Low Urine Secretion of Semaphorin3A in Lupus Patients with Proteinuria

Rimar Doron1,4, Lidar Merav2, Eiza Nasrin3, Sabag D Adi3, Toubi Elias3, Slobodin Gleb1, Rosner Itzhak1, Rozenbaum Michael1 and Vadasz Zahava3

Received 2 August 2021; accepted 20 September 2021

Abstract—Immune semaphorins are important in controlling both innate and adaptive immune responses. The regulatory role of semaphorin3A (sema3A) in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and other autoimmune diseases is widely reported. Decreased levels of serum sema3A were shown to correlate with SLE disease activity. The aim was to assess urine concentrations of sema3A in SLE patients and its correlation with renal involvement and disease activity. Urine levels of sema3A were analyzed in 38 SLE patients, 13 with renal involvement, and were compared to 10 healthy volunteers and 8 RA patients (disease control group). The excretion of urine sema3A was found to be significantly lower in SLE patients compared to healthy volunteers and RA patients (4.9 ± 3.9 ng/ml, 8.5 ± 2.7 ng/ml, 9.85 ± 1.7 ng/ml, respectively, \( p = 0.0006 \)). Urine sema3A was significantly lower in SLE patients with lupus nephritis than in patients without nephritis (4.0 ± 3.4 ng/ml vs. 6.5 ± 3.8 ng/ml, \( p = 0.03 \)). Urine sema3A inversely correlated with proteinuria and SLE disease activity. Urine sema3A is decreased in lupus patients and should be further evaluated as a possible biomarker for disease activity and renal involvement.

KEY WORDS: Semaphorin3A; VEGF; SLE; Lupus nephritis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease characterized by autoantibody production, immune complex deposition, and systemic immune dysregulation. Nearly every organ system can be affected in SLE, making diagnosis difficult [1, 2]. Renal involvement is one of the more serious manifestations of systemic lupus erythematosus (SLE) and is associated with increased morbidity and mortality. Active renal disease is defined with an active urine sediment (proteinuria and hematuria with dysmorphic casts), proteinuria of more than 500 mg/day, and elevated serum creatinine.
Renal flares occur in 27 to 66% of patients with lupus nephritis [3–5]. Despite advances in the management of patients with lupus nephritis, 10% of patients with SLE progress to renal insufficiency and end-stage renal disease [5]. In the face of increasing or non-resolving proteinuria, treatment should be escalated; yet in some cases, proteinuria is related to chronic damage (glomerular sclerosis) for which no additional treatment is needed. Increasing serum dsDNA concentration and decreasing complement level (mostly C3) may help to discriminate active disease from chronic damage. A number of serum and urine novel molecular biomarkers, such as urine levels of the chemokine monocyte chemoattractant protein-1 (MCP-1), neutrophil gelatinase–associated lipocalin (NGAL), and serum levels of tumor necrosis factor–like weak inducer of apoptosis (TWEAK), have been studied in lupus nephritis as well [6]. Alas, in some cases decision-making regarding immunosuppressive treatment and steroid tapering is challenging. Novel biomarkers are needed to guide lupus nephritis treatment.

Semaphorins are a large family of proteins that were initially reported to be involved in axon-guidance, malignancy spread, and angiogenesis. Some of these, mainly those belonging to a class of secretory proteins such as semaphorin3A (sema3A), were recognized as “immune semaphorins” and appreciated as playing important roles in regulating both innate and adaptive immune responses [7, 8]. Sema3A was shown to be a potent regulator of CD4+ T cell function, suppressing pro-inflammatory secretion and proliferation of T cells in normal homeostasis and in autoimmune diseases such as in rheumatoid arthritis (RA) [9, 10]. We have reported decreased serum levels of sema3A in SLE compared to normal individuals, correlating with SLE disease activity [11]. Anti-cardiolipin antibodies (ACLA) at medium or high titer (> 40 MPL U/mL) were associated with lower levels of serum sema3A. Additionally, recombinant sema3A injection to NZB/W mice was shown to improve SLE-like manifestations, decrease proteinuria, and increase survival [12].

