Glomerulonephritis (GN) is a progressive inflammation of the glomeruli that can be caused by a variety of underlying disorders. It is one of the primary causes of renal failure and end-stage renal disease that require dialysis and transplantation. Immunosuppressive therapy has been used to treat GN clinically, but the therapeutic benefits of this treatment are limited and the treatment *per se* can also cause renal damage.\(^1\)

Crescentic GN is also called rapidly progressive GN; if left untreated, it rapidly progresses into acute renal failure. Anti-glomerular basement membrane (anti-GBM) GN is a typical type of crescentic GN. It is characterized clinically by the rapid deterioration of renal function and histologically by mononuclear cell infiltration into the stroma, glomerular cell proliferation, crescent formation in the glomerulus with the deposition of anti-GBM antibodies.\(^2\)

To investigate this disease, we utilized an experimental model in Wistar-Kyoto (WKY) rats, in which injection of rabbit anti-rat GBM antibody induces severe proliferative and necrotizing GN with crescent formation that resembles human anti-GBM GN.\(^3\)

Previously, we identified the protein kinase casein kinase II (CK2) as a GN-related gene through microarray analysis performed on these anti-GBM GN model rats, and demonstrated that *in vivo* inhibition of the kinase ameliorates renal dysfunction and histological progression of the condition.\(^2\)

Furthermore, previous studies using the same model of anti-GBM GN have suggested that several genes are also relevant to the onset of anti-GBM GN.\(^4\)

The study reported herein shows a novel antinephritic effect observed in the anti-GBM GN rat model following administration of an emulsion containing tricaprylin, a medium-chain triacylglycerol. We compared the effects of the tricaprylin emulsion administration *in vivo* with that of a CK2 inhibitor administration. The results of the *in vivo* experiments demonstrated that the tricaprylin emulsion ameliorated renal dysfunction of the anti-GBM GN rat model effectively and showed a greater antinephritic effect than that of the CK2 inhibitor. To elucidate the detailed mechanism of action of the emulsion, we also performed a gene ontology (GO)-based microarray analysis of gene expression profile data obtained from the renal cortices of our experimental mice.

**MATERIALS AND METHODS**

**Animals** The experimental protocol for the present study was reviewed and approved by the Animal Care Committee of Kyoto University. Seven-week-old male WKY rats weighing 200 g were purchased from Charles River Laboratories Japan (Atsugi, Kanagawa, Japan) and used in all the experiments.
Animals were housed in a constant temperature room with a 12-h dark/12-h light cycle. The general condition and body weight of the rats were observed over the course of the experiments.

Isolation of Glomeruli Glomeruli were isolated from the renal cortices of the rats using the differential graded sieving method, which yields glomeruli with a purity greater than 90%. Briefly, kidneys were ground gently through 400-µm and then 200-µm stainless steel sieves. The fraction obtained was washed with phosphate buffered saline on a 90-µm mesh. The supernatant was then centrifuged at 905×g for 5 min at 4°C (MX-300; Tomy, Tokyo, Japan).

Anti-GBM GN To prepare anti-GBM antibodies, GBM antigen from rats was prepared from isolated glomeruli in accordance with the Krakower method. Five albino rabbits were immunized subcutaneously with GBM antigen emulsified with Freund’s complete adjuvant (Nihon Becton Dickinson, Tokyo, Japan). Three booster injections of the same antigen were given at two-week intervals. Four days after the final booster, blood was collected from the carotid artery under anesthesia. Anti-GBM sera were decomplemented by heating at 56°C for 30 min and absorbed with freshly harvested rat erythrocytes. Twenty-four WKY rats were divided into 4 groups of 6 rats (Control, anti-GBM GN, the tricaprylin emulsion-treated anti-GBM GN, and emodin-treated anti-GBM GN). The rats assigned to the GN groups were injected under anesthesia in the dorsal tail vein with 3 mL/kg anti-GBM serum that had been diluted 10-fold with saline. The day of injection was defined as day 0. The rats assigned to the control groups were injected intravenously with 3.0 mL/kg normal nonimmune rabbit serum diluted 10-fold with saline. The day of injection was defined as day 0. The rats assigned to the control groups were injected intravenously with 3.0 mL/kg normal nonimmune rabbit serum to allow comparison with rats in the GN groups.

