Published in final edited form as:

Clin Immunol. 2017 December ; 185: 109–118. doi:10.1016/j.clim.2016.08.015.

A systems approach to renal inflammation in SLE

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Abstract

Lupus disease and its complications including lupus nephritis (LN) are very disabling and significantly impact the quality of life and longevity of patients. Broadly immunosuppressive treatments do not always provide the expected clinical benefits and have significant side effects that contribute to patient morbidity. In the era of systems biology, new strategies are being deployed integrating diverse sources of information (molecular and clinical) so as to identify individual disease specificities and select less aggressive treatments. In this review, we summarize integrative approaches linking molecular disease profiles (mainly tissue transcriptomics) and clinical phenotypes. The main goals are to better understand the pathogenesis of lupus nephritis, to identify the risk factors for renal flare and to find the predictors of both short and long-term clinical outcome. Identification of common key drivers and additional patient-specific key drivers can open the door to improved and individualized therapy to prevent and treat LN.

Keywords

Systems biology; LN

Introduction

Lupus nephritis (LN) is a severe complication of SLE that affects from 20–75% of SLE patients depending on ethnicity and age, being more common in younger individuals and in individuals of African or Asian ancestry (reviewed (1, 2)). The introduction of broadly immunosuppressive therapies together with the improved management of hypertension and the wide availability of renal replacement therapy has drastically altered the natural course of diffuse proliferative LN such that it is no longer almost universally lethal. Nevertheless, even in the setting of clinical trials where compliance is monitored, response rates to current immunosuppressive therapies are <50% and up to 40% of patients sustain permanent renal injury with 5–10% requiring dialysis within 10 years (3–5). Poor outcome is more frequent in geographic areas with limited access to high quality medical care (6). In the US there are

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continuing and significant disparities in outcome between white and non-white patients with LN as a result of both genetic and environmental/socioeconomic factors (2, 5, 7).

Despite the accelerating pace of discovery in immunology and the development of large numbers of biologic agents that antagonize a multitude of immune pathways, there has not yet been a successful randomized controlled clinical trial of a biologic agent in LN (3, 8). Furthermore, several approaches that have been successful in mouse models have failed in clinical trials in humans (9, 10).

Reasons for the slow pace of therapeutic discovery in LN are myriad (11). First, the disease is heterogeneous and there are as yet no good biomarkers that predict disease onset, severity of disease course or response to a particular therapy. Thus it is currently not possible to subclassify patients for clinical trials. Second, the disease may follow a remitting and relapsing course such that multiple insults can be layered onto the kidney over an extended time period. This may confer resistance to single therapeutic interventions. Third, genetic factors unrelated to systemic inflammation can influence the severity of renal damage, such as polymorphisms in kallikrein genes (12) and ApoL1 (13). Fourth, clinical measures of disease remission are not always accompanied by histologic remission such that some patients may be undertreated. Fifth, new therapies are often tested in active disease with less attention to trials that prevent disease onset or flares – stages that may be more sensitive to particular types of intervention. Finally, delay in diagnosis, poor access to qualified health care providers and failure to comply with medications all have a substantial impact on renal outcomes.

It is therefore evident that better approaches are needed to define the disease process active in the kidneys of individual patients so as to target therapy to the mechanisms currently damaging the nephrons. In order to do this human tissue needs to be available so that new technologies can be applied and mechanistic pathways of renal damage identified. One strategy to enhance our understanding of a complex disease such as LN is the application of a systems approach. Systems biology has been essential in the understanding of complex datasets, starting from microarray technology (14) and progressing to the era of RNA sequencing technology, epigenetics, proteomics and lipidomics. Such an approach will enhance our understanding of the pathogenesis of LN, will improve patient stratification for clinical trials and will identify new therapeutic targets with the ultimate goal of offering customized medicine to LN patients. This review describes how systems biology is helping to identify the processes by which renal damage occurs in LN. We will focus on molecular profiling in both mouse models and humans and refer the readers to recent reviews of proteomics (15) and metabolomics (16, 17).

1. From LN Mouse models to Human LN

In our own work, we have used several bioinformatics tools to highlight important pathogenic mechanisms in three different mouse LN models and in human LN. Our work also highlighted the contribution of renal mononuclear phagocytic cells to disease progression in both mice and humans; this could be the basis for functional studies that lead to new therapeutic strategies. Finally, by identifying the renal transcriptional features

common to human LN and LN murine models, this work can help the scientific community to select the appropriate model to study pathways or pathogenic processes of interest.

1.1. The utility of mouse models in understanding the pathogenesis of LN

The murine and human immune systems are remarkably similar and there is no doubt that animal models can be useful for studying human disease and identifying new therapies (18–20). The translation of pre-clinical studies to a successful therapeutic for humans has occurred in a large number of autoimmune diseases including rheumatoid arthritis, psoriasis, inflammatory bowel disease, uveitis and multiple sclerosis.

There are multiple mouse models of LN, some of which share genetic similarities with humans (21, 22). This has both advantages and challenges. On the one hand it is possible to identify features of human disease that can be modeled and investigated at the mechanistic level in the appropriate mouse model. By contrast, the availability of multiple models has confused the interpretation of therapeutic studies since strategies that are effective in one mouse model may be ineffective or even deleterious in another. Reporting of positive results in only one mouse strain and failure to report negative results in others may result in substantial misunderstanding of the role of particular therapies. Furthermore, the application of therapies in mice may have different results even in the same strain depending on the timing. For example, the effect of gene knockouts can occur before disease initiation but may no longer be relevant during the effector stage. Similarly, therapeutic agents that prevent nephritis onset may no longer be effective once the initial renal insult has occurred (reviewed (11)). It is important, therefore, that mouse studies should include predetermined endpoints and be conducted in relevant models and at several disease stages if they are to be effective in guiding the development and application of human therapies.

