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Immunomodulatory Therapy Using Recombinant IFN-γ Revealed Opposite Effects in Different Stages of Experimental Murine Membranous Nephropathy

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Background: Therapeutic agents for membranous nephropathy (MN) remain ill-defined. Strongly Th2 polarized immune response participated in the pathogenesis of MN. Here, we assessed the therapeutic effects and mechanisms of Th1 cytokine, IFN-γ, therapy for experimental murine MN. **Methods:** MN was induced in BALB/c mice with intravenous injections of cationic bovine serum albumin. Three groups of mice were administered 50 μg/kg recombinant IFN-γ in the early stage (weeks 1 to 3), late stage (weeks 4 to 6) or PBS (weeks 1 to 6) intravenously three times a week starting from MN induction. Disease severity was verified by serum and urine metabolic profiles and by renal histopathology. The oxidative stress was measured by superoxide production using DHE stain while inflammatory status was measured by positive NF-κB cells. Th1/Th2 cell axis polarizations were also determined. **Results:** There were significant reduction of proteinuria, remarkable amelioration of glomerular lesions accompanied by decreased immune deposition, and complement activation in mice receiving rIFN-γ therapy in the early stage. The mice receiving rIFN-γ therapy in the late stage revealed deterioration of metabolic and renal histopathological parameters. Similarly, changes of inflammatory status, and oxidative stress also showed opposite patterns. Early rIFN-γ therapy may reverse the strongly Th2 immune response of MN to alleviate disease severity, while late rIFN-γ therapy exacerbated the inflammatory process of MN, resulting in greater disease severity. **Conclusions:** There were opposite effects of rIFN-γ therapy in different stages of MN. Immunomodulatory treatment using rIFN-γ in the early stage of MN shifts the Th1/Th2 immune response and may be considered as a potential therapeutic strategy of MN in the future.

Key words: immunomodulatory therapy, IFN-γ, membranous nephropathy

INTRODUCTION

Membranous nephropathy (MN), the most prevalent cause of nephrotic syndrome in adult humans, is characterized by an *in situ* immune-complex disposition over the subepithelial space and is recognized as an autoimmune-mediated glomerulonephritis (GN).[1,2] Approximately 30%-40% of patients with MN develop progressive renal impairment, which results in end-stage renal failure after 10 to 15 years.[3] Effector cells, immunoglobulins (Igs), inflammatory cytokines, complements, and oxidative stress all participate in the pathogenesis of MN.[4,5] Presently available immunosuppressive therapies are not always effective and often have many persistent side effects.[5] Therefore, the appropriate treatment of patients with MN is still open to debate.

Early studies using monoclonal antibodies to T cell subsets indicate that T cells are central to the induction and glomerular injury in Heymann nephritis (HN), a rat model of idiopathic MN.[6-8] The T lymphocyte-derived cytokines also regulate the cellular and humoral immune responses to nephritogenic antigens and modulate inflammatory events.[9,10] It is widely recognized that $CD4^+$ T cells can be differentiated into different subsets according to their cytokine profiles, including Th1, Th2, Th17 and Treg cells.[11,12]. Th1 and Th2 subsets are regulated

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reciprocally to maintain a balance, which plays an important role in immune-mediated GN.[13] Th1-predominant responses are strongly associated with proliferative and crescentic forms of GN while Th2 responses are associated with membranous patterns of injury.[14,15]. However, studies involving patients with idiopathic MN showed a consistently negative response for DTH effectors, an increase in IL-4 production by peripheral T helper cells; and a predominance of IgG4 (Th2-type subclass) as well as complement deposition in glomeruli; suggesting a Th2 response.[16-19] In our previous study, a progressive Th2 response and a subsequent compensatory induction of the Th1 response were found. Both peripheral and renal immune reactions strongly polarized toward Th2 type immune response in the process of cBSA-induced MN were demonstrated.[20] Furthermore, IFN-γ is not only a key Th1 cytokine, but has also been employed to improve hepatitis C-associated glomerular disease[21].

In this study, we used recombinant IFN-γ (rIFN-γ) treatment in different stages of experimental murine MN to evaluate the therapeutic effects and mechanisms. There were opposite effects of rIFN-γ therapy in different stages of MN. There were significant reduction of proteinuria, remarkable amelioration of glomerular lesions accompanied by decreased immune deposition, and complement activation in mice receiving rIFN-γ therapy in the early stage. The mice receiving rIFN-γ therapy in the late stage revealed deterioration of metabolic and renal histopathological parameters.

MATERIALS AND METHODS

Mice

All animal studies were approved by the Animal Care and Use Committee of the National Defence Medical Center (Taipei, Taiwan) and all experiments were conducted according to the guidelines of the National Institutes of Health. BALB/c female mice (4-6 weeks old, about 20 g body weight) were initially purchased from the National Laboratory Animal Center (Taipei, Taiwan) and were maintained under specific-pathogen-free conditions in the Laboratory Animal Center of the National Defense Medical Center.

