevant to the clinical effect.
- Develop a better understanding of human lupus and its relationship to lupus-like disease in mice. Test the ability of mouse models to predict effective therapeutics.
- Foster consortia for clinical trials and other means of getting sufficient numbers of patients to study, such as going outside the United States for trial participants when necessary.

Turning Research into Treatments: The Importance of Partnership

Turning laboratory research into therapeutics and then getting those therapeutics to market will require finding creative ways to partner with industry. Translating from the mouse to the human level is a difficult transition. Currently, it is not possible to develop an actual therapeutic or diagnostic outside industry. The major obstacle still remains getting it to the next level – manufacture and testing.

There are a few examples where NIH has provided funds to biotech companies to develop their product in clinical trials. However, available grants are probably insufficient for phase I studies, and the requirements to move a product forward exceed what can be done with an investigator and small biotechnology firms.

For small biotechnology firms, investing the funds for phase I is often too risky, so NIH developed a program – through its Roadmap for Medical Research – to allow the investigator to move a molecule from the laboratory to commercial development. Now it might be time to move to the next level – a program that would allow investigators to partner with industry representatives that is willing to provide funds to move these projects ahead.

Conclusion

There has never been more excitement in the government, academia and industry about lupus research and the prospects for improved diagnosis and therapies for this potentially devastating disease. Advances in technology as well as the development of partnerships – among the institutes, among centers and with patient advocacy groups and industry – promise to turn this excitement into new therapies to prevent the progression of lupus and its complications and perhaps even its onset.

Appendix A

The Future Directions of Lupus Research

Broad Goals and Priorities

Lay the Foundation for Lupus Prevention

Lupus prevention may become an attainable goal in the next decade. To achieve this goal it will be critical to advance and coordinate research efforts to:

- Identify populations at risk
- Identify risk of disease
 - Family studies
 - Genetics
- Evaluation of the best options for prevention

Identify Triggers of Disease

The interplay between genes and environmental factors needs to be dissected and defined in individual patients and in patient subsets. Critical to this goal will be to define triggers:

- Endogenous: genes, hormones
- Exogenous: infectious agents, chemical exposures, biopsychosocial factors

Define Target Organ Damage Mechanisms

The goal is to prevent progression of target organ damage. It will be critical to acquire technologies for the assessment of multiple and overlapping mechanisms of disease occurring over time that cause irreversible damage. These include identification of:

- Inherent target organ susceptibility factors
- Mechanisms of injury: immune, inflammatory, vascular
- Mechanisms of progression: repair failure, abnormal clearance, persistence of triggering agent
- Mechanisms of recovery
- Role of local cells
- Measurement of injury: non-invasive rapid assays

Understand Autoantibodies

The role of autoantibodies in disease and their use as biomarkers needs to be fully understood. Research priorities and opportunities exist on:

- Defining the genes that regulate tolerance to self and production of autoantibodies
- Role in pathogenesis via traditional and functional mechanisms
- Interaction with target organ targets
- Initiation/induction of tissue damage
- Targeting their effector function for therapeutic purposes
- Role and use as biomarkers

Expand Biopsychosocial Research

The goal is to increase our understanding of behavioral, psychological and societal factors that affect the course and the long term outcomes in the disease. Modifiable factors could be identified through research on:

- Epidemiology
- Impediments to access of care and maintaining status quo of therapies
- Quality of life
 - Self-report vs. physician report
- Interactions with institutions, government, etc., and their effect on long-term outcomes
- Interaction with other psychiatric disease
- Mind/body interactions
- Cognitive affliction
- Coping styles
- Support groups
 - Family
- Transition from pediatric to adult
- Disparities
 - Poverty, race/ethnicity
- Origin and mechanisms of fatigue

Discover and Validate Biomarkers

The goal during the next few years will be to use modern approaches to discovery and validation of existing biomarkers for lupus diagnosis, prognosis and evaluation of therapies. Promising biomarkers are likely to emerge from research on:

- Genetics
- Polymorphisms and gene products evaluated by novel technologies such as microarrays and proteomics
- Validation studies in well characterized patient cohorts
- Analysis of potential use of selected biomarkers and their combinations in clinical subsets