1.2. Choice of mouse models

Although no one model of LN can be considered definitive (23–26), we chose to study NZB/W, NZM2410 and NZW/BXSB mice because of differences in their autoantibody specificities (27) and renal pathology and because of their genetic similarities to human SLE. Sle1 (present in NZB/W and NZM2410 mice) is located in an area of chromosome 1 syntenic with human chromosome 1, a region with several identified SLE susceptibility loci (21). Similarly, the kallikrein locus found within Sle3 in NZW and NZM2410 mice and its ortholog in humans have both been associated with increased susceptibility to SLE nephritis (12). Finally, while the TLR7 reduplication of BXSB mice is not found in humans, polymorphisms of TLR7 that result in overexpression have been identified in human SLE patients (28, 29). NZB/W and NZM2410 mice both have lymphocyte hyperreactivity but NZM2410 make anti-chromatin rather than anti-DNA antibodies, develop glomerulosclerosis rather than proliferative disease and have less immune infiltrates in the kidneys (30). Male NZW/BXSB mice, with two copies of the TLR7 gene (31, 32), have mononuclear phagocyte and B cell activation in response to RNA antigens. They make anti-Sm/RNP and anti-cardiolipin antibodies and develop severe proliferative nephritis with exuberant infiltrates of macrophages and lymphoid cells (33).

1.3. Molecular profiling of kidneys from mice and humans reveals both shared and unique features

LN renal genome wide expression analysis allowed us to define the shared profiles of changes in gene expression and associated pathways between human LN biopsies (compared with normal human kidney biopsies) and kidneys (nephritic compared with young) from the three mouse models and showed striking concordance in nephritis-associated changes in gene expression between mice and humans (34). Those profiles were used to generate biological literature-based transcriptional networks. The **Tool for Approximate LargeE graph matching (TALE)** was used to align those networks to define the conserved functional relationships in both species. TALE is an algorithm which queries a large number of nodes (the genes) and edges (the connections between 2 genes) in complex networks (35). By allowing some mismatches in the network substructures while generating the overlap, it helps to prioritize the best conserved regulatory transcripts and connections between them. This tool allowed us not only to identify overlapping single genes having similar expression pattern in both species but also to translate the species-specific transcriptional networks to cross-species shared transcriptional networks.

A total of 20 nodes (including STAT1, LYN, CCL5, CXCL10, PTPRC, PSMB8), were regulated in the same direction in all three mouse models and human kidneys. Shared signatures within these nodes included those of lymphoid cell infiltration and activation, extracellular matrix formation and fibrosis, endothelial cell activation, fibrinolysis, mitochondrial dysfunction, defective angiogenesis and tubular injury. In addition we defined molecular pathways unique to each model that are shared with human LN. Examples include a regulatory and activated T cell signature in NZB/W mice, an IL-17 signature in NZW/BXSB mice, podocyte loss and metabolic stress in NZM2410 mice and macrophage/DC infiltration in NZB/W and NZW/BXSB mice. Surprisingly, the NZM2410 glomerulosclerotic mouse model shared the most renal transcriptional events with human LN kidneys and the NZW/BXSB model the least. This was surprising, but may reflect the ability of steroids to rapidly dampen inflammation since >90% of the patients had already received glucocorticoids at the time of biopsy.

In the same study (34), we also compared the signatures of LN biopsies with those of IgA nephropathy (IgAN) and hypertensive nephropathy (HT). As expected, more inflammatory genes were shared with the IgAN biopsies than with the HT biopsies but approximately one third of the transcripts regulated in LN biopsies were unique to LN. For example, the LN glomeruli had more downregulation of *VEGFA* and of mitochondrial genes (citrate synthase node) than did the IgAN or HT biopsies suggesting a greater degree of oxidative stress in the LN biopsies. Loss of VEGF was also a feature of progressive disease in the murine LN models and did not completely reverse after remission induction with immunosuppressive therapy (36) this suggests that some processes involved in chronic renal injury and disease progression may be resistant to systemic immune suppression and may require a different therapeutic approach to prevent poor long term outcomes in LN.

1.4. Renal macrophages and dendritic cells

A macrophage/DC signature was highlighted as a shared pathway among all three LN mouse models and human biopsies. This is of interest because CD68⁺ mononuclear phagocyte infiltration is associated with a poor prognosis in human LN (37–39). Several subtypes of these cells have been identified by immunohistochemistry in LN biopsies (40–42); the functions and origins of these cells are still unknown.

1.4.1. Phenotypic and functional analysis of renal mononuclear phagocytes in mouse lupus models—Macrophage/DC infiltration is a prominent feature of disease in NZB/W and NZW/BXSB mice. We therefore performed extensive phenotypic and functional characterization of renal macrophages and DCs at multiple disease stages in these two strains (43, 44). We identified and characterized two different macrophage populations in the kidneys of pre-nephritic mice. The dominant CD11b⁺/CX3CR1⁺/F4/80^{hi} resident population (43, 45–47) is monocyte derived and CSF dependent, and although it is derived from the fetal yolk sac it can also be repopulated from the bone marrow (48). A minor population of monocyte derived cells is F4/80^{lo}. We also identified three populations of renal dendritic cells including CD11c^{hi} myeloid dendritic cells, CD103⁺ dendritic cells and plasmacytoid dendritic cells (43, 44). Similar populations have recently been described in the kidneys of normal mice (49).