Experimental design

The mice were divided randomly into two groups: an experimental group and a control group. Animals in both groups were immunized with 0.2 mg of cationic bovine serum albumin (cBSA) emulsified in an equal volume of complete Freund's adjuvant[20,22]. Two weeks later, the experimental group (MN) received cBSA (13 mg/kg) intravenously three times per week every other day for six weeks, and the control group (NC) received pure saline following the same schedule. According to previous studies, 50 μg/kg recombinant IFN-γ was chosen as the therapeutic dosage.[23] The experimental mice were then divided randomly into three subgroups, each of which received one of the three treatments administered by intraperitoneal injections: 50 μg/kg recombinant IFN-γ in the early stage (weeks 1 to 3), late stage (weeks 4 to 6) or PBS (weeks 1 to 6) intravenously three times a week starting from MN induction. N = 3 in each subgroup. Homogenous cBSA was prepared as previously described [22]. Disease severity was verified by serum and urine metabolic profiles and by renal histopathology.

Serum and urine measurements

Urine samples were obtained for proteinuria screening using Labstix (Bayer Corp., Pittsburgh, PA). Grades 0 to 4 were assigned to the following ranges of urine protein concentrations: 0-30, 30-100, 100-300, 300-2000, and > 2000 mg/dL, respectively. Concentrations of serum creatinine, albumin, and total cholesterol were determined using commercially available kits (Roche Diagnostics, Indianapolis, IN).

Histological examination

Formalin-fixed and paraffin-embedded sections of kidney tissues were cut and stained with H & E according to a method described previously.[24] Frozen sections were air dried, fixed in acetone, and washed with phosphate-buffered saline (PBS) prior to incubation with FITC-conjugated goat anti-mouse IgG, and C_3 (Capple, Durham, NC) for the immunofluorescence study.[25] The pathological findings of MN were classified into four stages: stage I, subepithelial deposits; stage II, spike formation; stage III, incorporation of deposits into the basement membrane; and stage IV, disappearance of the deposits.[24] Immunofluorescence was quantified by analyzing the mean fluorescence intensity of each glomerular tuft in mice. Values were observed and evaluated semiquantitatively, as described previously.[26]

Reactive oxygen species (ROS) detection in kidney

In situ superoxide anion production was determined by dihydroethidium (DHE) labeling as described previously.[25] Briefly, 15-μm-thick frozen sections were incubated with 10 μmol/L of DHE (Molecular Probes, Eugene, OR) at 37°C for 30 minutes in a humidified chamber protected from light. Fluorescent images were