Move Forward Treatment and Therapy

New treatments for lupus are being tested by the private sectors. The goal of the NIH and academic research will be to ensure a continuous supply of new targets for intervention and adequate trial methodologies. Research likely to contribute to this goal will be focused on:

- Identification of targets for non-immunosuppressive therapies
- Target organ repair
- Collateral damage – musculoskeletal, cardiovascular, chronic fatigue, mild myalgia
- Prevention of collateral damage
- Treatment of symptoms (e.g., cognitive rehabilitation, control of fatigue)

Resources and Infrastructure

To achieve the goals of this plan, critical resources will be needed, and they will have to be implemented in creative and dynamic ways to ensure rapid progress. Implementation will require careful attention to achieving an adequate balance between investigator-initiated research and network/collaborative large projects. The following is a list of some of those resources. Their acquisition and maintenance will require collaborative approaches by the funding organization, the academic centers and the private sector.

- **Patient Collections**

Issues affecting the collection and sharing of patient cohorts that will have to be addressed include:

- Accessibility
- Control populations
- Registry maintenance facilitation
- Specimen collection with clinical trials
- More patients for intervention

- **Partnerships**

A collaborative environment that facilitates sharing of data, specimens and information will enhance research and research careers in lupus. For example, immunologists and rheumatologists should seek direction from other areas like reproductive medicine, neurophysiology, cell biology, and hematology. Some principles that may guide their development include:

- Free exchange between academic investigators and industry
- Free exchange between investigators
- Educate institutions on collaborative efforts
- Participation of patient groups

- **Training**
 - Education – physicians for new therapies
 - New investigators

Appendix B

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Appendix C

Glossary

Acquired immunity – immunity arising from exposure to antigens.

Allele – any of two or more alternative forms of a gene that occupy a specific site on a chromosome.

Annexin II – a calcium-dependent phospholipid-binding protein whose function is to help organize exocytosis of intracellular proteins to the extracellular domain.

Anti-dsDNA – anti-double-stranded DNA antibodies – antibodies directed against the DNA in the cell nucleus. This antibody is common in the serum of people with lupus but rare in people with other rheumatic diseases.

Anti-La antibodies – also called anti-SS-B antibodies. An antinuclear antibody characteristic of lupus and Sjögren's syndrome. In lupus, anti-La antibodies (and anti-Ro antibodies) increase the risk of having a child with neonatal lupus.

Anti-Ro antibodies – also called anti-SS-A antibodies. An antinuclear antibody characteristic of lupus and Sjögren's syndrome. In lupus, anti-Ro antibodies (and anti-La antibodies) increase the risk of having a child with neonatal lupus.

Antibodies – a molecule (also called an immunoglobulin) produced by B cells in response to an antigen. The binding of antibody to antigen leads to the antigen's destruction.

Antigen – a substance or molecule that is recognized by the immune system. The molecule can be from foreign material, such as a bacterium or virus, or the molecule can be from one's own anti-glutamate receptor antibodies and called a self-antigen.

Antiphospholipid syndrome – an immune disorder characterized by the presence of antiphospholipid antibodies (abnormal antibodies directed against phospholipids in cell membranes) in the blood. Problems associated with the syndrome include abnormal blood clotting, migraine headaches, repeat spontaneous abortions and low blood platelet counts (thrombocytopenia).

Apoptosis – a form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area. Apoptosis plays a crucial role in developing and maintaining health by eliminating old cells, unnecessary cells, and unhealthy cells; however, too much or too little apoptosis can result in disease.

Atherogenic – initiating, increasing or accelerating formation of deposits on the walls of the arteries.

Atherosclerosis – the build-up of plaque inside blood vessels, causing them to stiffen and harden. Commonly referred to as hardening of the arteries.

Autoantibodies – antibodies that are made against the body's own organs and tissues rather than foreign parts of bacteria or viruses.

Autoantigens – endogenous antigens that stimulate the production of self-directed antibodies, which can result in an autoimmune disease, such as lupus.

Autoimmune disease – a condition in which the immune system mistakenly attacks the body's own organs and tissues.

