N-Acetyl-Seryl-Aspartyl-Lysyl-Proline: mechanisms of renal protection in mouse model of systemic lupus erythematosus

Tang-Dong Liao,1 Pablo Nakagawa,1 Bransilava Janic,1 Martin D’Ambrosio,1 Morel E. Worou,1 Edward L. Peterson,2 Nour-Eddine Rhaleb,1 Xiao-Ping Yang,1 and Oscar A. Carretero1

1Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan; and 2Department of Public Health Sciences, Henry Ford Hospital, Detroit, Michigan

Submitted 4 February 2015; accepted in final form 27 February 2015

The production of cytokines, chemokines, and other proinflammatory factors that promote inflammation, fibrosis, and endothelial damage. Renal and cardiovascular morbidity are the leading causes of mortality in SLE (20). The most common form of kidney disease in SLE patients is glomerulonephritis with histological changes spanning from mild mesangial proliferation to severe inflammation, glomerular and tubulointerstitial fibrosis, vascular lesions, and end-stage renal disease (8, 57). Several different mouse models of SLE have been established for studying the underlying mechanisms of this autoimmune disorder, such as BXSB, NZBWF1, and MRL/lpr mice (48). BXSB and NZBWF1 models are most useful for studying SLE-related coronary artery disease and hypertension, respectively. Since our study focused on the kidney damage associated with SLE, here we used MRL/MpJ-Fasplpr/2J (Lupus) mice that develop an aggressive form of lupus nephritis with no hypertension (47). We used age-matched MRL-MpJ mice as controls (Ctrl).

N-acetyl-seryl-aspartyl-lysyl-proline, i.e., Ac-SDKP is a natural tetrapeptide that is released from its precursor thymosin β4, by prolyl oligopeptidase (12). Ac-SDKP is found in human plasma and circulating mononuclear cells (43) and is inactivated by ACE (3). Ac-SDKP was previously shown to have anti-inflammatory and anti-fibrotic properties (2, 59) and a decrease in endogenous Ac-SDKP levels promoted heart and kidney fibrosis (11). These Ac-SDKP anti-inflammatory and anti-fibrotic effects were shown to be in part the result of a decrease in macrophage infiltration and transforming growth factor (TGF)-β expression (59). Ac-SDKP role in inflammation and fibrosis was mainly explored in hypertension and heart failure animal models (29, 38). However, the role of Ac-SDKP in autoimmune processes is less clear. Indirect evidence on potential beneficial effect of Ac-SDKP in autoimmune diseases came from the recent clinical study on lupus nephritis showing that ACE inhibitors delayed the renal involvement and decreased risk of disease activity in SLE patients (15). On the other hand, autoimmune animal models were used only by two studies that both reported structural and functional improvement in response to Ac-SDKP. The first study used rat model of EAM where Ac-SDKP decreased the expression of proinflammatory cytokines, chemokines and cell adhesion molecules, and cardiac infiltration by macrophages, dendritic and T cells (35). The second study used lupus-prone mouse model where Ac-SDKP attenuated the progression of renal damage, decreased proinflammatory cell infiltration and fibrosis, and these actions were suggested to be achieved by downregulation of TNF-α and TGF-β pathways (54). While these data indicate that Ac-SDKP targets immune cells migration and tissue infiltration, it is still not clear what is the underlying mechanism


F1146

1931-857X/15 Copyright © 2015 the American Physiological Society http://www.ajprenal.org

**SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)** is an autoimmune chronic disease that can affect almost any organ of the body. SLE affects an estimated 1.5 million Americans, with 90% of them being women (9). Currently, SLE is a noncurable disease and the treatment is based on controlling the symptoms by immunosuppressive and anti-inflammatory therapies (20, 24). In lupus patients with nephritis and hypertension, angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers are added to the therapy (7).