Renal podocytes and epithelial cells in distal tubules in kidneys of wild mice models express and secrete sem3A. Though pathophysiology was not fully defined, secreted sema3A seems to be crucial for podocyte survival and for the integrity and function of the kidney glomerular filtrates [13]. The action of sema3A in the glomeruli of wild-type mice was found to be tightly related to VEGF and its receptor, which, in turn, counteracts damage to podocytes induced by sema3A [14]. Urine secretion of sema3A was reported to be significantly increased in animal models with diabetic nephropathy and was found to correlate with the extent of proteinuria and renal dysfunction [15]. In a recent study, urinary sema3A levels were suggested as a possible predictive marker in the development of contrast-induced acute renal injury [16]. The renal pathologies in which sema3A was evaluated were all related to a direct renal injury or persistent ischemia but not to immune-mediated injury. Urinary excretion of sema3A as a biomarker for renal injury of lupus nephritis has not been evaluated before.

**Aim of the Study**

To assess urine concentrations of sema3A in SLE patients and its correlation with renal involvement and disease activity.

**PATIENTS AND METHODS**

**Patients**

Consecutive SLE patients fulfilling 2012 SLICC criteria [1] were recruited and were compared to RA patients (disease control) and age- and gender-matched healthy volunteers. SLE patients with renal involvement per renal biopsy and with more than 0.5 g urinary protein per 24 h and active sediment were classified as active lupus nephritis and were compared to SLE patients free of renal involvement (no proteinuria and serum creatinine below 1.0 mg/dl). The study was approved by the local Research Ethics Board and all patients gave their informed consent.

**Methods**

Demographic characteristics and clinical manifestations of lupus patients were assessed by frontal interview and by physical examination. Medical records were examined for disease onset, kidney involvement, past history of vasculitis, arthritis, pericarditis, and CNS involvement. Laboratory results included complete blood count, serum creatinine, C3 and C4, autoantibodies (anti-nuclear antibodies-ANA, anti-dsDNA, anti-cardiolipin IgM and IgG), and 24-h urine collection for protein.

Disease activity was determined for each patient by the SLEDAI 2K score [17].

Complete renal response was defined as a UPCR of less than 0.5 and normal or near-normal (within 10%
if initially abnormal) GFR. Partial renal response was defined as at least 50% reduction in protein-to-creatinine ratio (UPCR), normal or near-normal (within 10% if initially abnormal) GFR, and less than 800 mg proteinuria per 24 h with non-active urine sediment [2].

Semaphorin3A Urine Analyses

Fifty milliliters of fresh urine samples were collected from all studied individuals. Urine was centrifuged and the supernatant then concentrated up to 50 times the initial concentration. Subsequently, all concentrated samples were stored at −20 °C until analyzed. Semaphorin3A was measured using a specific commercial human Sema3A ELISA kit (MBS732622, San Diego, CA, USA).

Statistical Analysis

Continuous data are presented as the mean ± SD. Categorical variables are presented as frequencies and percentages. Comparisons of sema3A levels between categories were made using 2-tailed t-tests. In order to determine the difference between the different patient groups, 1-way ANOVA and post hoc tests using Tukey’s procedure were performed. We further evaluated relationships between sema3A and disease-related covariates by a correlation test, reported via Pearson’s r. A two-tailed p-value of 0.05 or less was statistically significant. Statistical analysis was performed using the R software (The R Foundation for Statistical Computing 3.0.2 2013).

RESULTS

Thirty-eight SLE patients were recruited and compared to 8 RA patients (disease controls) and 10 healthy volunteers (Table 1). There was no difference between the three groups with respect to age (59 ± 18, 51 ± 19, 52 ± 15, respectively) or sex (percent women 87% vs. 75% vs. 80%). Disease duration was 5.2 ± 3.3 years. Medical therapy is shown in Table 1.