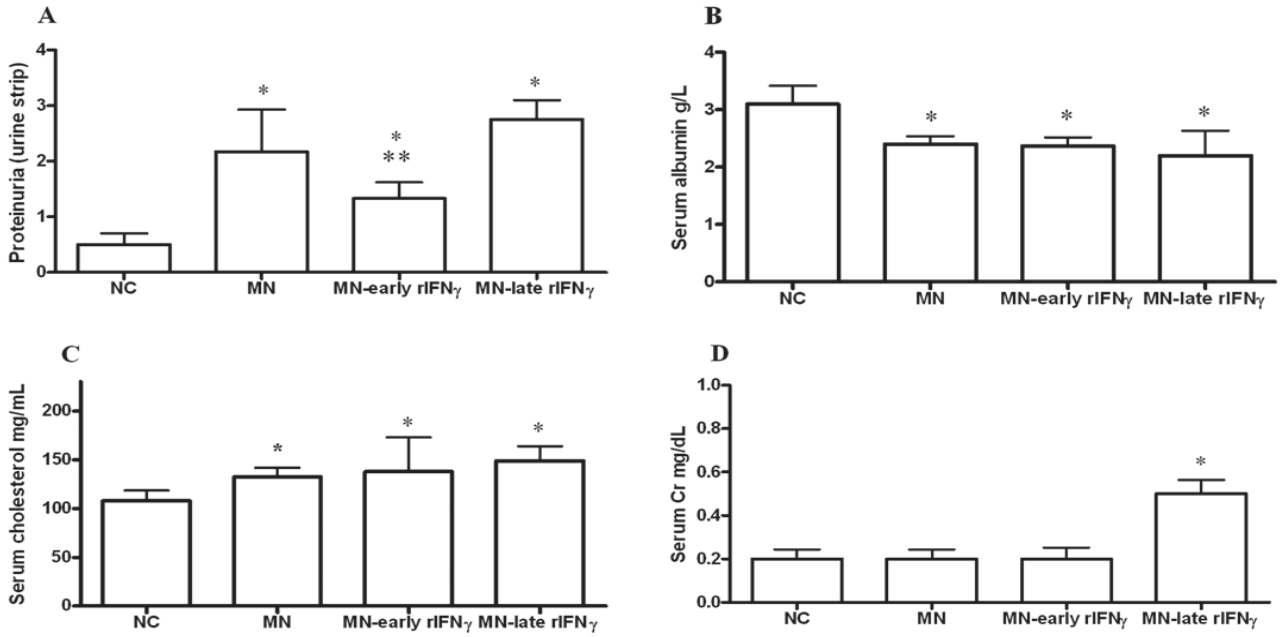

Fig. 1 Effects of rIFN-γ therapy on laboratory characteristics of mice with experimental MN. Urinary proteinuria (A), serum albumin (B), serum cholesterol (C) and serum creatinine levels (D) of the normal control group (NC), membranous nephropathy group (MN), mice with membranous nephropathy receiving early rIFN-γ treatment (MN-early rIFN-γ), and mice with membranous nephropathy receiving late rIFN-γ treatment (MN-late rIFN-γ).*$P < 0.05$ versus the control group. **$P < 0.05$ versus the MN group.

quantified by counting the percentage of positive nuclei per kidney cross-section.

Immunohistochemistry (IHC)

For detection of NF-κB-positive cells in glomeruli, IHC was performed on frozen sections that were fixed using cold acetone. Nonspecific antibody binding, endogenous avidin-binding activity and endogenous peroxidase activity were blocked using normal serum, the Biotin Blocking System (Dako Corporation, Carpinteria, CA) and 3% H_2O_2, respectively. Sections were then incubated with primary antibody to biotin-conjugated anti-mouse Phospho-NF-κB/p65 (Ser276) antibody (Cell Signaling, Danvers, MA), followed by incubation with avidin-horseradish peroxidase complex. The reaction products were visualized using freshly prepared diaminobenzidine tetrahydrochloride. Slides were counterstained with hematoxylin and mounted for microscopic examination.

Flow Cytometry and Intracellular Cytokine Staining

All mAb were purchased from BD Biosciences (San Jose, CA) or eBioscience (San Diego). Isolated splenocytes were stained with marker-specific antibodies: Allophycocyanin (APC)-conjugated anti-mouse CD4. For intracellular cytokine staining, splenocytes were stimulated with 20 ng/mL phorbol 12-myristate 13-acetate (PMA) plus 500 ng/mL ionomycin for 4 h at 37°C, the last 2 h in the presence of monensin (2 μM; Sigma-Aldrich). Cells were then washed with PBS that contained 2% FCS, fixed and permeabilized with saponin solution (eBioscience, San Diego, CA) and incubated with FITC-conjugated IFN-γ antibody and/or PE-conjugated anti-mouse IL-4 antibody. The stained cells were analyzed by FACS Caliber cell sorter using Cell Quest software (Becton Dickinson).

Statistical analysis

All data are expressed as the mean$\pm$SD. Statistical analysis was performed using the *t*-test when comparing two groups or by analysis of variance and Tukey's *post hoc* test when multiple groups were compared. Significance was defined as $P < 0.05$.

RESULTS

Effects of rIFN-γ treatments on general characteristics

Mice with experimental MN developed the characteristic symptoms of nephrotic syndromes, such as protei-

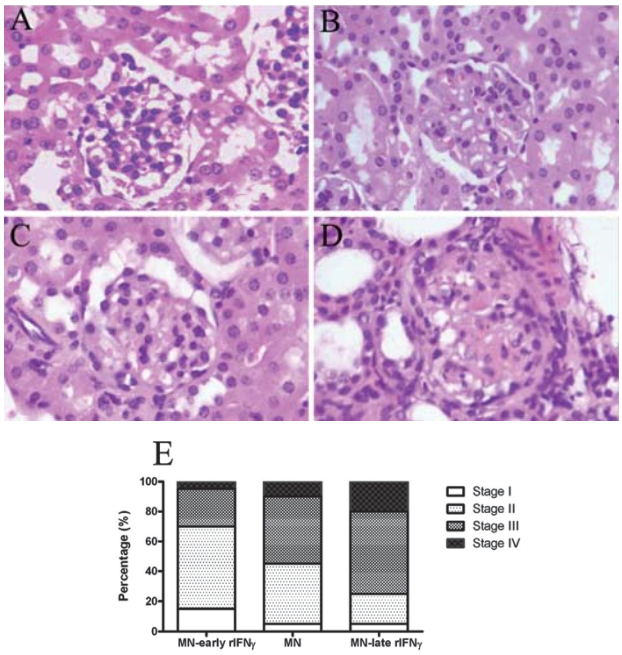

Fig. 2 Changes in histopathology of mice with experimental MN. Kidneys from mice of the NC group (A), MN group (B), MN group receiving early rIFN-γ treatment (C) and MN group receiving late rIFN-γ treatment (D) are shown with H&E staining. The pathological severity of the MN mice receiving early rIFN-γ treatment appeared to be milder than that of the MN mice and those receiving late rIFN-γ treatment mice (E). All images are at 400× magnification.