SLE is characterized by the development of auto antibodies against a variety of nuclear and cytoplasmic self-antigens and by deposition of immune complexes in the various tissues. These changes lead to complement activation and increase in the production of cytokines, chemokines, and other proinflammatory factors that promote inflammation, fibrosis, and end-organ damage. Renal and cardiovascular morbidity are the leading causes of mortality in SLE (20). The most common form of kidney disease in SLE patients is glomerulonephritis with histological changes spanning from mild mesangial proliferation to severe inflammation, glomerular and tubulointerstitial fibrosis, vascular lesions, and end-stage renal disease (8, 57). Several different mouse models of SLE have been established for studying the underlying mechanisms of this autoimmune disorder, such as BXSB, NZBWF1, and MRL/lpr mice (48). BXSB and NZBWF1 models are most useful for studying SLE-related coronary artery disease and hypertension, respectively. Since our study focused on the kidney damage associated with SLE, here we used MRL/MpJ-Fasplpr/2J (Lupus) mice that develop an aggressive form of lupus nephritis with no hypertension (47). We used age-matched MRL-MpJ mice as controls (Ctrl).

N-acetyl-seryl-aspartyl-lysyl-proline, i.e., Ac-SDKP is a natural tetrapeptide that is released from its precursor thymosin β4, by prolyl oligopeptidase (12). Ac-SDKP is found in human plasma and circulating mononuclear cells (43) and is inactivated by ACE (3). Ac-SDKP was previously shown to have anti-inflammatory and anti-fibrotic properties (2, 59) and a decrease in endogenous Ac-SDKP levels promoted heart and kidney fibrosis (11). These Ac-SDKP anti-inflammatory and anti-fibrotic effects were shown to be in part the result of a decrease in macrophage infiltration and transforming growth factor (TGF)-β expression (59). Ac-SDKP role in inflammation and fibrosis was mainly explored in hypertension and heart failure animal models (29, 38). However, the role of Ac-SDKP in autoimmune processes is less clear. Indirect evidence on potential beneficial effect of Ac-SDKP in autoimmune diseases came from the recent clinical study on lupus nephritis showing that ACE inhibitors delayed the renal involvement and decreased risk of disease activity in SLE patients (15). On the other hand, autoimmune animal models were used only by two studies that both reported structural and functional improvement in response to Ac-SDKP. The first study used rat model of EAM where Ac-SDKP decreased the expression of proinflammatory cytokines, chemokines and cell adhesion molecules, and cardiac infiltration by macrophages, dendritic and T cells (35). The second study used lupus-prone mouse model where Ac-SDKP attenuated the progression of renal damage, decreased proinflammatory cell infiltration and fibrosis, and these actions were suggested to be achieved by downregulation of TNF-α and TGF-β pathways (54). While these data indicate that Ac-SDKP targets immune cells migration and tissue infiltration, it is still not clear what is the underlying mechanism.
involved in attenuating this cellular response. Therefore, in the present study, we tested the hypothesis that in MRL/lpr mice lupus model Ac-SDKP prevents inflammation and renal end-organ damage by decreasing complement C5/C5a and C5b-9 activation, proinflammatory cytokines, chemokines, and cell adhesion protein expression, and as a consequence causes a decrease in macrophage and T cell infiltration in the kidney. The Ac-SDKP dose chosen in this study was previously shown to correlate to the increase in plasma Ac-SDKP levels caused by ACEi administration (25, 46).

MATERIALS AND METHODS

Animals. Female MRL/MpJ-Faslpr/2J (lupus) and age-matched control MRL-MpJ mice (Ctrl), 10 wk of age (Jackson Laboratory), were used in the studies. Before all surgical procedures, mice underwent analgesia and anesthesia with butorphanol (2 mg/kg sc) and pentobarbital sodium (50 mg/kg ip), respectively. This study was approved by the Henry Ford Hospital Institutional Animal Care and Use Committee and was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental protocols. Mice of 12 wk of age were randomly divided into four groups: 1) control mice receiving vehicle (Veh-Ctrl; n = 6), 2) control mice receiving Ac-SDKP (Ac-SDKP-Ctrl; n = 6), 3) lupus mice receiving vehicle (Veh-Lupus; n = 10), and 4) lupus mice receiving Ac-SDKP (Ac-SDKP-Lupus; n = 10). The vehicle (0.01N acetic acid saline) and Ac-SDKP (800 μg·kg⁻¹·day⁻¹) were infused subcutaneously for 20 wk via osmotic mini-pumps implanted in the back of mice. Systolic blood pressure (SBP) was monitored using computerized tail-cuff system (BP 2000, Visitech) (27). At the end of the treatment, glomerular filtration rate (GFR) was measured, urine and blood samples were collected, animals were euthanized, and kidney tissues were collected for further histological and protein analysis.