Drug Treatment Tricaprylin (1,2,3-trioctanoylglycerol; 100 mg/mL; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in chloroform and combined with L-α-phosphatidylcholine (35 mg/mL; Avanti Polar Lipids, AL, U.S.A.), Saline (Otsuka Pharmaceutical Factory, Tokyo, Japan) was added to the mixture after the chloroform had been evaporated completely. The resulting suspension was sonicated using an ultrasonic bath (30 s, 6 times) and filtered through a 0.22-µm membrane filter. Separately, distearoylphosphatidylethanolamine–polyethylene glycol 2000 (DSPE–PEG2000) (14 mg/mL; Sunbright DSPE-020CN, NOF Co., Tokyo, Japan) was dissolved in saline and incubated in a water bath for 3 h at 70°C to obtain micelles of PEG. The PEG micelles were added to the tricaprylin/L-α-phosphatidylcholine suspension and the mixture was incubated for 90 min at 70°C to obtain the tricaprylin emulsion. The tricaprylin emulsion, tricaprylin alone (100 mg/mL), L-α-phosphatidylcholine alone (35 mg/mL), or DSPE–PEG2000 alone (14 mg/mL) was administered intravenously at 0.5 mL/rat once a day after injection of the anti-GBM serum from day 1 to day 7 or day 14 at which time they were sacrificed. The CK2 inhibitor emodin (3-methyl-1,6,8-trihydroxyanthraquinone; Sigma, MO, U.S.A.) dissolved in 4% dimethyl sulfoxide (DMSO) and suspended in a 0.5% carboxymethylcellulose (CMC)–saline solution was administered intraperitoneally at 20 mg/kg of body weight within the same time period as the tricaprylin emulsion administration. At the end of the study, the rats were anesthetized, their blood was collected by cardiac puncture, and their organs were collected.

Proteinuria and Serum Creatinine (Scr) To analyze proteinuria, the rats were housed individually in metabolic cages for the 24-h urine collection. Urinary protein was measured by SRL, Inc. (Tokyo, Japan) using the Biuret method. Levels of blood urea nitrogen (BUN) and Scr were measured by SRL, Inc. using an automated analyzer (Hitachi, Tokyo, Japan).

Histological Evaluation of Renal Tissue Kidney tissues from each animal were processed for analysis by light microscopy and immunostaining. For light microscopy, the tissues were fixed in 10% neutral-buffered formalin (pH 7.4) and embedded in paraffin. Sections (4.0 µm) were then subjected to periodic acid-Schiff (PAS) staining, which were evaluated quantitatively by counting the total number of glomerular cells, and the total numbers of glomeruli with mesangiolysis and capillary balloononing, respectively, and measuring the areas of 20 randomly selected glomerular cross-sections using NIH Image (NIH, Bethesda, MD, U.S.A.). Glomerular crescent formation was assessed in a blinded manner. Crescent formation was defined as the presence of at least 2 non-tubular cell layers observed in the Bowman’s space. To describe the degree of crescent formation quantitatively, the crescentic score was used (number of glomeruli with crescent formation/30 glomeruli). The sections (4.0 µm) were also immunostained with a goat polyclonal antibody against Ki-67 (Santa Cruz Biotechnology, CA, U.S.A.), a mouse antibody against rat ED-1 (Chemicon International, Temecula, CA, U.S.A.), and a goat antibody against rat monocyte chemotactic protein-1 (MCP; Santa Cruz Biotechnology, TX, U.S.A.), using a VECTASTAIN Elite ABC Kit (Vector Laboratories, Burlingame, CA, U.S.A.). The color was then developed by incubation with a DAB Substrate Kit (Pierce, Rockford, IL, U.S.A.). The sections were counterstained with hematoxylin. The average numbers of Ki-67, ED-1, and MCP-1 positive cells in a glomerular cross-section were determined by enumerating the positive cells in 20 glomeruli in each section.