Activated macrophages can be classified into several functional subtypes (50, 51) but they have a high degree of plasticity *in vivo* and they may switch functions during the inflammatory and repair phases of acute renal injury (50, 52–56). Reparative macrophages are not always beneficial; prolonged exposure to these macrophages may foster excessive remodeling and damage (reviewed (50)). We found both expansion and activation of the dominant CD11b⁺/F4/80^{hi} renal macrophage population during active LN. Some of these cells appear to be derived from circulating Ly6C^{int}/Gr1^{int-lo} cells that are expanded in the peripheral blood of lupus prone mice (43). They are highly phagocytic and they express neither iNOS (M1) nor arginase (M2) upon stimulation with appropriate cytokines (44). During nephritis, F4/80^{hi} cells markedly upregulate expression of CD11b and they accumulate large numbers of both single membrane and double membrane vesicles resulting in a marked increase in cell size (43). In addition we showed a loss of mitochondria in these cells suggesting that they may have been subjected to oxidative stress. Importantly, we did not detect infiltration of Ly6C^{hi}/Gr1^{hi} inflammatory macrophages in perfused kidneys during any stage of LN in the three mouse models.

A large population of CD11c^{hi} myeloid dendritic cells infiltrates the kidneys during proliferative SLE nephritis and disappears during remission (43, 46). By contrast, there was no increase in renal pDCs and only a modest increase in CD103⁺ DCs (44). During active nephritis renal CD11c^{hi} DCs are abundant within lymphoid aggregates of NZB/W mice and within glomeruli of NZW/BXSB mice and are topographically separated from F4/80^{hi} macrophages in nephritic kidneys (33, 46). We found that they are derived from bone marrow CD11b⁺ cells and they express CCR7, as well as CD209 (DC-SIGN), a marker of one of the subsets of tubulointerstitial mononuclear phagocytes in human SLE nephritis (40, 41). Importantly, an increase of renal macrophages and DCs has recently been observed in

human glomerulonephritis biopsies and an increase in both CD141 (CD103+ equivalent) and myeloid DCs is observed in fibrotic biopsies (57).

1.4.2. Gene expression profiling of renal mononuclear phagocytes during active SLE nephritis—To determine the function of the activated macrophages in the kidneys of nephritic mice we performed gene expression analyses of F4/80^{hi} macrophages and dendritic cells from young and nephritic NZB/W mice (34, 43). These studies have allowed us to identify genes that are markers of increased macrophage infiltration as well as genes whose expression reflects alterations in cell function. Examples of molecules whose expression is highly upregulated in nephritic compared with young F4/80^{hi} macrophages include regulatory mediators such as ITGAM, IL10 and IL1rn, reparative mediators such as MMP14, Fn1 and pro-inflammatory molecules such as CXCL13, CD40, CCL7, IFN inducible genes and complement factor B. Thus, the traditional M1/M2 paradigm is insufficient to explain the macrophage phenotype of LN. Compared with CD11c^{hi} dendritic cells, F4/80^{hi} macrophages express genes associated with membrane organization and transport, metabolic processes and angiogenesis. By contrast CD11c^{hi} dendritic cells express genes associated with regulation of lymphocyte activation and cell death. Similar differences in gene expression of renal macrophages and DCs have been reported in the Sle1.TLR7 model (58).

1.4.3. Imputation of a macrophage gene expression profile in human LN biopsies—To further analyze the role of macrophages in LN, gene expression profiles of macrophages isolated from NZB/W kidneys were compared with murine and human LN kidney gene expression profiles. This strategy allowed us to attribute mRNA signatures to macrophages in the human samples even when such information was not initially derived from the human tissue. **Literature-based transcriptional network and pathway analyses tools** were used to integrate the resulting gene expression profiles in their biological context. Importantly, we established that there is considerable overlap of the NZB/W macrophage gene signature with that of human LN but that there may be some functional differences between macrophages in the glomerular and interstitial compartments (34). Shared genes involve innate immune activation pathways including an upregulation of MyD88, IRF7 and interferon induced genes.

The unique genes shared between NZB/W macrophages and human LN glomeruli are dominated by a PPAR γ transcriptional profile and are enriched in genes involved in protein ubiquitination. This is consistent with our observation that F4/80^{hi} cells from proteinuric NZB/W mice contain autophagocytic vesicles and suggests a role of these cells in intracellular proteolysis, presumably of oxidatively modified and ubiquitinated proteins. The glomerular macrophage profile also uniquely expresses a tissue repair signature. By contrast the interstitial macrophage/DC profile is dominated by a NF κ B transcriptional profile and is characterized by the expression of genes involved in coagulation and fibrinolysis (34). The advent of single cell RNASeq technology may allow further classification of macrophage subtypes in both mouse and human LN kidneys.

1.5. Sequential molecular events in disease onset, remission and relapse

Mechanisms of disease progression may be inferred from changes in gene expression over the course of disease. To determine which renal molecular pathways are associated with response to therapy and relapse we characterized the molecular genetic profile of kidneys from NZB/W mice at 5 sequential disease stages and at times after induction of remission with a combination of cyclophosphamide (CYC) and costimulatory blockade (36). We employed a machine learning algorithm (package named Melikerion whose source codes can be downloaded from <http://www.finndiane.fi/software/melikerion/>) called **Self-Organizing Map (SOM)**, to identify genes that changed coherently throughout the disease course including initial onset, remission and relapse. SOM helps to identify the biological processes driving coherent expression modules and more specifically to extract the fundamental patterns of gene expression inherent in serial data sets. SOM allowed us to identify two major gene clusters that are regulated during disease progression; the first, occurring at new proteinuria onset, identifies the inflammatory component of nephritis and the second, occurring during established disease (proteinuria of more than 2–3 weeks duration) reflects the metabolic disturbances and mitochondrial dysfunction that are associated with hypoxia. In NZB/W mice, our studies show that remission induction (proteinuria of 30 mg/dl on 2 occasions) reverses a large proportion of the abnormal renal gene expression profile of nephritis including markers of inflammatory cell infiltrates (e.g. CD11b, CCR7), inflammation (e.g. CCL2, TNF), endothelial cell activation (e.g. VCAM-1) and tubular damage (e.g. LCN2). Importantly however, a mitochondrial and metabolic signature recurs in NZB/W mice during progression towards relapse before the reappearance of proteinuria, perhaps reflecting an increased propensity to hypoxia conferred by the initial tissue insult. We further identified podocyte loss, renal tubular dysfunction, endothelial cell activation and tissue remodeling as the functional features associated with clinical relapse and consequent renal failure in both models (36).