Fig. 1. Serum anti-dsDNA antibody concentration. Serum concentration of anti-dsDNA antibodies was significantly elevated in the lupus mice receiving vehicle (Veh-Lupus), compared with control mice receiving vehicle (Veh-Ctrl). Ac-SDKP treatment did not decrease the serum concentrations of anti-dsDNA antibodies in neither, control mice receiving Ac-SDKP (Ac-SDKP-Ctrl) nor lupus mice receiving Ac-SDKP (Ac-SDKP-Lupus).

Fig. 2. Effect of Ac-SDKP on C5/5a and C5b-9 complement, monocyte chemotactic protein (MCP)-5, regulated on activation normal T cells expressed and secreted (RANTES), and macrophage colony stimulating factor (M-CSF) expression. Renal expression of C5/C5a (A), MCP-5 (B), RANTES (C), and M-CSF (D) was measured with mouse protein array kit. Data were expressed as means ± SE; n = 6. P < 0.01, Veh-Ctrl vs. Veh-Lupus mice; Ac-SDKP-Lupus vs. Veh-Lupus mice. E: glomerular C5b-9 expression detected by immunofluorescent staining. Green indicates positive C5b-9 staining. Tissue section images captured using ×40 objective. Scale bar = 25 μm. F: analysis of C5b-9 expression in lupus mice. Data were calculated as a percentage of the glomerular area and expressed as means ± SE; n = 6. P < 0.005, Veh-Ctrl vs. Veh-Lupus mice; P < 0.005, Ac-SDKP-Lupus vs. Veh-Lupus mice.
Urinary Ac-SDKP and albuminuria. Mice were allowed to adapt to metabolic cages for 24 h after which they underwent 24 h of fasting and urine collection. ACE inhibitor lisinopril (10^{-3} M) was applied to prevent Ac-SDKP degradation. Urine Ac-SDKP was measured using EIA KIT (SPI Biologatories), as previously described (30). Albuminuria was determined by ELISA kit (Cayman Chemicals).

GFR. GFR was measured as previously described (32). Data were expressed as microliters per minute per 100 mg of kidney weight (kidney wt).

Gomeral matrix analysis. Paraffin-embedded tissues sections (4 µm) were stained with periodic acid Schiff (PAS). Thirty five glomeruli within randomly chosen fields of renal cortex were imaged at ×400 magnification. The dark pink color was considered a positive staining representing the extracellular matrix. Glomerular matrix was analyzed by computerized image analysis system (Microsuite Biological imaging software, Olympus America, Center Valley, PA) and positive staining was expressed as the percentage of glomerular area. All of the images shown in this study were captured and analyzed using the same imaging system, unless otherwise specified.

Collagen deposition. Picrosirius red staining was used to quantify renal interstitial and perivascular collagen deposition (38, 53). Randomly chosen fields within corticomedullar junction were imaged at ×200 magnification. Interstitial collagen fraction was calculated as the ratio of the collagen-positive area to the imaging area.

Collagen content. A piece of apical renal cortex was used for hydroxyproline assay as described previously (39). Data were expressed as micrograms of collagen per milligram of dry weight (14).

Gomeral nephrin and complement C5b-9 expression. Frozen sections (6 µm) were stained with goat anti-nephrin antibody (1:50; R&D Systems) and rabbit anti-C5b-9 antibody (1:500; Abcam) and positive signals were visualized using Alexa 488-conjugated species’ appropriate secondary antibody. Areas of positive staining within the glomeruli were measured in each section and expressed as percentage of glomerular area (29).

Plasma anti-dsDNA antibodies. Plasma anti-dsDNA antibody levels were measured by ELISA kit according to the manufacturer’s protocol (Alpha Diagnostic International, San Antonio, TX).