In Vitro CK2 Kinase Assay CK2α was prepared as described previously. The CK2α kinase reaction was performed in 15 µL of reaction mix that contained 200 mm 3-(N-morpholino)propanesulfonic acid (MOPS) pH 7.2, 25 mm β-glycerophosphate, 5 mm ethylene glycol bis(2-aminoethyl)ether-N,N′,N″,N‴-tетраакисная кислота (EGTA), 1 mm sodium orthovanadate, 1 mm diethiothreitol, 13.5 mm MgCl2, 0.4 µM PKA inhibitor cocktail, 0.2 mm CK2 substrate peptide (RRRDDDSSDDD), 8.5 µm Unit CK2α enzyme, 0.09 mm ATP, 2.2 mm [γ-32P]ATP, 0.6 µL of inhibitor or 4% DMSO (Nacalai Tesque, Kyoto, Japan) vehicle as a control. One unit of kinase activity is defined as the amount of enzyme that will catalyze the transfer of 1 nmol of phosphate to RRDDDSDDD per min at 37°C. After incubation at 37°C for 10 min, the reaction was terminated by the addition of 10 µL of 40% trichloroacetic acid. Aliquots of 5 µL of the reaction mix were transferred to a 96-well P81 UniFilter plate (Whatman, Kent, U.K.), and each well was washed with 0.2 mL of 0.75% phosphoric acid solution 20 times. After incubation for 30 min in 0.02 mL of Microscinti-0 (PerkinElmer, Inc., CT, U.S.A.), residual radioactivity was measured using a TOP count NXT Scintillation and Luminiscence Counter (PerkinElmer, Inc.,).
tained a protease inhibitor cocktail and a phosphatase inhibitor cocktail (Sigma). Supernatant was prepared by centrifugation at 10000g, 4°C for 10 min (MX-300; TOMY). Protein concentration was determined using supernatant (diluted, 1:5000) in accordance with the Bradford method. Aliquots of supernatant that contained 0.05 mg of protein were used to assay the CK2 activity. The endogenous CK2 activity was assayed by the same protocol for the in vitro CK2 kinase assay.

Histological Evaluation of Testis Terminated deoxyribo nucleotidyl transferase mediated deoxyuridine triphosphate nick-end labeling (TUNEL) analysis of testis was performed in accordance with Gavrieli’s method. The average number of TUNEL-positive cells in a testis section was determined by enumerating the number of TUNEL-positive cells per seminiferous tubule in each section.

RNA Extraction and Microarray Processing Total RNA was extracted from tissue using ISOGEN (Nippon Gene, Tokyo, Japan). RNA integrity was checked on an Agilent 2100 Bioanalyzer (Agilent Technologies, Tokyo, Japan). Microarray analysis was performed as described previously. Briefly, genome-wide mRNA expression profiles were obtained by microarray analysis with the Affymetrix GeneChip Rat (230 2.0) Array, in accordance with the manufacturer’s instructions. Normalized data are available at the Gene Expression Omnibus (GEO) website (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE46295.

Analysis of Microarray Data The microarray data obtained from the in vivo model and GEO (GSE32583 and GSE32591) were examined and visualized using R/Bioconductor. The expression levels of our microarray data were calculated from probe intensities and arrays normalized using the mas5 method. Only probe sets with normalized signals >20 were defined as expressed and selected for analysis. The expression of individual genes was defined as altered when comparison of the average normalized signal intensities using expression of individual genes was defined as altered when level of urinary protein excretion increased almost 15-fold at day 14 (Fig. 1A). Administration of emodin (20 mg/kg/d), a CK2 inhibitor, in the anti-GBM antibody-injected rats alleviated the development of proteinuria significantly (Fig. 1A), a CK2 inhibitor, in the anti-GBM antibody-injected rats alleviated the development of proteinuria significantly (Fig. 1A), in contrast to the control rats, the anti-GBM antibody-injected rats developed severe proteinuria, with the level of urinary protein excretion increased almost 15-fold at day 14 (Fig. 1A). Administration of emodin (20 mg/kg/d), a CK2 inhibitor, in the anti-GBM antibody-injected rats alleviated the development of proteinuria significantly (Fig. 1A), which is in agreement with our previous study. Moreover, administration of the tricaprylin emulsion (0.5 mL/d) attenuated the proteinuria to a greater extent than that of emodin (Fig. 1A). Consistently, levels of BUN and SCr at day 14 were elevated in the anti-GBM antibody-injected rats as compared with the control rats, and the increase was reduced by administration of the tricaprylin emulsion (0.5 mL/d) or (20 mg/kg/d)
emodin (Figs. 1B, C).