B cells – also called B lymphocytes. A type of white blood cell that matures in the bone marrow. When stimulated by a particular antigen, B cells (also called B lymphocytes) differentiate into plasma cells that synthesize the antibodies that circulate in the blood and react with the specific antigens.

Carotid intima-medial thickness (IMT) – a measure of the thickness of the carotid artery wall. A measurement used to determine evidence of atherosclerosis.

Central tolerance – the process by which potentially autoreactive immune system cells are eliminated before they can mature and be released to circulate in the body.

Chemokines – one of a large group of cytokines (chemical substances released by several types of cells in the body) that attract white blood cells. Chemokines are involved in a wide variety of processes, including inflammation that occurs in lupus.

Cohort – in epidemiology, a group of individuals who share a common characteristic. In cohort studies, subjects are followed over time to study information about the *incidence* of a disease and the *relative risk* of incurring the disease (the ratio of disease incidence in subjects exposed to certain risk factors against those not exposed).

Congenics – a technique by which a mouse strain is derived by backcrossing mice carrying a locus of interest to mice of another inbred strain for successive generations.

Cyclophosphamide – a highly toxic immunosuppressive drug, used in the treatment of severe lupus organ involvement. Brand name: Cytoxan®.

Cytokines – chemical substances released by several types of cells in the body that have varied effects on many cells of the body. For example, some cytokines can cause growth and activation of the immune system cells.

Cytomegalovirus (CMV) – a member of the herpes virus family that affects most people at some time during their lives. Most people experience no symptoms or mild symptoms

(such as enlarged lymph nodes, fever and fatigue), but the virus can be serious or life-threatening in a newborn or a person with a weakened immune system.

Cytoskeleton – the scaffolding structure of the cell cytoplasm (the substance of a cell outside of the nucleus).

Dendritic cells – a special type of antigen-presenting cell that activates T lymphocytes and stimulates the growth and differentiation of B cells. Dendritic cells are found in the lymph nodes and spleen.

Endothelial cells – the flat epithelial cells found on the luminal surface of blood vessels, lymph vessels and the heart. This continuous layer of cells is called the endothelium.

Epidemiology – the science concerned with the study of the factors determining and influencing the frequency and distribution of diseases and their causes in a defined human population.

Epstein-Barr virus (EBV) – a virus in the herpes family that is best known for causing infectious mononucleosis. Research suggests it is an environmental factor in the development of lupus.

Estrone – a naturally occurring weak estrogenic hormone that is secreted by the ovaries and often synthesized and used in hormone replacement therapy.

Etiology – the study of the causes of disease.

Fc receptor – a receptor on hematopoietic cells that will bind to the constant end of immunoglobulin after the antibodies have bound to antigens. The Fc receptors therefore increase the affinity phagocytic cells have on microbes, causing the engulfing and ingestion of bacteria or other foreign bodies by phagocytes and subsequent killing of the pathogen.

Genome – complete genetic complement of an organism.

Genotype – the genetically inherited characteristics of an individual.

Glomeruli – tiny ball-shaped structures in the kidneys composed of capillaries involved in the filtration of the blood to form urine.

Glomerulonephritis – nephritis accompanied by inflammation of the capillary loops in the glomeruli of the kidney.

Heterogeneous – dissimilar; composed of unlike parts. Lupus is a heterogeneous disease in that it can have diverse symptoms and manifestations, which can be different in different people.

Hippocampus – an area of the forebrain that helps regulate emotion and memory.

Human leukocyte antigen (HLA) – one of a group of proteins found on the surface of white blood cells and other cells that play an important part in the body's recognition of self and in the immune response to foreign substances. These antigens vary from person to person. HLA is used in conjunction with a letter and number (e.g., HLA-B27) to designate a specific allele at a given HLA locus.

Humoral immunity – the component of the immune system involving antibodies that are secreted by B cells and circulate as soluble proteins in blood plasma and lymph.

Hydroxychloroquine – a member of the family of drugs called antimalarials. Originally developed for the treatment of malaria, hydroxychloroquine is now commonly used in the treatment of lupus. Brand name: Plaquenil®.