Proinflammatory protein array. Kidney cortex tissue samples were analyzed for protein expression levels of 29 inflammatory mediators using “Proteome Profiler Mouse Chemokine Array Kit,” according to the manufacturer’s protocol (R&D Systems). This array included complement C5/C5a, monocytic chemotactic protein 5 (MCP-5), regulated on activation normal T cells expressed and secreted (RANTES), and macrophage colony stimulating factor (M-CSF). Data were expressed as arbitrary units (AU) representing the optical density (OD) values of protein of interest divided by the positive control OD values.

Intercellular adhesion molecule-1 expression. Kidney protein extracts (120 µg/sample) were analyzed by Western blot. Antibodies used were primary anti-intercellular adhesion molecule (ICAM)-1 antibody (1:4,000; R&D Systems), primary anti-GAPDH antibody (1:50,000; Cell Signaling Technology), and the appropriate peroxidase-conjugated secondary antibody (1:20,000; Santa Cruz Biotechnology). Positive signals were visualized using ECL-plus detection system (Amersham Biosciences). Data were expressed as the ratio of ICAM-1 to the GAPDH.

Macrophage and T cell infiltration. Cryosections (6 µm) were used for the immunohistochemistry to detect macrophages (rat anti-mouse CD68, 1:200, AbD Serotec) and CD3+ T cells (hamster anti-mouse CD3, 1:200, AbD Serotec). Detection system was ABC kit (Vecstain Elite ABC peroxidase kit, Vector Lab) and 3-amino-9-ethylcarbazole substrate. Data were expressed as number of cells per millimeter squared.

Facial lesions score. Facial lesions assessment was performed independently by three different unbiased investigators. Facial rash was scored according to the following scale: 1 for normal, 2 for mild, 3 for moderate, and 4 for severe.

Data analysis. All data are expressed as means ± SE. ANOVA and nonparametric Wilcoxon tests were used to compare mean values of the various parameters between different groups. Hochberg’s method for multiple comparisons was used to adjust the alpha level of significance (22).

RESULTS

Serum anti-dsDNA antibody concentration. Serum concentrations of anti-dsDNA antibodies were significantly elevated in vehicle-treated lupus mice (Veh-Lupus), compared with vehicle-treated control nonlupus mice (Veh-Ctrl) animals. Ac-SDKP treatment did not decrease the serum concentrations of anti-dsDNA antibodies in neither, Ac-SDKP-treated control, nonlupus mice (Ac-SDKP-Ctrl), nor Ac-SDKP-treated lupus mice (Ac-SDKP-Lupus; Fig. 1).

Complement C5/5a and C5b-9, MCP-5, RANTES, and M-CSF expression in the kidney. Protein array analysis of C5/C5a, MCP-5, RANTES, and M-CSF, and immunohistochemistry analysis of C5b-9 revealed that the expression levels of these factors were significantly higher in the Veh-Lupus mice, compared with Veh-Ctrl mice (P < 0.001 and P < 0.005, respectively). These levels were significantly lower in Ac-SDKP-Lupus mice, compared with Veh-Lupus mice (P < 0.01). No effect of Ac-SDKP treatment on the expression levels of these factors was detected in Ac-SDKP-Ctrl mice (Fig. 2, A–F).

Fig. 3. Effect of Ac-SDKP on intercellular adhesion molecule (ICAM)-1 expression. A: renal tissue Western blot analysis of ICAM-1 and GAPDH. ICAM-1 band density was quantified after normalization with GAPDH band density. B: ICAM-1 expression. Data were expressed as means ± SE; n = 6. P < 0.002, Veh-Ctrl vs. Veh-Lupus mice; P < 0.001, Ac-SDKP-Lupus vs. Veh-Lupus mice.
ICAM-1 expression in the kidney. Veh-Lupus mice expressed significantly higher amount of ICAM-1, compared with Veh-Ctrl mice ($P < 0.002$; Fig. 2, A and B). Ac-SDKP-Lupus mice had significantly lower amount of ICAM-1, compared with the Veh-Lupus mice ($P < 0.001$), and these ICAM-1 amounts were comparable with the basal ICAM-1 expression observed in Veh-Ctrl mice. These basal ICAM-1 levels in Ac-SDKP-Ctrl mice were not affected by Ac-SDKP treatment (Fig. 3, A and B).