**Histological Analysis** We examined the effects of treatment with the tricaprylin emulsion and emodin on renal histology by PAS staining (Fig. 2A). The most prominent change in the anti-GBM GN rats was severe glomerular crescent formation. The degree of crescent formation was lower in the emodin-treated anti-GBM GN rats than in the vehicle-treated ones. Furthermore, the tricaprylin emulsion inhibited crescent formation more effectively than emodin (Fig. 2A upper). The total glomerular cell number was increased significantly in the anti-GBM GN rats as compared with the control rats and this increase was prevented by administration of the tricaprylin emulsion or emodin (Fig. 2B). The anti-GBM GN rats had the highest crescentic score, and this was reduced significantly to an intermediate level by administration of emodin and nearly to zero by administration of the tricaprylin emulsion (Fig. 2C).

To evaluate the cell proliferation, we conducted immunohistological staining for Ki-67, a marker of cell proliferation (Fig. 2A lower). As shown in Fig. 2D, the number of Ki-67-positive cells in the glomeruli of anti-GBM GN rats was augmented significantly as compared with control rats, and the increase was inhibited markedly by administration of the tricaprylin emulsion or emodin (Fig. 2D, p<0.001).

We also examined the level of macrophage infiltration in the glomeruli after administration of the tricaprylin emulsion or emodin by immunostaining for ED-1 and MCP-1, which are markers of rat macrophages (Fig. 3A). The number of ED-1 and MCP-1 positive cells was increased markedly in nephritic glomeruli of the anti-GBM GN rats as compared with the normal glomeruli of the controls. Administration of either the tricaprylin emulsion or emodin inhibited the accumulation of ED-1 and MCP-1 positive cells in glomeruli significantly (Figs. 3B, C).

As emodin is reported to show testicular toxicity, we performed TUNEL analysis to examine whether the tricaprylin emulsion also had the toxicological effect on testis. The result of the TUNEL assay elucidated that very few TUNEL-positive cells were detected in the testis of the anti-GBM GN rat with the tricaprylin emulsion administration (Figs. 3D, E).

**Expression Profile Analysis** To elucidate the molecular mechanisms of the antinephritic effects of the tricaprylin emulsion and emodin on anti-GBM GN rats, we performed gene expression profile analysis. As shown in Fig. 4A, the expression of 3616 genes was altered in the anti-GBM GN rat model as compared with the control rats (annotated as “GBM vs. C”). Administration of emodin resulted in 1453 genes being affected (annotated as “E vs. C”). Administration of the tricaprylin emulsion led to 770 genes being affected (annotated as “TC vs. C”). Thus, the number of genes whose expression changed in anti-GBM GN was reduced remarkably by administration of the tricaprylin emulsion or emodin. A Venn diagram (Fig. 4A) shows the relationships among the genes that were expressed differentially in the three pairwise comparison groups. Among the 3616 genes...
whose expression was altered in the anti-GBM GN rats, 2336 genes were expressed at normal levels after administration of the tricaprylin emulsion or emodin, which shows that there is a significant difference in the gene expression pattern between the anti-GBM GN rat group and the treated groups.

To investigate the biological processes with which the affected genes are associated, we performed a GO-based microarray analysis. Furthermore, to understand the effects of the tricaprylin emulsion administration in more detail and to compare the pathogenesis of different types of nephritis, we listed the data for human specimens and animal models of another autoimmune nephritis-LN, which are registered in the public domain microarray database (GEO), including data for the NZB/W mouse pre-LN, the NZB/W mouse LN profile, and the human LN profile. 23 We calculated the $p$ values of each GO to reflect the degree of the GO overrepresentation and visualized them in a heatmap. As shown in Fig. 4B, we mapped the $p$-values obtained from the hypergeometric test in the independent pairwise comparisons between each test sample and its corresponding control for the different GO terms (Left heatmap, Value: $-\log_{10} P$, increase: right, decrease: left in each pairwise comparison group. If only one of the $-\log_{10} P$ values in a given GO term was $>5$, the GO term was listed). The overrepresentation of the GO terms in “GBM vs. C” was decreased in “TC vs. C” (Fig. 4B). To estimate the discrimination between our anti-GBM GN model and other models of nephritis (Left heatmap in Fig. 4B), the degree of similarity among all the samples in the analysis was calculated on the basis of the correlation coefficients between the columns of $p$ values and a dendrogram was generated using Ward’s method.
(Fig. 4C). The dendrogram revealed a similarity between the rat anti-GBM GN model and the severe form of mouse LN model (LN III vs. C) at a linkage distance of 0.11.