Hypoxia – deficiency in the amount of oxygen reaching body tissues.

Immune tolerance – the safeguards that the immune system naturally possesses to protect from harming self.

Immunogenic – producing an immune response.

Immunoglobulin A (IgA) – the class of antibodies produced predominantly against ingested antigens, found in body secretions, such as saliva, sweat and tears, and functioning to prevent attachment of viruses and bacteria to epithelial surfaces.

Immunopathogenic – originating or producing immunologic disease.

In vitro – in glass, as in a test tube. An *in vitro* test is one that is done in a glass or plastic vessel in a laboratory, outside of a living organism.

In vivo – within a living organism.

Interferons – natural proteins produced by the immune system in response to a challenge by a foreign agent. In humans, there are four major classes of interferon: alpha, beta, gamma and omega. Interferon alpha and beta have many subtypes.

Knockout mice – a mouse that is genetically engineered so that one more of its genes have been made inoperable, providing a way to learn about a gene that has been sequenced but has unknown or incompletely known function.

Knock-in mice – a mouse that has had a gene from another organism or a stem cell line inserted into a specific locus on its genome.

Low-density lipoprotein (LDL) – a complex of lipids and proteins, which transports cholesterol from the liver to the tissues of the body. High levels are associated with an increased risk of atherosclerosis and coronary heart disease.

Lupus Federal Working Group (LFWG) – The LFWG exchanges information and coordinates federal efforts in lupus research and education. The group is comprised of representatives from all relevant DHHS agencies and other federal departments with an interest in lupus.

Lipopolysaccharide – a large molecule that contains both lipid and a carbohydrate. Lipopolysaccharides are a major suprastructure of gram-negative bacteria that contribute to the structural integrity of bacteria and protect them from host immune defenses.

LJP 394 – an experimental lupus drug designed to tolerize or shut down pathogenic B cells, arresting their production of antibodies to double-stranded DNA (dsDNA). LJP 394 is currently in clinical trials to determine whether treatment with the drug is more effective than placebo in delaying the time to major lupus flare.

Lupus – used specifically in this plan to mean systemic lupus erythematosus. A chronic, relapsing, inflammatory, and often febrile multisystemic autoimmune disorder. It can affect one or several parts of the body including the joints, skin, blood vessels, kidneys, lungs, heart or brain. Another lupus is *discoid or cutaneous lupus*, which mainly affects the skin.

Lymphocytes – small white blood cells that are critical components of the immune system. There are several types of lymphocytes: B cells are primarily involved in the production of antibodies; T cells release chemicals that activate and direct the movements of other cells to help fight infection or attack foreign matter; and natural killer cells attack cells that are infected by microbes.

Macrophage – a white blood cell that is a mononuclear phagocyte, capable of ingesting cellular debris and/or foreign material.

Major histocompatibility complex (MHC) molecules – molecules that are found on cell surfaces and display antigen; the antigen-MHC molecules may then interact with a T-cell receptor.

Metabolomics – systematic study of the unique chemical fingerprints that specific cellular processes leave behind that can give an instantaneous picture of the physiology of a cell.

Methylation – the addition of a methyl group to a cytosine residue to convert it to 5-methylcytosine.

Microchimerism – the presence in an individual of a population of cells derived from another human being.

Microcirculation – the flow of blood or lymph through the smallest vessels of the body, such as the venules, capillaries, and arterioles.

Monocytes – white blood cells that have single nuclei.

Mononuclear phagocytes – Monocytes. There are two principal types of phagocytes – mononuclear (monocytes) and neutrophils, both of which are types of white blood cells.

Murine – having to do with rats or mice.

Mutagenesis – the development of a sudden structural change within a gene or chromosome of an organism (mutation), resulting in the creation of a new character or trait not found in the parent.

Natural killer (NK) cells – a major component of the innate immune system that attack cells that are infected by microbes but do not attack the microbes themselves.

Neonatal lupus – a lupus-like disease in a baby due to the transfer through the placenta of lupus antibodies from the mother who has lupus to the fetus. Features may include a decrease in red and white blood cells and platelets and a skin rash. Problems can also develop in the electrical system of the baby's heart (congenital heart block), necessitating the placement of a cardiac pacemaker.