The 38 SLE patients were further divided into two groups: (a) 25 patients suffering from arthritis, skin, and hematological involvement but with no renal involvement and (b) 13 patients with renal involvement, of whom 7 were with active nephritis class IV (mean 24 h urinary protein 2900 ± 210 mg/day and mean serum creatinine level of 1.8 ± 0.16 mg/dl) and 6 patients (3 with class IV nephritis and 3 with class V nephritis) who were treated and achieved complete or partial renal response (mean 24 h urinary protein 750 ± 190 mg/day and mean serum creatinine level of 0.8 ± 0.10 mg/dl). In patients with no renal involvement, the SLE disease activity score (SLEDAI) was 5.6 ± 2.9, lower than in patients with renal involvement 16 ± 10 (p < 0.03).

Sema3A Urine Levels

Urinary excretion of sema3A was significantly lower in SLE patients when compared to healthy volunteers and RA patients: 4.9±3.9 ng/mL, 8.5±2.7ng/mL, 9.855±1.7ng/mL respectively, p < 0.05 (Fig. 1a). SLE patients with nephritis (n = 13) had lower urine sema3A concentration than patients without renal involvement: 4.0 ± 3.4 ng/ml vs. 6.5 ± 3.8 ng/ml, p = 0.03 (Fig. 1b). Further analysis revealed that within the group with lupus nephritis, patients with active disease (n = 7) had a lower urinary sema3A level than those in partial remission (n = 6): 5.2 ± 1.4 ng/ml vs. 1.8 ± 1.5 ng/ml, p = 0.05 (Fig. 1c).

Urinary Sema3A inversely correlated with level of proteinuria r = − 0.43 p = 0.01 (Figs. 2 and 3) and SLEDAI 2K, r = − 0.3, p = 0.04 (Fig. 2), but not with serum creatinine concentration, disease duration, autoantibodies, ACLA IgG titers, or serum complement levels.

DISCUSSION

This is the first study to assess urine sema3A secretion in SLE patients. We have demonstrated a significantly lower level of sema3A in the urine of lupus patients compared to RA and healthy volunteers and an inverse correlation with disease activity (SLEDAI 2K) and proteinuria (due to lupus nephritis). In contrast, former studies reported higher secretion of urine sema3A in patients with diabetic nephropathy or contrast-media–induced injury. While SLE manifests classical immune-mediated inflammation, contrast-media–induced renal injury is related to ischemic damage. The normal function of kidney podocytes, collecting tubules, and endothelial cells is physiologically maintained by a balance between sema3A and vascular endothelial growth factor (VEGF) expression and secretion. Thus, excess or lack of either protein may disrupt glomerular filtration barrier homeostasis [18]. Tight regulation of sema3A expression and secretion is therefore required for the maintenance of normal glomerular function. The pathophysiology of increased sema3A secretion in ischemic renal injury has been studied. Increased sema3A secretion in
urine and decreased VEGF receptor expression was shown to be associated with nephropathy-glomerular endothelial damage and proteinuria — in diabetic mice [13]. In an additional report, exposure of podocytes to recombinant sema3A decreased mRNA expression of plexinA1, A2, and A3 (VEGF receptors) and induced dose-response podocin downregulation [14]. Sema3A was also reported to induce a 10-fold increase in podocyte apoptosis and to decrease significantly the activity of the Akt survival pathway [19].