To provide a detailed mechanistic insight into the role of the tricaprylin emulsion and the CK2 inhibitor towards anti-GBM GN, we performed hierarchical clustering based on the correlation coefficients between the rows of p-values of pairwise GO terms and generated a dendrogram (left in Fig. 4B). According to the horizontal hierarchical clustering, we identified several characteristic clusters in the heatmap (Left heatmap in Fig. 4B). Also, to better understand the horizontal hierarchical clustering, in an additional heatmap (Right heatmap in Fig. 4B), we displayed the p values of the same GO terms from the left heatmap in the GO analyses of the gene groups in the specific regions of the Venn diagram (2336 hollow star, 849 solid circle in Fig. 4A), which were annotated as “GBM+E+TC−”; 225 (solid circle) was annotated as “GBM+TC−”; and 72 (solid square), which were annotated as “GBM+TC−” and “E+TC−” and “TC”, respectively. Shown bold in the frame in Fig. 4B, we found that half of the GO terms in cluster a were fibrosis- and ion transport-related and all the GO terms but one in cluster b were cell cycle-related. The GO terms in cluster a and b were only overrepresented in “GBM vs. C,” other than “E vs. C” or “TC vs. C” and, moreover, the overrepresented GO terms in the list annotated as “GBM+E−TC−” of the right heatmap were predominantly located in the two identified clusters (cluster a and b in Fig. 4B). Furthermore, we depicted the GO terms in the two identified clusters and their hypergeometric test p values with different color bars for each pairwise comparison group (Supplementary Figs. 1A, B). To reveal detailed relationships among the GO terms in the cluster, we organized the terms in a directed acyclic graph24 (Supplementary Figs. 2A, B) and noticed that in cluster b, most (10 of 11) of the terms were in one subset and associated with the cell cycle process (left side in Supplementary Fig. 2B). To provide more functional information, we selected the altered genes in the GO categories of cluster b, mapped and visualized them on the KEGG/pathway database (Supplementary Fig. 3). The most significantly over-expressed genes in these GO categories were mainly
The tricaprylin emulsion, 0.5 mL/d; Emodin, 20 mg/kg/d; Tricaprylin, 0.5 mL/d; PEG2000, 0.5 mL/d; Egg lecithin, 0.5 mL/d. (B) Results of the in vitro CK2 kinase assay. Data are means±S.E.M. n=3 animals. ***p<0.001 (vs. anti-GBM).

Identification of Active Components and Effect on CK2 Kinase Activity of the Tricaprylin Emulsion

The tricaprylin emulsion consists of the medium-chain triacylglycerol tricaprylin, egg lecithin, and the water-soluble polymer DSPE-PEG2000. To investigate the antinephritic effects of the individual components in the emulsion, the effect of each component was tested. We measured proteinuria at day 7 in the anti-GBM GN rat model after administration of each component of the emulsion separately. The results showed that tricaprylin (0.5 mL/d) limited the progression of proteinuria significantly (Fig. 5A).

To investigate whether the antinephritic effect of the emulsion was related to renal CK2 activity, the effects of the tricaprylin emulsion and emodin on renal CK2 kinase activity were examined in vitro and endogenously. In the in vitro CK2 kinase assay, we detected no significant inhibition of CK2 activity by the tricaprylin emulsion in contrast to emodin (Fig. 5B). However, measurement of the endogenous CK2 activity in the renal cortex showed that the enhanced CK2 activity in the anti-GBM GN was not observed in both treatment modalities (Fig. 5C). Therefore, the combined results of the two assays suggest that the tricaprylin emulsion might affect CK2 gene expression and/or enzymatic activity in a manner, which differs from the direct inhibition by emodin.