1.6. Biomarker identification

Remission induction therapy is most effective in NZB/W mice when it is administered at proteinuria onset rather than after the mitochondrial and metabolic signature has been established. Our finding that most changes in renal gene expression occur at clinical disease onset suggests that irreversible damage may progress rapidly, underscoring the need for early biomarkers of renal flare. We analyzed the time dynamics of renal molecular changes during LN progression and remission in the NZB/W mouse model using **singular value decomposition (SVD)**, an unbiased approach to identify genes whose expression correlated most tightly with disease stage (36). SVD is a technique which identifies specific patterns from multivariate time course data, in our case genome wide expression data at 5–7 different disease stages (36). We then identified the following genes that are also highly expressed in human LN biopsies.

1.6.1. Genes that are upregulated in prenephritic kidneys and fluctuate with remission and progression towards relapse—include CCL5, Ly86, CD52 and FCGR3; of these, increasing urinary CCL2 (MCP1) is currently the most promising available biomarker for disease flare (59, 60), whereas failure to normalize urinary CCL5 during remission is associated with an increased risk for subsequent flare (61). Ly86 is

expressed by plasma cells and renal macrophages; interestingly, serum levels of soluble Ly86 correlate with disease progression in MRL/lpr mice (62). CD52 and FCGR3 are markers for infiltrating renal cells.

1.6.2.. Many of the genes that are regulated at proteinuria onset, reverse with remission induction and become abnormal again during progression towards relapse—are associated with macrophages/DCs. These include C1q components that are highly upregulated in human renal biopsies and are uniquely produced by tissue macrophages, PLAC8, a dendritic cell molecule required for anti-bacterial responses (63) and Laptm5 a macrophage molecule involved in the cytokine response to TLR stimulation (64).

1.6.3. Genes whose expression is detected only in proteinuric mice—include those involved in endothelial cell activation, tubular injury, tissue remodeling and innate immune processes. Of these, CXCL10 and VCAM-1 are soluble proteins that have already been identified as potential urinary biomarkers for human LN (65, 66). Other genes in this group whose expression can be measured in the serum and/or urine include TIMP1 and EGF (see below).

1.7. Summary

We established that each of the three murine models we have studied in detail share both common and unique molecular features with the human disease. LN onset is characterized by infiltration with inflammatory cells, whereas disease progression is associated with a mitochondrial and metabolic profile that may reflect increasing tissue hypoxia. Furthermore the renal tissue appears to be more sensitive to hypoxic stress during relapse than during the initial flare, perhaps reflecting the failure to fully correct endothelial cell viability. Macrophage/DC infiltration and activation is a key shared feature of LN in mice and humans (34, 36, 43, 67). Infiltration with these cells is associated with poor prognosis in humans. We have identified two major renal mononuclear phagocyte cell sub-populations that are activated during LN (43, 44) and several macrophage/DC functional pathways that are shared between mouse and human LN (14, 34, 36, 43). Which of these pathways are protective and which are pathogenic remains to be determined. Finally, our studies suggest a panel of potential biomarkers that can be tested in patients.

2. Linking genotype to phenotype

Genome wide association studies (GWAS) have been extremely successful in identifying susceptibility loci for many disease phenotypes, including SLE and related endophenotypes (68, 69) and have led to successful therapeutic approaches in other diseases (70, 71). Single nucleotide polymorphisms (SNPs) that are linked to disease phenotype may be found within as well as outside coding regions and can impact the disease trait via associated transcript expression changes. Delineating the genetic factors that lead to LN among patients with SLE may facilitate our understanding of the etiology of LN, provide improved prediction of risks of progression and ultimately motivate novel therapeutic targets. In an example of this concept, Liu et al. show that decreased transcriptional expression of kallikrein genes in

mouse anti-GBM models increases the susceptibility to nephrotoxic glomerulonephritis, as well as conferring an increased risk for LN in humans (72). This was confirmed by Li et al, who showed that the protective effect occurs through inhibition of the inflammation and oxidative stress pathways (73).

The first large-scale genome-wide investigation of LN was recently published and provided evidence of multiple biologically plausible LN susceptibility loci (74). We showed that **linking renal transcriptional profiles from LN biopsies with gene candidates identified by GWAS** using systems genetics strategies can facilitate identification of molecular mechanisms driving LN. We examined gene expression profiles from LN micro-dissected glomerular and tubulointerstitial compartments of biopsies for the regions showing the strongest association with LN as identified by GWAS. Several genes identified in GWAS studies were significantly regulated in the glomerular and tubulointerstitial compartments from renal biopsies including PDGFRA, ID4, COL4A1 and ITGAM, suggesting that these genes may be involved in LN pathogenesis.

The PDGFRA (Platelet-Derived Growth Factor Receptor Alpha) gene encodes a tyrosine kinase receptor. Its ligand PDGF is expressed by renal mesangial cells, endothelial cells, macrophages and smooth muscle cells and is involved in renal fibrosis in different forms of glomerulonephritis through inflammation and activation of renal interstitial and mesangial cells (75). Our molecular studies did not confirm any regulation of the PDGFRA gene in nephritic kidneys of the three mouse models. This is consistent with the finding that SNPs associated to PDGFRA gene and linked to the dysregulation of its associated pathway may not have homolog genetic modification in the mouse models. Nevertheless, inhibition of the PDGF pathway using a tyrosine kinase receptor antagonist (Imatinib) prolonged the survival of both NZB/W and MRL/lpr mice through antiproliferative and antifibrogenic effects (76).