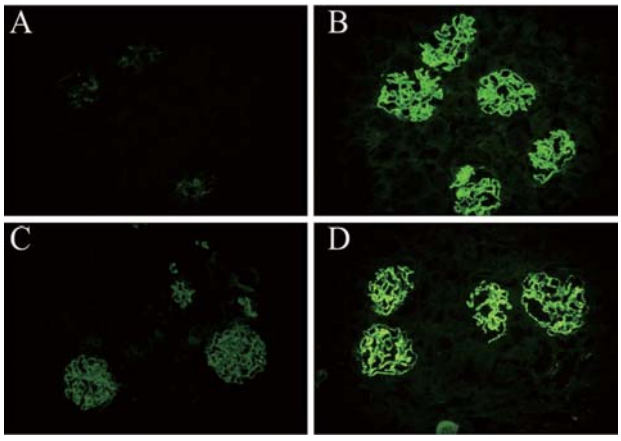

Fig. 3 Immunofluorescent staining for immunoglobulin G in experimental MN mice. Immunofluorescent staining for IgG in the kidneys of mice from the BC group (A), MN group (B), MN group receiving early rIFN-γ treatment (C), and MN group receiving late rIFN-γ treatment (D) are shown. All images are at 200× magnification

nuria, hypoalbuminemia and hypercholesterolaemia (Fig. 1). In MN mice that had received early rIFN-γ treatment, a marked attenuation of proteinuria was observed. However, no therapeutic effect was observed in MN mice that had received late rIFN-γ treatment. Furthermore, renal function deteriorated severely in MN mice receiving late rIFN-γ treatment, as evidenced by elevated serum creatinine level.

Effects of rIFN-γ treatments on histopathological findings

Pathological findings in MN mice included diffuse thickening of the glomerular basement membrane compared with that of the normal control group (Fig. 2A-E). The pathological severity of early rIFN-γ mice appeared to be milder than that of MN mice (Fig. 2C). However, MN mice receiving late rIFN-γ treatment demonstrated severe renal damage proved by much immune cell infiltration, glomerusclerosis and fibrosis (Fig. 2D). All experimental groups showed positive immunofluorescent staining for IgG, with a discrete beaded appearance along the glomerular capillary wall (Fig. 3A-D). The immunofluorescence intensity in early rIFN-γ mice was lower than that in MN mice (Fig. 3B and C) (Table 1). However, the immunofluorescence intensity increased in late rIFN-γ mice compared with that in MN mice (Fig. 3B and D). Immunofluorescent staining for C3 also presented as intense granular fluorescence along the glomerular capillary wall, with a pattern similar to that of IgG (Fig. 4A-D).

Effects of rIFN-γ treatments on ROS production in kidney cells

To detect more specifically and locally the ROS production in the kidney, we analyze *in situ* superoxide anion radical production using DHE assay in fresh-frozen sections of renal tissue. DHE fluorescence levels were at low levels in normal mouse kidneys (Fig. 5A). We obtained a significantly increased DHE fluorescence in MN kidneys, implicating in situ superoxide anion production (Fig. 5B). Compared with those in MN mice, lower DHE fluorescence levels were observed after MN mice were treated with early rIFN-γ (Fig. 5C). The administration of late rIFN-γ increased the DHE fluorescence levels (Fig. 5D), indicating exacerbated ROS production when compared with that in MN mice. Therefore, these results further demonstrated that early rIFN-γ treatment de-

Table 1. Changes of histological severity in kidneys from mice receiving rIFN treatment.

Severities ＼ Group	NC	MN	MN-early rIFN	MN-late rIFN
Total fluorescence score for IgG	16.15±7.68	247.50±33.82*	153.49±38.36*#	258.14±20.47*
Total fluorescence score for C3	12.32±9.37	237.15±23.37*	98.67±19.82*#	265.42±10.83*
DHE positive cells / fields	15.25±1.83	54.44±3.39*	32.18±4.77*#	109.18±13.0*#
NF-kB positive cells / fields	0.63±0.74	5.86±0.65*	3.00±0.76*#	8.00±1.07*#

NC: normal control, MN: membranous nephropathy, DHE: dihydroethidium, *$P < 0.05$ versus the NC group, #$P < 0.05$ versus the MN group