DISCUSSION

Current treatment of anti-GBM GN is still limited mainly to plasmapheresis coupled with immunosuppressive treatment.11 We had shown previously that the inhibition of CK2 gene expression and enzymatic activity is effective in the treatment of our anti-GBM GN rat model.12 By chance, we found that a tricaprylin emulsion, which we had attempted initially to use as a vehicle for administration of the CK2 inhibitor, was also an effective treatment in the anti-GBM GN rat model. We also verified that tricaprylin itself, a medium-chain triacylglycerol, played the critical role in the antinephritic effect. Some earlier studies had reported ameliorating effects on renal disease of the dietary supplementation with long-chain triacylglycerols (fish oil) in both murine and human,20 and our results provide the first evidence that medium-chain triacylglycerols can significantly attenuate proteinuria and the progression of crescentic GN.

To clarify the mechanism of action of the tricaprylin emulsion on the anti-GBM GN model, we conducted a detailed GO-based microarray analysis. We applied GO because it provides a consistent description of genes or gene products in which biological features are annotated in a species-independent manner and it enables us to identify changes beyond the level of individual genes, and also to compare different species. The method of microarray analysis used in this paper has the following advantages: 1) Reliability owing to the use of only data and source codes that have been published; 2) The ability to compare gene expression profile data with the public microarray database; 3) Availability of differential microarray data between different animal species or experimental platforms; 4) Ability to identify the affected biological processes by GO analysis rather than affected individual genes alone. This method provides an effective means of utilizing the
published microarray data, and a fast and low-cost strategy to further progress in this area.

The method enabled us to make a crosswise comparison between multiple models of nephritis and clinical samples by utilizing previously published GeneChip data. Our microarray analysis revealed that rat anti-GBM GN and mouse LN showed a similar pattern of GO overrepresentation (Fig. 4C). At the molecular level, previous studies had reported that several effector molecules or genes might determine the degree of tissue damage and renal dysfunction in both anti-GBM GN and LN.29,30 Hence, we suggest that our result may provide strong evidence to support the hypothesis that these two diseases may share some common pathological changes, either in the same animal species or between different species.

The progression of the experimental GN is always relevant to the onset of the glomerular fibrosis, the increasing cell proliferation in the crescent, the enhanced apoptosis of the glomerular cells, and the augmented inflammation in the glomeruli.2 From our microarray analysis, we did identify a series of characteristic clusters of GO terms related to fibrosis, cell cycle, cell apoptosis, and inflammatory response overrepresented in our anti-GBM GN datasets (Fig. 4B, Table 1). It is noteworthy that the overrepresentation of GO terms related to cell cycle disappeared in anti-GBM GN by administration of the CK2 inhibitor or the tricaprylin emulsion in contrast to control. Previously, we have reported that administration of the CK2 inhibitor suppressed the glomerular cell proliferation in the experimental GN model.23 In the emodin-treated anti-GBM GN rats, we verified the suppression of elevated renal CK2 activity (Figs. 5B, C) and increased glomerular cell proliferation (Fig. 2A lower) induced by the anti-GBM antibody. Similar to the histological experiment, we also detected that the overrepresented pattern of the GO terms in anti-GBM GN, in which most of the over-expressed genes were relevant to cell proliferation (Supplementary Fig. 3), was not observed with administration of emodin (cluster b in Fig. 4B). However, we found that the CK2 activity was not directly inhibited by the tricaprylin emulsion in the in vitro experiment, but decreased endogenously in the tricaprylin emulsion-treated anti-GBM GN rats (Figs. 5B, C). This suggests that administration of the tricaprylin emulsion may influence upstream pathways that diminish the enhanced renal CK2 activity in vivo, attenuating glomerular cell proliferation (Fig. 2A lower) in anti-GBM GN, which is also consistent with the result of cluster b in our microarray analysis (cluster b in Fig. 4B). Like emodin administration, the cell cycle-related GO terms in cluster b were not overrepresented in the tricaprylin emulsion-treated anti-GBM GN (cluster b in Fig. 4B). Moreover, the GO terms in the other clusters, which were related to the glomerular fibrosis, inflammation, and cell apoptosis, were overrepresented in the emodin administration, but not in the tricaprylin emulsion administration, suggesting other antinephritic effects of the tricaprylin emulsion (Table 1). The histological experiments showed ameliorating effects of the tricaprylin emulsion administration on the glomerular fibrosis (Fig. 2A upper) and the inflammatory response (Fig. 3A) in the anti-GBM GN rats, which was greater than that of emodin administration. We indicate that the antinephritic effect of the tricaprylin emulsion would be manifold, while that of the CK2 inhibitor may be mainly relevant to the cell cycle, which may be mediated by the inhibition of CK2 activity. Of note, from the GO analysis of “GBM+E+TC+,” we found that the overrepresented GO terms were predominantly immune response-related (Right heatmap in Fig. 4B), which suggested that the action of the tricaprylin emulsion may be significant, not completely effective, and carried out after the onset of anti-GBM GN. In addition, we noticed that the fibrosis-related GO terms were only overrepresented in our anti-GBM GN rat datasets, and not in the NZB/W mouse LN datasets (Left heatmap in Fig. 4B). The NZB/W mouse is characterized by hypercellular renal lesions and fibrinoid necrosis with no glomerular fibrosis,23 which is in agreement with the result of cluster a in our microarray analysis of the NZB/W mouse LN datasets. Consistently, our microarray analysis may provide a strong mechanistic clue for the understanding of experimental crescentic GN, especially the experimental anti-GBM GN and the pharmacological effects of the tricaprylin emulsion.