As in human LN kidneys, ID4 was significantly down-regulated and ITGAM highly up-regulated in the kidneys of all three murine models. ID4 is a member of the helix-loop-helix family of transcriptional regulators. Loss of Id4 in B cells enhances cell proliferation triggered by CpG oligonucleotides and decreases sensitivity to dexamethasone-mediated apoptosis (77). ID4 regulates cell differentiation in multiple cell types and also enhances estrogen production and signaling (78); how this influences the course of LN is currently unknown. ITGAM encodes for the alpha chain of the CD11b (complement receptor 3) adhesion molecule that is expressed on myeloid cells and has both protective and pro-inflammatory functions in inflammation (79, 80). ITGAM polymorphisms are associated both with lupus susceptibility and severity and recent functional studies have determined that the dominant polymorphism R77H decreases ligand binding to CD11b under flow conditions and may compromise the inhibitory functions of the molecule by altering the affinity of the molecule for its ligands (79, 80). We found that the increase in expression of CD11b in all three murine models is due both to an increase in CD11b expressing cell infiltrates and an increase in CD11b expression on renal macrophages. How this increase in CD11b expression alters macrophage function in the setting of LN is not known. Collagen genes, including COL4A1 were over-expressed in the NZB/BXSB model, suggesting that this is the most appropriate model to study renal fibrosis.

In summary, the identification of gene candidates by GWAS can point to fruitful areas for further investigation. As we demonstrate here, the mouse models may model the human disease and may therefore be useful for mechanistic studies to further delineate the function of particular gene candidates.

3. The utility of intrarenal transcripts in predicting progression to CKD – from human LN to mouse models

Despite the use of immunosuppressive therapies, the risk of progression of LN to renal failure remains unacceptably high. A recent publication from Ju et al. showed that risk for progression of CKD in three different patient cohorts with several different renal diseases, is associated with a shared interstitial molecular signature of 68 genes (81).

To determine whether these 68 genes can be used in pre-clinical studies to model disease and therapeutic responses, we analyzed microarray data of kidneys from NZB/W, NZM2410 and NZW/BXSB mouse lupus strains at various disease stages and after remission induction. Of the 68 human genes, 64 had a corresponding mouse gene ID and 61 were represented on the mouse microarray chip. Of these 49 (80.3%) were regulated in the same direction (in nephritic compared to young) as in humans in at least one strain (38 in NZM2410 mice, 44 in NZW/BXSB mice, 33 in NZB/W mice) (Supplementary Table 1). 28 of the 49 genes were common to all three strains (Table 1). Few genes were unique to a single strain; of these, collagen genes were uniquely expressed in nephritic NZW/BXSB mice (Figure 1A).

To determine which genes could distinguish new onset proteinuria (within the last 48 hours) from established disease (proteinuria of >2–3 weeks duration) and could therefore be used as potential biomarkers of progression to CKD, we compared the profiles of newly proteinuric NZB/W mice to those of NZB/W mice with established disease. Of the 68 genes comprising the shared molecular signature of human CKD progression 33 were expressed in nephritic NZB/W mice. Of these 9 (Table 2), including Epidermal Growth Factor (EGF - Figure 1B) and tissue inhibitor of metalloproteinase 1 (TIMP1) became abnormally regulated only during established disease, confirming their association with CKD progression. TIMP1 regulates the turnover of extracellular matrix by inhibiting the proteolytic activity of matrix metalloproteinases (MMPs). A decrease in matrix degradation during chronic tissue injury may enhance tissue fibrosis. EGF is one of three growth factors that bind to the EGFR, a transmembrane tyrosine kinase receptor that is expressed in the kidney epithelium and interstitium and maintains epithelial integrity. EGF and EGFR may be beneficial in promoting tubular regeneration during acute renal injury. By contrast during chronic renal disease renal EGF is diminished and EGFR signaling through its other ligands HB-EGF and TGF alpha may be pro-fibrotic (82). Thus a decrease in EGF and an increase in TIMP1 may be useful biomarkers of a switch to a profibrotic phase of LN disease progression. Of the other 7 genes (Table 2), A2M (α 2macroglobulin), a soluble protein which binds many cytokines and growth factors, THBS1 (thrombospondin-1), an anti-angiogenic component of the extracellular matrix whose renal expression correlates inversely with renal capillary number (83, 84) and SERPING, a plasma protein inhibitor of the complement cascade are also soluble proteins; their utility as biomarkers for CKD progression remain to be tested.

To determine whether expression of the CKD associated genes is reversible we examined their expression in kidneys of NZB/W mice after immunosuppressive therapy. Most of the CKD associated genes normalized after immunosuppression but tended to drift back to their abnormal values during impending relapse (Figure 1B and Supplementary Table 1). This suggests that expression of these biomarkers in the serum or urine could be tested for their ability to predict relapse prior to the onset of proteinuria.

The study of Ju et al. (81) showed that down-regulation of renal EGF mRNA levels can predict progression of chronic kidney disease (CKD). Transcriptional data from two cohorts, the ERCB discovery cohort and C-PROBE validation cohort included data from 30 and 16 LN patients respectively. Figure 1C shows a significant correlation of eGFR with the mRNA levels of EGF in both groups of LN patients, suggesting that these findings apply to lupus patients as well as to the whole cohort. Furthermore the downregulation of renal EGF expression is associated with an increase in urinary EGF that can predict fibrosis/tubular atrophy and the loss of GFR. While these findings need to be confirmed in larger cohorts, they suggest that a systems approach in human renal disease can identify new clinically applicable biomarkers that may have direct impact on patient care.

