

ВОЛЧАНОЧНЫЙ НЕФРИТ И ВЫДЕЛЕНИЕ ПОЧЕЧНЫХ ЛЕЙКОЦИТОВ

Махима Сара Мэтью

Доцент кафедры дерматологии

Самаркандский государственный медицинский университет

Аннотация

По крайней мере, у 20% пациентов с системной красной волчанкой (СКВ), которые подвержены риску развития терминальной стадии почечной недостаточности (ТПН), наблюдается повреждение почек, что является серьезным осложнением. Поскольку инфильтрация иммунных клеток в почки тесно связана с волчаночным нефритом, предотвращение этой инфильтрации может замедлить прогрессирование заболевания. Эффективные и повторяемые процедуры изоляции необходимы для исследования функции инфильтрирующих почки лейкоцитов.

Ключевые слова: биопсия почек; стратификация; суррогатные биомаркеры; волчаночный нефрит; персонализированная медицина.

LUPUS NEPHRITIS AND THE ISOLATION OF KIDNEY LEUKOCYTE

Mahima Sara Mathew

Associate professor in the Department of Dermatology

Samarkand State Medical University

Abstract

At least 20% of patients with Systemic Lupus Erythematosus (SLE) who are at risk of developing end-stage renal disease (ESRD) experience renal injury, a serious complication. Because immune cell infiltration into the kidneys is closely linked to lupus nephritis, preventing this infiltration may slow the progression of the disease.

Effective and repeatable isolation procedures are necessary to investigate the function of kidney-infiltrating leukocytes.

Keywords: renal biopsy; stratification; surrogate biomarkers; lupus nephritis; personalized medicine.

The introduction

As it progresses to severe pathology and renal failure, nephritis—a common and dangerous manifestation of many autoimmune diseases—significantly raises the risk of morbidity and mortality.

Depending on the related autoimmune disease, nephritis can have different specific forms and underlying mechanisms (Anders & Rovin, 2016; Davidson, 2016; McGaha & Madaio, 2014; Yu et al., 2017). The deposition of immune complexes in the glomeruli, which causes local inflammation and attracts pro-inflammatory immune cells, is frequently the cause of renal injury. The mechanisms underlying autoimmune nephritis have been better understood thanks to experimental models of SLE. For example, autoreactive antibodies that target elements like the glomerular basement membrane (Reynolds, 2011) or megalin (Spicer et al., 2007) are administered to inducible models to replicate disease pathogenesis. These mice have an increased inflammatory response to IgG-containing immune complexes and hyperactive B cell responses due to the lack of the inhibitory Fcγ receptor for IgG. FcγRIIb deficiency causes spontaneous autoimmune pathology, which is similar to human SLE in that it results in splenomegaly, anemia, systemic immune activation, anti-nuclear antibody production, and fatal nephritis (Bolland & Ravetch, 2000; Bolland et al., 2002; Tarasenko et al., 2007).

Evaluation of Renal Function: Analysis of Proteinuria

Experimental animals are usually chosen at advanced stages of kidney damage, when immune cell infiltration is more noticeable, in order to efficiently isolate kidney-infiltrating leukocytes. Blood creatinine or blood urea nitrogen (BUN) levels are two ways to measure kidney function in live animals. However, as needed in studies of disease progression, repeated blood sampling over long periods of time can introduce variability, especially in autoimmune models. Frequent blood draws may also change the animal's blood composition and raise the risk of infections.

Materials and methods

Data Gathering

To determine how many leukocytes have entered each kidney:

Samples are examined using flow cytometry. To guarantee reliable comparisons, all samples must be processed using the same parameters, such as the same acquisition time and sample volume.