Of all the components in the emulsion, tricaprylin, a kind of medium-chain triacylglycerol, has been used as a pharmaceutical additive for many years, whereas DSPE–PEG2000

| Pathological feature | Biological process | Anti-GBM GN | Emodin-treated anti-GBM GN | TC-treated anti-GBM GN | GBM+E+TC+ | GBM+E+TC− | GBM+E−TC− | LN mouse/human |
|---------------------|-------------------|-------------|--------------------------|-------------------|-----------|----------|---------|-------------|
| Cell proliferation  | Cell cycle (in cluster b) | ↑ | — | — | — | ↑ | ↑/↑ | |
|                     | Collagen fibril organization (in cluster a) | ↑ | ↑ | — | — | ↑ | ↑ | / — |
|                     | Extracellular structure organization (in cluster c) | ↑ | ↑ | — | — | ↑ | ↑ | / — |
| Glomerular fibrosis | Cytokine/chemokine production (in cluster d_1/2/7) | ↑ | ↑ | — | — | ↑ | ↑ | / — |
|                     | Leukocyte cell activation/proliferation/locomotion/migration (in cluster d_1/2/3/4/7/9/11) | ↑ | — | ↑ | ↑ | — | ↑/↑ | |
| Cell apoptosis      | Cell apoptosis (in cluster d_5/6) | ↑ | ↑ | — | — | ↑ | ↑ | / — |

TC, the tricaprylin emulsion; solid arrow, significantly overrepresented; hollow arrow, overrepresented; bar, not overrepresented.
has been developed to use for micelle formation to direct the delivery of therapeutic moieties to a targeted tissue. In the present study, tricaprylin was shown to have a notable inhibitory effect on the progression of proteinuria for the first time (Fig. 5A). Several studies have reported that long-chain triacylglycerols can attenuate proteinuria in renal dysfunction. Several possible mechanisms have been suggested for long-chain triacylglycerol-induced prevention of renal dysfunction, such as suppression of sterol regulatory element-binding protein and inhibition of the activation of nuclear factor-kappa B (NF-κB) in the kidney tissue. We also detected the attenuation of enhanced expression of NF-κB in anti-GBM GN by administration of this medium-chain triacylglycerol emulsion from our microarray data. Moreover, medium-chain triacylglycerols or fatty acids were reported to interact with multiple proteins to affect various signaling pathways, such as G protein-coupled receptor GPR84 and peroxisome proliferator activated receptor γ. Further study is needed to identify the critical signaling pathways that are stimulated by medium-chain triacylglycerols, particularly the tricaprylin emulsion or tricaprylin alone, to induce the antinephritic effect on anti-GBM GN. Given the current limited and insufficient pharmacotherapy for GN, the tricaprylin emulsion or even tricaprylin alone is of interest as a potentially effective treatment for GN.

In summary, we demonstrated a strong antinephritic effect of the tricaprylin emulsion on anti-GBM GN in vivo and identified tricaprylin as the major active component in the emulsion. Moreover, we analyzed the detailed mechanism by which the emulsion ameliorated anti-GBM GN using in silico approach and developed an effective method of evaluating the pharmacological effects of the emulsion on nephritis by comparing microarray data between different animal species or experimental platforms.

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