In summary, mice with LN have a strikingly similar pattern of CKD-related gene expression to humans and these genes can be used to track therapeutic responses. Downregulation of EGF and upregulation of TIMP1 mark progressive disease in mice as in humans and could potentially be used to monitor responses to new therapies in a manner that is relevant to human disease. The fibrosis signature is best modeled in NZW/BXSB mice, as the relevant collagens are relatively restricted to this mouse strain.

4. Longitudinal molecular profiling of peripheral blood cells in LN

As data sets become larger to include expression profiles from many samples and even to integrate clinical data into the analysis, new analytic tools are being developed to reduce the complexity of the data. A schematic overview is presented Figure 2.

4.1. Modular analysis of total PBMCs

Recently, Chaussabel et al have developed a method for “modular transcriptional repertoire analyses” of peripheral blood mononuclear cells of patients with a variety of diseases including lupus (85, 86). From this work, they characterized “Blood modules”. Briefly, they clustered the genes from a collection of publicly available gene expression datasets derived from PBMCs of patients with 8 different pathological conditions and identified the genes that were coordinately regulated in all eight datasets (“M1 modules), 7 of 8 datasets (“M2 modules) or 6 of 8 datasets (“M3 modules”), etc. up to M6 regardless of disease. From each of these modules, progressively smaller submodules were then identified (M1.1, M1.2 etc) for a total of up to 260 identifiable modules. Although this process was driven only by the data, many of the modules could be identified as related to a particular cell type, pathway or functional process. When healthy patients were compared with untreated SLE patients using an initial set of 28 modules), 5 modules were overexpressed (plasma cells, interferon, neutrophils, myeloid and erythrocytes) and 6 were underexpressed (ribosomal proteins, cytotoxic cells, T cells and undetermined).

Chiche et al. (87) then applied the full 260 modular analyses to longitudinal blood molecular profiling of total PBMCs from a large cohort of pediatric lupus patients and correlated this data with clinical activity and disease severity. They identified three different modular interferon signatures that were differentially associated with mild, moderate or severe disease, showing that the interferon response is complex. One of these interferon modules appeared to correlate with renal flares.

Banchereau et al (88) extended this analysis to more patients and used the “**weighted gene co-expression network analysis**” (WGCNA) method (89) to ask whether gene expression patterns correlate with disease activity. When applied to gene expression datasets, WGCNA allows gene expression modules to be related to external information such as clinical data in order to find biologically interesting modules e.g. those associated with disease severity or organ specific manifestations. Using WGCNA to analyze each patient over time together with assignment on the basis of the modular analysis, they clustered the patients into 7 phenotypic subgroups, each with a different combination of the 5 overexpressed modules (plasma cells, interferon, neutrophils, myeloid and erythrocytes) identified in the original studies. One of these phenotypic groups, PG4, all had LN. In these patients the SLEDAI correlated with IFN response, myeloid lineage, and plasmablasts and the patients also had high numbers of neutrophils. While extensive validation of these findings is needed, and application to adults has not yet been performed, the authors hypothesize that subphenotyping based on peripheral blood signatures could be useful to predict increasing disease activity in SLE patients and anticipate treatments accordingly.

Application of these complex studies to a clinically meaningful and inexpensive laboratory test is challenging. Using the genes identified in the 7 phenotypic subgroups Banchereau et al. modified their analysis to include only 797 genes that were able to successfully stratify the patients. This makes it feasible to design a simplified assay for clinical samples that can be used to prospectively test the proposed hypotheses. By characterizing SNPs previously known to be associated with SLE (e.g. ITGAM, BANK1), the authors also identified a set of non-overlapping SNPs that associated with either a lymphoid/B cell signature or a myeloid/neutrophil signature in the peripheral blood. Since the presence of a myeloid/neutrophil signature was associated with more disease activity and the presence of LN, genetic testing might also have some utility in predicting subsequent disease course.

4.2. Application of blood modules to renal tissue

Using the renal molecular profiles of the LN ERCB cohort, we determined whether the modules described by Banchereau et al for peripheral blood could stratify the LN patients. Approximately 45% and 35% of the 797 genes were regulated in the glomerular and tubulointerstitial compartments of LN renal biopsies respectively. In both compartments, plasmablasts (89%) and interferon response (68% and 74%) signatures were the most represented. The myeloid lineage/neutrophil signature was next with a representation of 54% and 39% in the glomeruli and tubulointerstitium respectively. The lymphoid lineage was also regulated in the glomeruli (35%), whereas the erythropoiesis signature was found at low levels only in the tubulointerstitium (8%).

None of the described modules significantly segregated the ERCB cohort according to the WHO classification, chronicity index or activity index. These data show that the disease activity-associated blood gene expression signatures do not necessarily reflect the renal molecular events. In other words: “PBL are critical but are not the only drivers in SLE nephritis” (90).

4.3. Modular analysis of PBMC subsets

The findings of Banchereau et al. using total PBMCs may be skewed by the altered distribution of cell subsets in lupus patients. Furthermore, meaningful changes in gene expression in small PBMC subsets may not be detectable. Other work has therefore applied transcriptome analysis to isolated PBMC subsets in autoimmune diseases including lupus. Using **hierarchical cluster analysis of individual genes**, McKinney et al described a specific steroid responsive CD8+T cell signature that segregated 2 subgroups of untreated active lupus patients according to disease severity (91). Hierarchical clustering using gene expression datasets allows visual segregation of subsets of patients based on similarities of their gene expression pattern. ITGA2, NOTCH1 and PTPN22 genes alone could segregate the patients (not the diseases). Such classification may allow “tailored therapy”, by identifying the patients that are most at risk of flare.