Table 1 Thirty-eight SLE Patients Recruited and Compared to 8 RA Patients (Disease Controls) and 10 Healthy Volunteers

| SLE (n = 38) | RA (n = 8) | Healthy controls (n = 10) | p value |
|--------------|-----------|---------------------------|---------|
| Lupus w/o nephritis (n = 25) | Lupus nephritis (n = 13) | | |
| Age (years) | 60 (± 19) | 55 (± 15) | 51 (± 19) | 52 (± 15) | 0.12 |
| Female (%) | 23 (92%) | 10 (76%) | 6 (75%) | 8 (80%) | 0.59 |
| ANA | 25 (100%) | 13 (100%) | 1 |
| DSDNA | 17 (68%) | 13 (100%) | 0.007 |
| C3 (80–160 mg/dL) | 96 ± 36 | 79 ± 48 | 0.86 |
| C4 (15–45 mg/dL) | 1 ± 9 | 18 ± 16 | 0.9 |
| Active nephritis | NA | 7 (54%) | |
| Complete or partial renal response | NA | 6 (46%) | 0.93 |
| ACLA igG > 40 MPL U/mL | 8 (32%) | 5 (38%) | 0.02 |
| Creatine | 0.6 ± 0.12 | 0.8 ± 0.2 | 0.002 |
| eGFR | 60 ± 5 | 55 ± 8 | 0.02 |
| Cutaneous | 25 (100%) | 8 (62%) | 0.001 |
| Alopecia | 14 (56%) | 2 (13%) | 0.05 |
| Arthritis | 25 (100%) | 6 (46%) | 0.0003 |
| serositis | 13 (52%) | 3 (23%) | 0.2 |
| Cytopenia | 19 (76%) | 4 (31%) | 0.02 |
| SLEDAI 2 K | 5.6 ± 2.9 | 16 ± 10 | 0.0004 |

Treatments

| | | | |
| Prednisone use | 23 (92%) | 12 (92%) | 0.16 |
| Prednisone dose (mg/day) | 7 ± 3.4 | 4.3 ± 3.8 | 0.9 |
| Hydroxychloroquine | 20 (80%) | 10 (77%) | 0.9 |
| Methotrexate | 5 (20%) | 1 (7%) | 0.6 |
| Azathioprine | 8 (32%) | 2 (15%) | 0.54 |
| Belimumab | 9 (36%) | 3 (23%) | 0.71 |
| Mycophenolate mofetil | 2 (8%) | 6 (46%) | 0.02 |
| Rituximab | 5 (20%) | 1 (7%) | 0.6 |
| Abatacept | 1 (7%) | 1 (7%) | 0.8 |
| Tocilizumab | 1 (7%) | 1 (7%) | 0.9 |

SLE systemic lupus erythematosus, RA rheumatoid arthritis, ACLA anticardiolipin antibodies igG > 40 MPL U/mL, SLEDAI systemic lupus erythematosus disease activity score, IVIG intravenous immunoglobulins

*pPartial renal response — at least 50% reduction in UPCR and normal or near-normal (within 10% if initially abnormal) GFR and less than 800 mg proteinuria per 24 h with non-active urine sediment

In a recent study, increased sema3A urinary secretion was associated with increased phosphorylated mTOR proteins leading to renal tubular injury and nephrotoxicity [20]. While these studies may support the role of increased urinary sema3A in ischemic nephropathy, immune-mediated inflammation may have a different renal pathophysiology. High expression of VEGF was reported to play role in the development of lupus nephritis by inducing ongoing angiogenesis and endothelial inflammation in the glomeruli of
Low Urine Secretion of Semaphorin3A in Lupus Patients with Proteinuria

SLE patients. Increased renal tubular VEGF expression is associated with advanced glomerular damage and fibrosis [21]. One may speculate that this could occur in part because of low sema3A secretion and the consequent lack of its effect in counterbalancing and neutralizing that of VEGF.