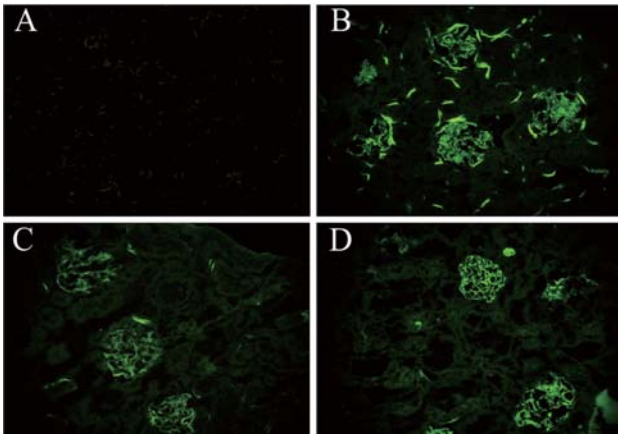

Fig. 4 Immunofluorescent staining for complement in experimental MN mice. Immunofluorescent staining for complement C3 in the kidneys of mice from the NC group (A), MN group (B), MN group receiving early rIFN-γ treatment (C), and MN group receiving late rIFN-γ treatment (D) are shown. All images are at 200× magnification.

creased while late rIFN-γ treatment increased superoxide production in kidney cells during MN.

Effects of rIFN-γ treatments on NF-κB-positive cells in kidneys

Activation of NF-κB is required for an optimal response to a variety of proinflammatory stimuli that result in inflammation. Therefore, we compared NF-κB activation in the glomeruli of MN mice that received early and late rIFN-γ treatment (Figure 6A-D). Kidneys from normal control mice showed little or no staining for activated NF-κB p65 (Figure 6A). In contrast, the numbers of positive NF-κB staining cells were marked in MN

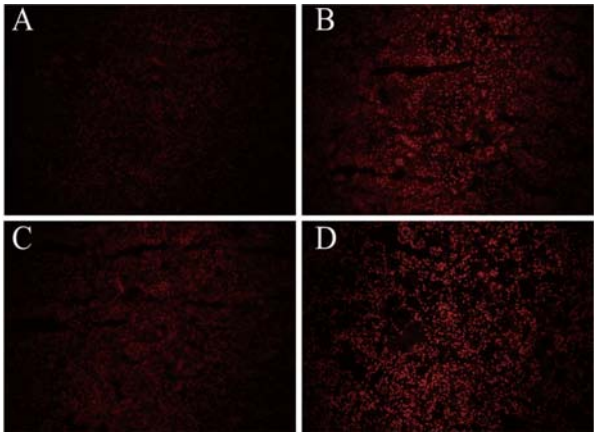

Fig. 5 Superoxide anion production in kidney cells. Fluorescence micrographs of DHE-positive cells in the kidneys of mice from the NC group (A), MN group (B), MN group receiving early rIFN-γ treatment (C), and MN group receiving late rIFN-γ treatment (D) are shown. All images are at 200× magnification.

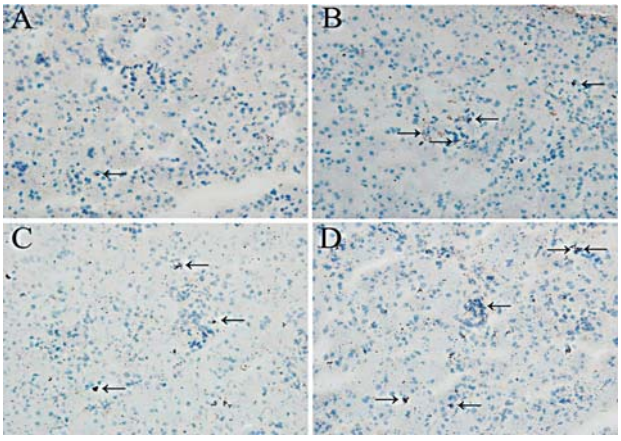

Fig. 6 NF-κB levels in kidneys cells. Kidneys from the NC group (A), MN group (B), MN group receiving early rIFN-γ treatment (C), and MN group receiving late rIFN-γ treatment (D) using IHC with phosphor-NF-κB. All images are at 200× magnification.

mice, indicating that the inflammatory response participates in Ig-mediated glomerular injury during MN (Figure 6B). Fewer positive NF-κB staining cells were observed after MN mice were treated with early rIFN-γ (Fig. 6C). The administration of late rIFN-γ increased the numbers of positive NF-κB staining cells in kidneys, indicating exacerbated ROS production when compared with MN mice (Fig. 6D). Therefore, these results further demonstrated that early rIFN-γ treatment decreased while late