In order to understand the underlying biological mechanisms leading to this patient classification, the same group applied the WGCNA method to a gene expression dataset from isolated CD8 and CD4+ T cells from a cohort of active SLE patients having the scientific advantage of being “treatment naïve” (91). This method allowed these investigators to identify a set of specific clinical outcome associated T-cell expression modules. **Gene set enrichment analysis (GSEA)** was applied to the genes from the CD8 modules segregating the patients according to their outcome. This tool highlighted a CD8 T cell exhaustion signature in the group having less risk of relapse/less flares, including the IL7R gene and a number of co-inhibitory molecules. The CD8 exhaustion signature was associated with a decrease in costimulatory genes in CD4 T cells. Importantly this same gene signature is inversely associated with vaccine and anti-viral responses and can be reversed by strategies that enhance co-inhibitory signals such as anti-PD1. These studies highlight the role of exhausted T cells in suppressing immunity to infections or cancers while protecting against autoimmunity and suggest a potential therapeutic strategy for preventing flares.

Most recently, modules of IFN-1 associated genes specific of leukocyte subsets were also identified by Flint et al using again the WGCNA method on several autoimmune diseases including SLE. A specific CD8 and CD4 T-cell interferon signature was found in SLE which distinguishes this disease from other autoimmune diseases (92).

4.4. Summary

In summary, methods are now developing to analyze large data sets in an agnostic manner and to integrate clinical data including outcomes and responses to therapy into the data sets so as to identify gene expression modules of biological interest, particularly those associated with more severe disease or disease flares. Functional analysis of these modules such as the

interferon sub-signatures, the exhaustion signature and the signature in the PG4 group described above is now required. The utility of these approaches for stratifying patients for clinical trials, predicting flares, monitoring treatment responses and personalizing drug interventions remains to be determined and is an area of ongoing research.

5. Conclusions

Constantly evolving technologies are opening new doors to deeper analyses and enhanced interpretation of the human transcriptome, especially of complex organs containing multiple specialized cell types such as the kidney. This leads to new opportunities to characterize markers of disease progression as well as to identify therapeutic targets. Using RNASeq technology, rat nephron segment transcriptome profiles were generated and can be used as a reference set for defining cell types (93, 94). Analysis of the epigenetic landscape of renal tissue is also of significant interest in LN with the potential for identification of therapeutic targets such as HDAC inhibitors and biomarkers such as miRNAs (95).

Recent resources related specifically to renal gene expression data are also publicly available to the research community. Nephroseq™ (<https://www.nephroseq.org>, freely available) and transMART (access restricted to the researchers involved in the particular cohort studies) (96) are two platforms with “pre-processed” data integrating gene expression profiling and clinical information, which allow exploration and a “customized” meta-data analysis of kidney diseases, including LN.

Several clinical trials have failed in LN despite extensive research and the application of rational therapeutic approaches supported by pre-clinical data. While inadequate clinical design may have in part contributed to the failure of these trials, it is clear that our conceptual framework for approaching the pathogenesis and treatment of LN is incomplete. In 2014, the National Institute of Health (NIH), industry and non-profit organizations joined forces to initiate the Accelerating Medicine Partnership (AMP) project (<https://amp-ralupus.stanford.edu/>), whose goals are to characterize new specific diagnostic and therapeutic targets through a better understanding of the mechanisms driving the disease, particularly the contribution of individual renal cell types. This study is novel with respect to the extensive network of investigators and the infrastructure assembled to ensure patient enrollment and sample acquisition, and the application of state-of-the-art technologies for high resolution analysis of kidney, blood and urine to dissect disease pathogenesis and tissue repair, focusing on both inflammatory mechanisms and tissue response. The impact of this study will be the discovery of new therapeutic targets and new data to guide therapeutic decision-making. In addition, in the course of this project the AMP will provide all clinical and academic researchers access to databases with processed and analyzed data via Immport (<https://import.niaid.nih.gov>).

Solving the problem of LN will require disease registries, biospecimen repositories of well phenotyped and genotyped patients, and clinical trial networks (97). Flow of data obtained using high-throughput analytical platforms and from translational research efforts such as AMP to clinical trialists and to basic researchers studying animal models will be essential in

developing a comprehensive approach to improving detection and treatment of LN resulting in improved long-term patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding This work was supported by the National Institute of Health [grant number R01 DK085241-01] and the O'Brien Renal Center Core [P30DK081943].

Abbreviations

SLE	Systemic Lupus Erythematosus
LN	Lupus Nephritis
RNA	Ribonucleic Acid
Anti-sm RNP	Anti-smith RiboNucleo Protein
TALE	Tool for Approximate Large graph matching
IgAN	IgA Nephropathy
HT	Hypertensive
SOM	Self-Organizing Map
SVD	Singular Value Decomposition
GWAS	Genome Wide Association Study
CKD	Chronic Kidney Disease
WGCNA	Weighted gene co-expression network analysis
AMP	Accelerating Medicine Partnerships

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- The NZB/W, NZM2410, NZWBXSB murine models share common and unique molecular features with human LN disease.
- Renal macrophages and dendritic cells are key players in LN.
- Mice with LN have a strikingly similar pattern of CKD-related gene expression to humans and these genes can be used to track therapeutic responses.
- The identification of gene candidates by GWAS can help to link genotype to phenotype in LN.
- New analytic tools are being developed to reduce the complexity of the data generated from molecular profiling of peripheral blood cells in LN to yield new hypotheses, new biomarkers and new therapeutic strategies.

Figure 1.

Analysis of 68 intra-renal transcripts predicting progression to CKD in humans. **A.** Comparison of the 68 CKD genes regulated in the same direction in human CKD and three mouse models (in nephritic compared to young, non—proteinuric mice). **B.** EGF mRNA expression data in NZB/W mice at sequential stages of the disease progression, after complete remission and during impending relapse. **C.** Correlation of tubulointerstitial EGF mRNA with eGFR in the ERCB and C-PROBE human LN cohorts.