We suggest several possible explanations for our finding of decreased urinary secretion of sema3a in SLE: (1) Local immune-mediated inflammation of endothelial cells and of tubules in kidneys of SLE patients is responsible for the lower sema3A production and secretion. (2) Treg cells are one of the important sources for sema3A secretion. The density of FoxP3+ Treg cells was found to be decreased in kidney biopsies of patients with tubular-interstitial nephritis [22]. Reduced expression of Treg cells in the glomeruli of SLE patients may result in loss of one of the important sources of sema3A. (3) Low serum sema3A levels were previously reported to correlate with SLE disease activity and thus may account for the decreased secretion by the kidneys of these patients [11]. Other mechanisms by which sema3A maintain glomerular/tubular normal function and the physiological level of urine sema3A required for this function remain to be determined. As noted above, sema3A is required for the suppression of ongoing angiogenesis by inhibiting VEGF activity and by activation of inhibitory intracellular pathways that inhibit VEGF signal transduction [23]. Sema3A has also been shown to be a regulator of glomerular vascular development. Deletion of sema3A resulted in defects in renal vasculature, excess endothelial cells within glomerular capillaries, and the development of proteinuria. This suggests that a physiological sema3A amount is required for the maintenance of a normal filtration barrier [24].

Fig. 1  a Urinary excretion of sema3A was significantly lower in SLE patients when compared to healthy volunteers and RA patients. b SLE patients with nephritis (n = 13) had lower urine sema3A concentration than patients without renal involvement. c Patients with active disease (n = 7) had a lower urinary sema3A level than those in complete or partial remission.

Fig. 2 Urinary Sema3A inversely correlated with level of proteinuria SLEDAI.
The limitations of this study include the small number of recruited patients and (mainly patients with lupus nephritis) only one timepoint of urine collection for each patient, with inadequate power to asses and correlate many important SLE parameters. Additionally, sema3A serum level and urinary excretion of VEGF and its receptors were not assessed and should be evaluated in parallel in future studies.

In summary, the regulatory role of sema3A in SLE and other immune-mediated diseases is well established in the literature. The present study is the first to describe low urine sema3A in SLE patients compared to controls, with an association with lupus nephritis, suggesting a role of sema3A in this target organ. Future studies with larger cohorts are needed to assess the use of urine sema3A as a potential marker for renal involvement in SLE.

**AUTHOR CONTRIBUTION**

All the authors contributed to the manuscript and reviewed and approved it as presented here.

**DECLARATIONS**

**Ethics Approval** Ethics committee approval was obtained

**Consent to Participate** All patients signed an informed consent form.

**Consent for Publication** We hereby transfer full rights for publication of this manuscript to Inflammation Journal. The manuscript has not been published elsewhere.

**Conflict of Interest** The authors declare no conflict of interest.

**REFERENCES**

1. Petri, M., A.M. Orbai, G.S. Alarcon, et al. 2012. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis & Rheumatism* 64: 2677–2686.
2. Houssiau, F.A., C. Vasconcelos, D. D’Cruz, et al. 2004. Early response to immunosuppressive therapy predicts good renal outcome in lupus nephritis: lessons from long-term followup of patients in the Euro-Lupus Nephritis Trial. *Arthritis & Rheumatism* 50: 3934–3940.
3. Palmer, S.C., D.J. Tunnicliffe, D. Singh-Grewal, et al. 2017. Induction and maintenance immunosuppression treatment of proliferative lupus nephritis: a network meta-analysis of randomized trials. *American Journal of Kidney Diseases* S0272–6386 (17): 30036–7.
4. Weening, J.J., V.D. D’Agati, M.M. Schwartz, et al. 2004. On behalf of the international society of nephrology and renal pathology society working group on the classification of lupus nephritis. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney International* 65: 521–530.
5. Ward, M.M. 2009. Changes in the incidence of end-stage renal disease due to lupus nephritis in the United States, 1996–2004. *The Journal of Rheumatology* 36: 63–67.
6. Misra, R., and R. Gupta. 2015. Biomarkers in lupus nephritis. *International Journal of Rheumatic Diseases* 18: 219–232.
7. Neufeld, G., Y. Mumblat, T. Smolkin, et al. 2016. The role of the semaphorins in cancer. *Cell Adhesion & Migration* 10: 652–674.
8. Nishide, M., and A. Kumanogoh. 2018. The role of semaphorins in immune responses and autoimmune rheumatic diseases. *Nature Reviews Rheumatology* 14: 19–31.
9. Catalano, A. 2010. The neuroimmune semaphorin3A reduces inflammation and progression of experimental autoimmune arthritis. *The Journal of Immunology* 185: 6373–83.
10. Cozacov, R., K. Halasz, T. Haj, and Z. Vadasz. 2017. Semaphorin3A: a key player in the pathogenesis of asthma. *Clinical Immunology* 184: 70–72.
Low Urine Secretion of Semaphorin3A in Lupus Patients with Proteinuria