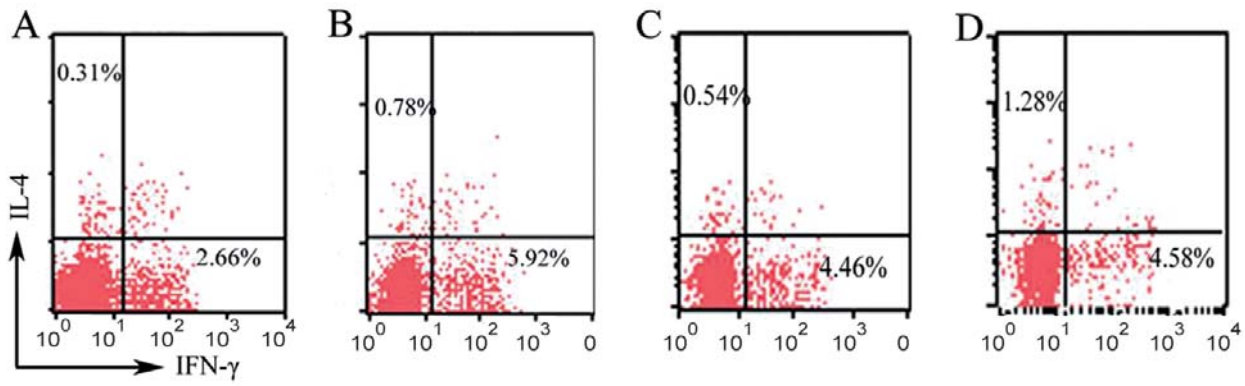

Fig. 7 Splenic lymphocytes of mice were stained with marker-specific antibodies for flow cytometric analysis: PE-conjugated anti-IFN-γ; FITC-conjugated anti-IL4; and APC-conjugated anti-mouse CD4. The proportions of Th1 ($CD4^+$ IFN-γ^+ T) cells and Th2 ($CD4^+IL4^+$ T) cells in the spleens of mice from the NC group (A), MN group (B), MN group receiving early rIFN-γ treatment (C), and MN group receiving late rIFN-γ treatment (D) are shown.

rIFN-γ treatment increased the inflammatory status in kidney cells.

Effects of rIFN-γ treatments on Th1/Th2 cell polarization by ICS

We analyzed the Th1 ($CD4^+$ IFN-γ^+ T) cells and Th2 ($CD4^+IL4^+$ T) cells in the spleens from NC, MN and MN mice treated with rIFN-γ (Fig. 7). Elevated populations of $CD4^+$ IFN-γ^+ and $CD4^+IL4^+$ T cells were found in MN mice. We found that early rIFN-γ therapy may decrease the Th2 immune response in the spleens to alleviate disease severity, while late rIFN-γ therapy may increase the Th2 immune response to exacerbate the inflammatory process of late MN, thus causing more severe disease.

DISCUSSION

This is the first study that found significant reduction of proteinuria, remarkable amelioration of glomerular lesions accompanied by decreased immune deposition, and complement activation in mice receiving rIFN-γ therapy in the early stage of MN. Mice receiving rIFN-γ therapy in the late stage revealed deterioration of metabolic and renal histopathological parameters. There were opposite effects of rIFN-γ therapy in different stages of MN. Immunomodulatory treatment using rIFN-γ in the early stage of MN shifts the Th1/Th2 immune response and may be considered as a potential therapeutic strategy for MN.

The central pathogenesis of MN involves the formation of subepithelial immune deposits and the subsequent production of glomerular injury through complement-dependent processes, oxidants, and cytokines, resulting in the development of massive proteinuria.[4,5,27,28] Early rIFN-γ treatment caused the effective attenuation of proteinuria and reduced significantly the glomerular immune deposits in mice with experimental MN. Furthermore, early rIFN-γ therapy also revealed effect of immunomodulatory Th2 response. A dramatic reduction in the generation of highly reactive ROS in early rIFN-γ-treated MN could be causally related to proteinuria in experimental MN, suggesting that oxidative stress plays a pathogenic role in damaging the glomerular filtration barrier during MN. Proinflammatory cascades, which can enhance the inflammatory response and induce the ROS production, also contribute to renal cell destruction.[5] Both the direct action of early rIFN-γ on systemic immunomodulatory effect, which decreases the Th2 humoral response and the concomitant reduction in immunodeposition, and local effects resulted from subsequent reduction in inflammation, complement activation, and oxidative stress in the MN glomeruli may have contributed to the attenuation of renal damage and proteinuria in MN.[29]

As compared with the finding that Th1-predominant nephritogenic immune responses are associated with severe proliferative and cresentic GN, Th2 immune responses are associated with membranous nephropathy.[13-15] During the MN process, there is a progressive increase in B cells combined with the production and deposition of IgG1 predominant anti-cBSA immunoglobulin during the early

phase of MN, indicating that the initial immune response involves primarily the humoral-mediated mechanism.[24] On the other hand, Rituximab, an anti-CD20 monoclonal antibody inhibiting B-cell differentiation and immunoglobulin secretion, has recently been employed to reduce proteinuria and prevent disease progression in patients with idiopathic MN, further confirming the pathogenic role of B cells in MN.[30-32] In our previous study, there is a significant increase in IL-4-producing $CD4^+$ T cells, but only a slight increase in IFN-γ-producing $CD4^+$ T cells in the early phase of MN mice[20]. In addition to assisting in antibody production, the T helper cell subsets also affect and direct cellular immune mechanisms in GN[9]. Interestingly, we also found a subsequent increase in Th1 expression in the late phase of MN. This increase in Th1 cells may play a role in the counter-regulation of the Th2 response, implying an association between the characteristics of the disease process and the kinetics of the Th1/Th2 responses during MN. Hence, the functional dichotomy between the Th1 and Th2 lymphocyte subsets play a regulatory role in the disease and both the humoral- and cell-mediated immune responses may participate in the pathogenesis of MN.