Figure 2.
Schematic overview of recent longitudinal molecular profiling analyses of peripheral blood cells in lupus, including methods for analysis and integration of clinical data.

Table 1

List of the 28 genes changed in nephritic compared to young mice (no autoantibodies, no proteinuria) in all three mouse models and in human CKD.

Gene	Human CKD compared to controls	NZB/W		NZM2410		NZWBXSB	
		Fold-change	q-value	Fold-change	q-value	Fold-change	q-value
C1qa	up	3.44	0.0000	3.51	0.0000	2.70	0.0000
Casp1	up	1.98	0.0000	1.52	0.0017	1.36	0.0012
Clu	up	1.75	0.0047	3.05	0.0000	1.99	0.0000
Egf	down	0.61	0.0056	0.68	0.0181	0.55	0.0000
Emp3	up	1.63	0.0000	1.55	0.0022	1.85	0.0000
Fgl2	up	2.71	0.0015	2.25	0.0016	1.92	0.0000
H2-Ab1	up	3.32	0.0000	6.28	0.0000	1.93	0.0000
H2-DMa	up	1.97	0.0000	2.95	0.0000	1.71	0.0000
H2-DMb2	up	2.38	0.0007	8.18	0.0000	1.72	0.0000
Ifi204	up	2.36	0.0097	2.64	0.0013	1.93	0.0000
Laptn5	up	2.65	0.0000	2.42	0.0000	1.79	0.0000
Lgals1	up	1.46	0.0064	3.06	0.0000	1.90	0.0000
Psbm9	dn	2.55	0.0026	2.51	0.0024	1.74	0.0008
Psbm8	dn	2.60	0.0000	4.35	0.0000	2.06	0.0000
Auxa1	up	2.32	0.0000	3.59	0.0000	2.49	0.0000
Lyz2	up	4.52	0.0000	4.51	0.0000	2.60	0.0000
Rcn1	up	1.77	0.0000	2.34	0.0003	1.93	0.0000
Serpine2	up	1.55	0.0026	1.84	0.0004	1.61	0.0000
Tgfb1	up	3.17	0.0000	2.96	0.0000	2.94	0.0000
Thbs2	up	1.31	0.0369	1.49	0.0156	1.46	0.0000
Timp1	up	2.16	0.0031	5.04	0.0000	4.41	0.0000
Tyrobp	up	3.53	0.0000	2.87	0.0000	2.37	0.0000
Vcam1	up	2.57	0.0000	10.73	0.0000	2.72	0.0000
Vim	up	1.84	0.0000	2.93	0.0000	2.55	0.0000
Wfdc2	up	1.50	0.0422	2.01	0.0000	2.24	0.0000

Gene	Human CKD compared to controls	NZB/W		NZM2410		NZWBXSB	
		Fold-change	q-value	Fold-change	q-value	Fold-change	q-value
Rnase6	up	1.77	0.0064	1.71	0.0104	1.36	0.0008
Actn1	up	1.33	0.0611	2.01	0.0003	1.70	0.0000
H2-Ea-ps	up	2.97	0.0000	4.90	0.0000	1.63	0.0002

Microarrays data from the 3 mouse strains were generated using Affymetrix 430.2 and are available on Gene Expression Omnibus at <http://www.ncbi.nlm.nih.gov/geo/> (accession numbers GSE27045, GSE32583, GSE44691 and GSE49898).References: Berthier CC et al, J of Immunol, 2012 and Bethunaickan R et al, PlosOne, 2013).

Nine human CKD predicting genes which regulation is significantly modified in 36 weeks old NZB/W (established proteinuria) mice compared to onset of proteinuria (23 weeks old animals).

Table 2

Gene symbol	Gene name	Short description (http://www.ncbi.nlm.nih.gov)	Human CKD compared to controls	23 weeks onset of proteinuria compared to young		36 weeks established proteinuria compared to young	
				Fold-change	q-value	Fold-change	q-value
EGF	Epidermal growth factor	Encodes a protein involved in the growth, proliferation and differentiation of numerous cell types	down	1.04	0.4756	0.61	0.0056
A2M	Alpha 2 macroglobulin	Protease inhibitor and cytokine transporter	Up	1.08	0.5263	1.51	0.0394
PDLIM1	PDZ and LIM domain 1	Encodes a cytoplasmic protein associated with the cytoskeleton	Up	1.24	0.0635	1.17	0.0180
THBS2	Thrombospondin 2	Glycoprotein that mediates cell-to-cell and cell-to-matrix interactions; potent inhibitor of tumor growth and angiogenesis	Up	1.10	0.3717	1.31	0.0369
SERPINC1	Serpin family G member 1	Plasma protein involved in the regulation of the complement cascade	Up	1.22	0.3385	1.33	0.0150
ACTN1	Actinin alpha 1	Cytoskeletal protein	Up	1.05	0.5488	1.33	0.0611
PSMB9	Proteasome subunit beta 9	Member of the immunoproteasome family. Gene located in the MHCII	Up	1.38	0.0707	2.55	0.0026
CLU	Clusterin	Secreted chaperone protein involved in cell death, tumor progression, neurodegenerative disorders	Up	1.40	0.2503	1.75	0.0047

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Gene symbol	Gene name	Short description (http://www.ncbi.nlm.nih.gov)	Human CKD compared to controls	23 weeks onset of proteinuria compared to young		36 weeks established proteinuria compared to young	
				Fold-change	q-value	Fold-change	q-value
TIMP1	Tissue inhibitor of metalloproteinase 1	Natural inhibitor of the matrix metalloproteinases (MMPs), a group of peptidases involved in degradation of the extracellular matrix	Up	1.63	0.2227	2.16	0.0031

Genes with q-value<0.05 are considered significant.