Neonatal-onset multisystem inflammatory disease (NOMID) – a systemic inflammatory disease with onset during the first two years of life, which is due in the majority of cases to a defect in the CIAS1 gene, an interleukin-1-dependent gene.

NIAMS – National Institute of Arthritis and Musculoskeletal and Skin Diseases.

NIH – National Institutes of Health.

NZB × NZW – New Zealand Black × New Zealand White – an inbred strain of mouse that is prone to lupus and useful for studying the disease.

Opsonization – the process by which bacteria are altered by opsonins, products of complement activation in blood serum, which causes them to become more susceptible to the action of phagocytes.

Pathogenesis – the processes that occur in the development of a disease.

Peripheral tolerance – the process by which potentially autoreactive cells are controlled after they reach the bloodstream.

Phenotype – the characteristics of an individual (or group) that can be seen and that result from the interaction of its genetic constitution and environmental factors.

Plasmacytoid dendritic cells (pDCs) – one of four types of dendritic cells. pDC morphology, surface markers, their migration *in vivo* and the ability to rapidly produce

large amounts of type I interferons (IFN-alpha/beta) in response to toll-like receptor (TLR) triggering sets them apart from other dendritic cell subsets.

Podocytes – an epithelial cell of the renal glomerulus, attached to the outer surface of the glomerular capillary basement membrane by cytoplasmic foot processes. These cells provide an additional barrier in the filtration of the blood in the kidney.

Polygenic disease – a disease that is caused by the action of more than one gene. Lupus is considered a polygenic disease, because there are likely many genes involved in its development. But lupus is not purely genetic; environmental factors are probably required to trigger the disease in genetically susceptible people.

Polymerase chain reaction (PMR) – a technique used in molecular genetics that allows scientists to analyze any short sequence of DNA or RNA without having to clone it.

Polymorphism – the presence of multiple alleles at a specific locus of a chromosome.

Prevalence – a statistical concept referring to the number of cases of a disease that are present in a particular population at a given time.

Prostaglandin – a hormone-like substance involved in a wide range of body functions, such as the contraction and relaxation of muscles, the regulation of blood pressure and inflammation, the dilation and constriction of blood vessels and the formation of the protective mucous lining of the stomach.

Proteomics – state-of-the-art methods that combine genomics, molecular biology and protein chemistry.

Proteinuria – the presence of excessive amounts of protein in the urine.

Prothrombin – a protein in blood plasma that is converted into thrombin during clotting.

Rheumatoid arthritis – a chronic systemic disease primarily of the joints, marked by inflammatory changes in the synovial membranes and articular structures.

Ro – (or anti-Ro) also known as SS-A, a type of antinuclear antibody that is common in Sjögren's syndrome.

Sclerotic – affected by sclerosis (a thickening or hardening of body tissue) usually from the excessive formation of fibrous interstitial tissue.

Sequela – a pathologic condition resulting from an earlier disease or injury.

Sm – (or anti-Sm) a type of antinuclear antibody reported in about one-third of lupus patients.

Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) – a concise measure of disease activity with excellent test-retest reliability and high responsiveness to clinically important changes in the disease.

T cell – a type of lymphocyte. T cells have T cell receptors and, sometimes, costimulatory molecules on their surfaces. Different types of T cells help to orchestrate the immune response and can issue orders for other cells to make cytokines and chemokines.

Thrombin – an enzyme in the blood formed from prothrombin that aids in blood clotting.

Thrombocytopenia – any disorder in which there are not enough blood platelets (cells in the blood that help blood to clot), often leading to abnormal bleeding.

Thrombophilia – a disorder of the blood-forming system in which there is an increased tendency to clot.

Thrombosis – the formation of a blood clot in a blood vessel.

Toll-like receptors (TLRs) – a family of receptors that provide critical links between immune stimulants produced by microorganisms and the initiation of host defenses.

Transgenic – the experimental insertion of a segment of DNA from one genome onto the DNA of a different genome. This technique is used to make genetically modified mice.