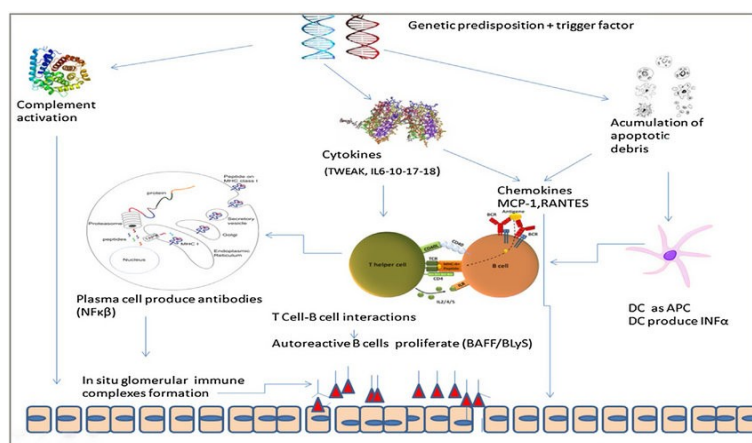
Analyzing Flow Cytometry

FlowJo (Tree Star) or comparable analytical software is used to analyze the results of flow cytometry.

- To mark circulating leukocytes, a CD45.2 BV421-conjugated antibody is used.
- Blood samples help determine the proper gating:

Mice without injections serve as negative controls, while mice with injections serve as positive controls.

The presence of immune cells in kidney tissue can be precisely measured thanks to this method, which helps differentiate between circulating and infiltrating leukocytes.



Lupus Nephritis Pathogenesis: Numerous genes have been linked to lupus nephritis (LN) by genomic research, but each gene only slightly raises the risk of systemic lupus erythematosus (SLE). Epigenetic, hormonal, and environmental factors all contribute to immune system dysregulation, which further complicates the disease's progression. The creation of immune complexes in the kidney, particularly between circulating antichromatin antibodies and extracellular chromatin in the glomeruli, is the most likely starting point for LN. Increased turnover of inflammatory cells and poor neutrophil and macrophage clearance of apoptotic cells are the causes of these autoantibodies.

Result and discussion

1. The Marker of Proteinuria

The degree of leukocyte infiltration in the kidney is closely linked to the severity of proteinuria in the FcγRIIb[KO] model. While female mice typically develop proteinuria earlier, male mice typically start to show symptoms around 5 months of age.

2. Managing Reagents Sensitive to Light

Light sensitivity is exhibited by fluorochrome-conjugated antibodies. As soon as the animals are injected, reduce their exposure to light to maintain their integrity.

3. Selection of Antibodies and Fluorochromes

When examined using the LSR II flow cytometer (BD Biosciences), the CD45.2 BV421 and CD45.2 PE-Cy7 antibodies produced the most distinct separation signals among the tested options.

The growth of lymphoid tissue in the tubulointerstitial space, the kidney's own production of local antibodies, and the activation of the complement system all exacerbate renal inflammation. By increasing MHC class II expression, dendritic cell (DC) activation improves antigen presentation and initiates the production of interferon (IFN). T cells are subsequently stimulated, and B cells are encouraged to differentiate into plasma cells that release antibodies. Elevated interferon levels and increased leukocyte mRNA expression are common in SLE patients.

In response to immune complexes and complement components, leukocytes and kidney cells release proinflammatory cytokines and chemokines, which prolong the inflammatory cycle and lead to recurrent episodes of nephritis.

In conclusion

It is crucial to use accurate and effective techniques for assessing immune cell infiltration in the kidney because autoimmune diseases, especially lupus glomerulonephritis in murine models, are complicated and variable. This protocol provides a reliable and controlled system for studying renal leukocyte infiltration using the FcγRIIb knockout (FcγRIIb[KO]) mouse model. According to this model, kidney damage and immune cell infiltration are strongly correlated, and the degree of proteinuria is consistent with this relationship. Simple urine tests can be used to easily monitor proteinuria levels.

References:

1. Hans-Joachim Anders & Brad Rovin. A pathophysiology-based approach to the diagnosis and treatment of lupus nephritis
2. Anders HJ, Jayne DR, Rovin BH. Hurdles to the introduction of new therapies for immune-mediated kidney diseases. *Nat Rev Nephrol.* 2016;12(4):205–216.
3. Anders HJ, Fogo AB. Immunopathology of lupus nephritis. *Semin Immunopathol.* 2014;36(4):443–459.

4. S. Bolland *et al.* Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis
5. T.L. McGaha *et al.* Lupus nephritis: Animal modeling of a complex disease syndrome pathology. *Drug Discovery Today: Disease Models*. (2014)
6. S. Bolland *et al.* Genetic modifiers of systemic lupus erythematosus in FcγRIIB^{-/-} mice. *Journal of Experimental Medicine*. (2002)