Macrophage and T cell infiltration in the kidney. Quantitative immunohistochemistry analysis showed significantly higher numbers of infiltrating macrophages and T cells in Veh-Lupus mice, compared with Veh-Ctrl mice ($P < 0.01$). In Ac-SDKP-Lupus mice, numbers of infiltrating cells were significantly lower, compared with Veh-Lupus mice ($P < 0.01$). In the Ac-SDKP-Ctrl mice, macrophage or T cell infiltration was not altered by treatment with Ac-SDKP (Fig. 4, A–D). Representative images showed macrophage (Fig. 4A) and T cell kidney infiltration (Fig. 4B).

GFR and albuminuria. At 32 wk of age, Veh-Lupus mice had significantly lower GFR, compared with Veh-Ctrl mice ($P < 0.01$). In Ac-SDKP-Lupus mice, GFR was significantly higher, compared with Veh-Lupus mice ($P < 0.01$). Ac-SDKP had no effect on GFR in Ac-SDKP-Ctrl mice (Fig. 5A). Veh-Lupus mice also developed significantly higher albuminuria, compared with Veh-Ctrl ($P < 0.01$) and treated Ac-SDKP-Lupus ($P < 0.01$) animals. In the AcSDKP-Ctrl mice, Ac-SDKP had no effect on albumin in the urine (Fig. 5B).

Glomerular nephrin expression. In Veh-Ctrl or Ac-SDKP-Ctrl mice, positive strong staining for nephrin expression was detected. In the Veh-Lupus mice, nephrin staining was decreased, compared with Veh-Ctrl mice, and it was partially restored in response to Ac-SDKP in Ac-SDKP-Lupus (Fig. 5C). Quantitative analysis demonstrated that Veh-Lupus mice had significantly lower levels of glomerular nephrin expression, compared with Veh-Ctrl ($P = 0.002$). In Ac-SDKP-Lupus mice, nephrin levels were significantly higher, compared with the Veh-Lupus mice ($P = 0.001$). In Ac-SDKP-Ctrl, Ac-SDKP had no effect on nephrin expression (Fig. 5D).

Glomerular matrix analysis. Veh-Lupus mice developed glomerular matrix depositions, detected by PAS staining as dark purple regions within the glomeruli. The percentage of
glomerular area positive for PAS was significantly higher in Veh-Lupus compared with Veh-Ctrl and Ac-SDKP-Lupus animals (P < 0.01). In Ac-SDKP-Ctrl mice, Ac-SDKP had no effect on the percentage of PAS staining in the glomeruli (Fig. 5, E–F).

Renal interstitial collagen. Deposits of interstitial collagen were analyzed by picrosirius red staining. Veh-Lupus mice had significantly higher percentage of the collagen-positive areas, compared with Veh-Ctrl (P < 0.01). In Ac-SDKP-Lupus mice, the percentage of these positive areas was significantly lower, compared with Veh-Ctrl (P < 0.01). Ac-SDKP had no effect on renal interstitial collagen in Ac-SDKP-Ctrl mice (Fig. 6, A and B).

Renal collagen content. In Veh-Lupus mice, collagen content, measured by the hydroxyproline assay, was significantly higher, compared with Veh-Ctrl mice (P < 0.05). In Ac-SDKP-Lupus mice, collagen content was significantly lower compared with the Veh-Lupus mice (P < 0.05). Ac-SDKP had no effect on collagen content in Ac-SDKP-Ctrl mice (Fig. 6C).

Facial lesions. Veh-Lupus mice developed a symmetrical facial skin rash at age of 25 wk. The lesions persisted until the end of the study period (32 wk of age). Ac-SDKP-Lupus mice had little or no facial lesions during the entire treatment period (Fig. 7A). Quantitative assessment demonstrated significantly higher lesion index score in Veh-Lupus mice, compared with Veh-Ctrl mice (P < 0.01). In Ac-SDKP-Lupus mice, this score was significantly lower compared with Veh-Lupus mice (P < 0.01). Ac-SDKP had no effect on the skin rash in Ac-SDKP-Ctrl mice (Fig. 7B).