There was no prominent infiltration of immune cells, and only a small number of T-lymphocytes infiltrated into the MN kidney. Hence, the resident renal cells are the major source of several inflammatory cytokines in MN.[33-36] It is likely that earlier intervention may be more effective and has specific effects on MN pathogenesis. In particular, blocking initial Th2 humoral immune response and late immune complexes induced subsequent inflammatory process that might attenuate the severity of disease. In our study, early IFN-γ treatment decreased the pro-inflammatory status, while late IFN-γ exacerbated the inflammatory process. Apart from the regulation of Th1/Th2 immune response, IFN-γ also has effects of inflammation, complement activation, and oxidative stress, which have been proposed to be major pathogenic factors in MN. In addition, it was also found that IL-4 administration prior to the onset of protienuria, rather than the early rIL-4 treatment, prevented protienuria in HN, indicating the importance of cytotoxic T cell in mediating the final effector phase of glomerular injury in HN.[7,37] This may partially explain why early IFN-γ exerted a therapeutic effect in alleviating, while late IFN-γ exacerbated experimental MN in our study. There were opposite effects of rIFN-γ therapy in different stages of MN. If these issues are taken into account, there is substantial potential for the immunomodulatory treatment using anti-cytokine therapies for MN.

Cytokines play important roles in the pathogenesis of many diseases and several studies have identified many strategies using cytokines as possible targets for disease treatment. Suppressing Th2 responses by administrating cytokines in asthma have been tried though the outcomes are diverse. IL-12, a Th1 cytokine, could reduce blood and number of airway eosinophils but had no significant effects on asthmatic response in mild allergic asthmatics.[38] On the other hand, long-term IFN-α administration suppressed Th2 cytokine production and improved lung function, medication requirements, asthma symptoms and hospitalizations in severe asthmatics.[39,40] IFN is a glycoprotein synthesized by immune cells or fibroblasts, functioning to eradicate infection or malignant cells. Exogenous IFN has a number of therapeutic uses. The most commonly used agent is IFN-α, which is employed to treat hepatitis C and B viruses and various malignancies. IFN-β is utilized to treat multiple sclerosis, whereas IFN-γ has been used as a treatment for chronic granulomatous disease. We have also used rIFNγ as an immunomodulatory agent for the treatment of autoimmune MN. Chronic IFN therapy may complicate clinical renal diseases, which are associated with podocyte injury including minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS).[41,42] The mechanism of podocyte injury with IFN is not fully understood. Direct IFN effect on the podocyte altering cellular proliferation and increased podocyte oxidative capacity have been proposed.[42] Furthermore, IFN may enhance synthesis of pathogenic cytokines, such as IL-6 and -13, which are permeability factors in FSGS and MCD. The deterioration of renal damage in late IFN-r treatment may result from the direct side effects of IFN on kidneys. Although the therapeutic effects of early rIFNγ treatment have been demonstrated in murine MN, whether it can be applied to humans requires further investigation.

In conclusion, we have demonstrated that there were opposite effects of rIFN-γ therapy in different stages of MN. IFN-γ may participate in the MN process. Immunomodulatory treatment using rIFN-γ in the early stage of MN shifts the Th1/Th2 immune response and may be considered as a potential therapeutic strategy for MN in the future.

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DISCLOSURE

All authors declare no competing financial interests.