11. Vadasz, Z., T. Hai, K. Halasz, et al. 2012. Semaphorin3A is a marker for disease activity and a potential immunoregulator in systemic lupus erythematosus. *Arthritis Research & Therapy* 14: R146.

12. Bejar, J., O. Kessler, A.D. Sabag, E. Sabo, O.B. Itzhak, G. Neufeld, et al. 2018. Semaphorin3A: a potential therapeutic tool for lupus nephritis. *Frontiers in Immunology* 634.

13. Tufro, A. 2014. Semaphorin3A signaling, podocyte shape, and glomerular disease. *Pediatric Nephrology* 29: 751–5.

14. Tapia, R., F. Guan, I. Gershin, J. Teichman, G. Villegas, and A. Tufro. 2008. Semaphorin3A disrupts podocyte foot processes causing acute proteinuria. *Kidney International* 73 (6): 733–40.

15. Mohamed, R., P. Ranganathan, C. Jayakumar, et al. 2014. Urinary semaphorin3A correlates with diabetic proteinuria and mediates diabetic nephropathy and associated inflammation in mice. *Journal of Molecular Medicine (Berl)* 92: 1245–56.

16. Ning, L., Z. Li, D. Wei, et al. 2018. Urinary semaphorin3A as an early biomarker to predict contrast-induced acute kidney injury in patients undergoing percutaneous coronary intervention. *Brazilian Journal of Medical and Biological Research* 51: e6487.

17. Gladman, D.D., D. Ibanez, and M.B. Urowitz. 2002. Systemic lupus erythematosus disease activity index 2000. *The Journal of Rheumatology* 29: 288–91.

18. Aggarwal, P.K., D. Veron, D.B. Thomas, et al. 2015. Semaphorin3A promotes advanced diabetic nephropathy. *Diabetes* 64 (5): 1743–59.

19. Guan, F., G. Villegas, J. Teichman, P. Mundel, and A. Tufro. 2006. Autocrine class 3 semaphorin system regulates slit diaphragm proteins and podocyte survival. *Kidney International* 69: 1564–9.

20. Song, M.F., Y. Yang, Z.W. Yi, et al. 2018. Sema3A as a biomarker of the activated mTOR pathway during hexavalent chromium-induced acute kidney injury. *Toxicology Letters* 15: 226–35.

21. Avihingsanon, Y., T. Benjachat, A. Tassanarong, P. Sodsai, V. Kittikovit, and N. Hirankarn. 2009. Decreased renal expression of vascular endothelial growth factor in lupus nephritis is associated with worse prognosis. *Kidney International* 75 (12): 1340–1348.

22. Rytkonen, S.H., P. Kulmala, H. Autio-Harmainen, et al. 2018. FoxP3+ T cells are present in kidney biopsy samples in children with tubulointestinal nephritis and uveitis syndrome. *Pediatric Nephrology* 33: 287–293.

23. Hakroush, S., M.J. Moeller, F. Theilig, et al. 2009. Effects of increased renal tubular vascular endothelial growth factor (VEGF) on fibrosis, cyst formation, and glomerular disease. *The American Journal of Pathology* 175: 1883–95.

24. Reidy, K.J., G. Villegas, J. Teichman, et al. 2009. Semaphorin3A regulates endothelial cell number and podocyte differentiation during glomerular development. *Development* 136: 3979–89.

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.