Urine Ac-SDKP concentration. Concentration of Ac-SDKP in urine collected for 24 h was significantly higher in Ac-SDKP-Ctrl and Ac-SDKP-Lupus mice, compared with their respective vehicle-treated controls. Urine Ac-SDKP concentration in Veh-Lupus mice was higher than in Veh-Ctrl mice, most likely due to renal damage; however, the observed difference between these two groups of animals did not reach statistical significance (Table 1).

SBP, body weight, and organ weight. Twenty weeks after treatment with Ac-SDKP no difference was observed in SBP among the groups. Kidney weight-to-body weight ratio increased significantly in Veh-Lupus mice, compared with the Veh-Ctrl mice. In Ac-SDKP-Lupus mice, kidney weight-to-body weight ratio was decreased compared with Veh-Lupus mice; however, the difference did not reach significance. Left ventricle-to-body weight ratio was not different among the groups (Table 1).

DISCUSSION

In this study, we examined the possible mechanisms by which Ac-SDKP decreases nephritis in mouse model of SLE (20). We found, as previously reported by Tan et al. (54), that in lupus mice Ac-SDKP treatment decreases renal nephritis, inflammation and fibrosis. These beneficial effects occurred
without changes in blood pressure and anti-dsDNA antibody concentrations. We hypothesized that Ac-SDKP beneficial effects in lupus are due to a significant decrease in: 1) C5/C5a, 2) C5b-9, 3) RANTES, 4) M-CSF, 5) MCP-5, and 6) ICAM-1, and these decreases could be responsible for the decrease in inflammation (macrophage and T cell kidney infiltration). Since Ac-SDKP neither altered the levels of auto anti-ds DNA antibodies nor, as previously reported, did it have any effect on C3 complement and IgGs deposition (54), these data indicate that in lupus mouse model Ac-SDKP may affect molecules downstream from immune complex formation and complement C3 activation. Therefore, to further dissect the mechanisms of Ac-SDKP action, we analyzed complement C5a and C5b-9, as well as their downstream effectors. Previous studies implicated that overexpression of complement C5a plays a role in the pathogenesis of autoimmune diseases, such as SLE (23). In addition, blocking of C5a receptor significantly decreased the severity of renal damage in mouse lupus models (4, 58), while deletion or blocking C3 or C3a receptor had no effect on murine lupus nephritis development (50, 51). In our study, Veh-Lupus mice expressed significantly higher levels of C5b-9, as well as their downstream effectors. Previous studies implicated that overexpression of complement C5a plays a role in the pathogenesis of autoimmune diseases, such as SLE (23). In addition, blocking of C5a receptor significantly decreased the severity of renal damage in mouse lupus models (4, 58), while deletion or blocking C3 or C3a receptor had no effect on murine lupus nephritis development (50, 51). In our study, Veh-Lupus mice expressed significantly higher levels of C5a, compared with Veh-Ctrl mice. However, the levels of C5a were significantly lower in Ac-SDKP-Lupus animals. C5a is a strong anaphylatoxin for immune cells that increases their chemotaxis and cytokine release (28). Since our data showed that in Ac-SDKP-Lupus mice renal infiltration with macrophages and T cells was of less magnitude than in Veh-Lupus mice, it is possible that this effect was in part due to C5a downregulation. In addition to generating C5a, complement activation involves the formation of C5b-9, i.e., membrane attack complex (MAC). Although it is still unclear which of the complement activation products are most important in lupus nephritis, C5b-9 was clearly demonstrated in glomeruli and peritubular basement membranes of kidneys from SLE nephritis patients (6). Our data showed that Veh-Lupus mice expressed significantly higher levels of C5b-9 deposits that were significantly reduced in response to Ac-SDKP treatment. As MAC, C5b-9 inserts into the phospholipid bilayers and causes cell lysis (5). However, MAC was also shown to trigger cellular reactions that can produce renal injury (45), mainly by mediating proinflammatory response of resident glomerular cells (49) and macrophages (21) and these are the actions that were most likely downregulated in response to Ac-SDKP treatment.

In addition, we showed that the levels of proinflammatory RANTES, MCP-5, and MCSF chemokines/cytokines were also significantly reduced in Ac-SDKP-Lupus mice. These chemokines/cytokines are produced mainly by macrophages and are increased in the kidneys of lupus mice before the occurrence of proteinuria and renal damage (33, 40). The observed decrease in RANTES, MCP-5, and M-CSF levels in response to Ac-SDKP is probably related to the combination of the decrease in the numbers of infiltrating macrophages and the decrease in macrophage cytokine expression. In addition, both effects may be due to Ac-SDKP affecting macrophages directly or via the decrease in C5a/C5b-9, or due to the combination of both. Our previous work showed that Ac-SDKP inhibited macrophage differentiation, activation, migration, and release of TNF-α (52).

MCP-5 and M-CSF have been previously associated with renal damage in lupus (13, 16). In mouse lupus model, MCP-5 kidney expression was increased (55) and 8-wk treatment with...
Ac-SDKP inhibited this expression (54). At the same time, depletion of M-CSF improved renal function and decreased systemic illness in lupus mice (17).

In addition to precipitating the reduction in macrophage influx, decrease in RANTES may also explain the significantly lower numbers of kidney-infiltrating T cells in Ac-SDKP-Lupus mice. RANTES is a strong stimulator of T cell chemotaxis, proliferation and cytokine production, and its depletion in lupus mice markedly decreased numbers of CD4−/CD8− T cells (56). Here, we document for the first time the decrease in RANTES levels and consequently numbers of kidney-infiltrating T cells in the kidneys of lupus mice in response to Ac-SDKP. We previously showed that in rat model of autoimmune myocarditis Ac-SDKP prevented tissue infiltration of T helper cells (35). The overall decline in chemokine/cytokine levels in response to Ac-SDKP treatment most likely further decreases T cells and macrophage influx as well as their proliferation, survival, and differentiation.

Besides induction by chemokines, recruitment of immune cells to the inflammatory sites strongly depends on the interaction and binding to endothelial cell adhesion molecules. We analyzed the expression of ICAM-1, an endothelial cell adhesion molecule important in autoimmune processes, especially for migration of autoreactive T cells (44). Here, we demonstrated that in Ac-SDKP-Lupus mice ICAM-1 expression was significantly lower than in Veh-Lupus mice. Again, this ICAM-1 downregulation may be in part due to the decrease in C5a levels, since in endothelial cells ICAM-1 was shown to be upregulated in response to C5a via NF-κB pathway (1). Our previous study in rat model of autoimmune myocarditis also showed significantly lower levels of ICAM-1 in Ac-SDKP-treated animals (34).

Inflammation, glomerular, and interstitial fibrosis cause a decrease in renal function in lupus. In MRL lupus mouse, loss of renal function develops between 12 and 24 wk of age (20). Here, we show that 32-wk-old Veh-Lupus mice developed significant albuminuria and a decrease in GFR that was improved after treatment with Ac-SDKP for 20 wk. Our results are in agreement with the recent report showing that, in this model of lupus, treatment with Ac-SDKP ameliorates progression of lupus nephritis (54). In lupus, glomerulosclerosis is the main reason for GFR decline and our data showed significant increase in extracellular matrix within the glomeruli of Veh-Lupus mice. These deposits were significantly lower in Ac-SDKP-Lupus mice. Recent studies implicated that glomerular sclerotic changes in lupus are accompanied with a decrease in the expression of podocyte slit diaphragm proteins, such as nephrin (41). Indeed, we detected significant decrease in nephrin expression in Veh-Lupus mice that was partially prevented with Ac-SDKP treatment. Thus, significant increase in nephrin expression together with ~50% decrease in glomerular matrix deposits and renal collagen expression are the most probable reason for the observed improvement in GFR and decrease in proteinuria in Ac-SDKP-Lupus mice, where GFR values were three times higher (65 ml/min) than in Veh-Lupus (19 ml/ml).

It is important to note that if in patients treated for renal failure the GFR improved by 200%, the therapy outcome would be considered as extremely successful. Tissue injury in kidneys of the lupus patients is not restricted to glomeruli only, but it affects interstitial tissue as well (37). Here, we demonstrated that interstitial collagen fraction increased significantly in the kidneys of Veh-Lupus mice and that Ac-SDKP treatment significantly prevented the occurrence of these collagen deposits. These results were consistent with what we previously reported on the anti-fibrotic effect of Ac-SDKP in various disease models including autoimmune myocarditis (35). However, the exact mechanisms of Ac-SDKP-mediated decrease in fibrosis are still not clear. As in other diseases characterized by fibrosis, TGF-β has been associated with renal fibrosis (31) and one of the mechanisms of Ac-SDKP anti-fibrotic effects may be a decrease in collagen formation via suppression of TGF-β pathway. Since proinflammatory cytokines other than TGF-β can stimulate fibrosis, especially through promoting fibroblast/epithelial/endothelial mesenchymal transition (10), downregulation of C5-9-induced cytokine pathways may be an additional mechanism contributing to the observed decrease in renal fibrosis in response to Ac-SDKP.

### Table 1. Urinary Ac-SDKP, SBP, and body and kidney wt

<table>
<thead>
<tr>
<th>Groups</th>
<th>Veh-Ctrl</th>
<th>Ac-SDKP-Ctrl</th>
<th>Veh-Lupus</th>
<th>Ac-SDKP-Lupus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Ac-SDKP,</td>
<td>ng/24 h</td>
<td>54.7 ± 5.7*</td>
<td>185.2 ± 19.9</td>
<td>90.6 ± 17.1</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>113 ± 3</td>
<td>108 ± 3</td>
<td>112 ± 2</td>
<td>113 ± 1</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>41.6 ± 1.9</td>
<td>41.2 ± 2.2</td>
<td>38.7 ± 0.9</td>
<td>37.3 ± 0.7</td>
</tr>
<tr>
<td>KW/BW, ng/10 g</td>
<td>109.2 ± 1.5</td>
<td>111.9 ± 3.7</td>
<td>142.0 ± 9.3</td>
<td>122.6 ± 3.5</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6–10. SBP, systolic blood pressure; KW/BW, kidney weight/body weight (wt). †P < 0.001 Veh-Ctrl vs. Ac-SDKP-Ctrl.  ‡P < 0.001 Ac-SDKP-Lupus vs. Veh-Lupus. †P < 0.001 Veh-Ctrl vs. Veh-Lupus.
In addition to renal and cardiovascular diseases, skin lesions occur in 90% of patients with SLE (42). The most intensively studied model for cutaneous lupus is the MRL/lpr mouse that usually develops spontaneous skin lesions by 5 mo of age (18, 26). Here, we demonstrated that 20-wk treatment with Ac-SDKP significantly decreased severity of facial skin lesions in these mice. Skin lesions in lupus mice were characterized by T cell-rich infiltrates (19) and shown to strongly depend on local ICAM-1 expression (36). Therefore, it is possible that the observed Ac-SDKP effect on skin lesions in part relies on the above postulated mechanisms involving Ac-SDKP modulation of renal T cell migration and ICAM-1 expression.

In summary, Ac-SDKP prevented renal damage and dysfunctions in the lupus mouse model. These protective Ac-SDKP effects were in part manifested as a prevention of inflammation and fibrosis. Ac-SDKP inhibited renal T cell and macrophage infiltration and proinflammatory chemokine expression, and collagen production. In addition, our data demonstrate for the first time that in lupus mouse models Ac-SDKP prevented an increase in C5a, C5b-9, and ICAM-1 expression. C5a was shown to be important in lupus development and is possibly playing an essential role in perpetuating inflammatory cell infiltration and activation, endothelial cell activation including the expression of ICAM-1, and proinflammatory cytokine/chemokine and collagen production, and downregulation of these actions may be central to the mechanisms involved in the observed Ac-SDKP anti-inflammatory effects that likely involve alteration of inflammasome (proinflammatory cytoplasmic complex) function and/or signaling cascades such as NF-κB pathway. Ac-SDKP treatment may be a novel and useful therapeutic strategy for the treatment of progressive autoimmune diseases.

GRANTS

This work was supported by National Institutes of Health Grant HL028982.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

EFFECT OF Ac-SDKP ON LUPUS