REFERENCES

1. Cattran DC. Idiopathic membranous glomerulonephritis. Kidney Int 2001;59:1983-1994.
2. Glassock RJ. Diagnosis and natural course of membranous nephropathy. Semin Nephrol 2003;23:324-332.
3. Cattran D. Management of membranous nephropathy: when and what for treatment. J Am Soc Nephrol 2005;16:1188-1194.
4. Cunningham PN, Quigg RJ. Contrasting roles of complement activation and its regulation in membranous nephropathy. J Am Soc Nephrol 2005;16:1214-1222.
5. Nangaku M, Shankland SJ, Couser WG. Cellular response to injury in membranous nephropathy. J Am Soc Nephrol 2005;16:1195-1204.
6. Penny MJ, Boyd RA, Hall BM. Role of T cells in the mediation of Heymann nephritis. ii. Identification of Th1 and cytotoxic cells in glomeruli. Kidney Int 1997;51:1059-1068.
7. Penny MJ, Boyd RA, Hall BM. Permanent CD8(+) T cell depletion prevents proteinuria in active Heymann nephritis. J Exp Med 1998;188:1775-1784.
8. Quiza CG, Leenaerts PL, Hall BM. The role of T cells in the mediation of glomerular injury in Heymann's nephritis in the rat. Int Immunol 1992;4:423-432.
9. Holdsworth SR, Kitching AR, Tipping PG. Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis. Kidney Int 1999;55:1198-1216.
10. Kelly CJ, Frishberg Y, Gold DP. An appraisal of T cell subsets and the potential for autoimmune injury. Kidney Int 1998;53:1574-1584.
11. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996;383:787-793.
12. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996;17:138-146.
13. Tipping PG, Kitching AR. Glomerulonephritis, Th1 and Th2: what's new? Clin Exp Immunol 2005;142:207-215.
14. Schena FP. Cytokine network and resident renal cells in glomerular diseases. Nephrol Dial Transplant 1999;14 Suppl 1:22-26.
15. Ring GH, Lakkis FG. T lymphocyte-derived cytokines in experimental glomerulonephritis: testing the Th1/Th2 hypothesis. Nephrol Dial Transplant 1998;13:1101-1103.
16. Oliveira DB. Membranous nephropathy: an IgG4-mediated disease. Lancet 1998;351:670-671.
17. Kuroki A, Iyoda M, Shibata T, Sugisaki T. Th2 cytokines increase and stimulate B cells to produce IgG4 in idiopathic membranous nephropathy. Kidney Int 2005;68:302-310.
18. Masutani K, Taniguchi M, Nakashima H, Yotsueda H, Kudoh Y, Tsuruya K, Tokumoto M, Fukuda K, Kanai H, Hirakata H, Iida M. Up-regulated interleukin-4 production by peripheral T-helper cells in idiopathic membranous nephropathy. Nephrol Dial Transplant 2004;19:580-586.
19. Hirayama K, Ebihara I, Yamamoto S, Kai H, Muro K, Yamagata K, Kobayashi M, Koyama A. Predominance of type-2 immune response in idiopathic membranous nephropathy. Cytoplasmic cytokine analysis. Nephron 2002;91:255-261.
20. Wu CC, Chen JS, Chen SJ, Lin SH, Chen A, Chang LC, Sytwu HK, Lin YF. Kinetics of adaptive immunity to cationic bovine serum albumin-induced membranous nephropathy. Kidney Int 2007;72:831-840.
21. Jefferson JA, Johnson RJ. Treatment of hepatitis C-associated glomerular disease. Semin Nephrol 2000;20:286-292.
22. Chen JS, Chen A, Chang LC, Chang WS, Lee HS, Lin SH, Lin YF. Mouse model of membranous nephropathy induced by cationic bovine serum albumin: antigen dose-response relations and strain differences. Nephrol Dial Transplant 2004;19:2721-2728.
23. Hayashi T, Ishida Y, Kimura A, Iwakura Y, Mukaida N, Kondo T. IFN-gamma protects cerulein-induced acute pancreatitis by repressing NF-kappa B activation. J Immunol 2007;178:7385-7394.
24. Wu CC, Chen JS, Lin SH, Chen A, Sytwu HK, Lin YF. Experimental model of membranous nephropathy in mice: sequence of histological and biochemical events. Lab Anim 2008;42:350-359.
25. Wu CC, Lu KC, Chen JS, Hsieh HY, Lin SH, Chu P, Wang JY, Sytwu HK, Lin YF. HO-1 induction ameliorates experimental murine membranous nephropathy: anti-oxidative, anti-apoptotic and immunomodulatory effects. Nephrol Dial Transplant 2008;23:3082-

3090.
26. Tsai PY, Ka SM, Chang JM, Chang WL, Huang YJ, Hung LM, Jheng HL, Wu RY, Chen A. Therapeutic potential of DCB-SLE1, an extract of a mixture of Chinese medicinal herbs, for severe lupus nephritis. Am J Physiol Renal Physiol 2011;301:F751-F764.
27. Kerjaschki D. Pathomechanisms and molecular basis of membranous glomerulopathy. Lancet 2004;364:1194-1196.
28. Ronco P, Debiec H. Molecular pathomechanisms of membranous nephropathy: from Heymann nephritis to alloimmunization. J Am Soc Nephrol 2005;16:1205-1213.
29. Wu CC, Lu KC, Lin YF, Chen JS, Huang CF, Chen CC, Lin SH, Chu P, Sytwu HK. Pathogenic role of effector cells and immunoglobulins in cationic bovine serum albumin-induced membranous nephropathy. J Clin Immunol 2012;32:138-149.
30. Cohen CD, Calvaresi N, Armelloni S, Schmid H, Henger A, Ott U, Rastaldi MP, Kretzler M. CD20-positive infiltrates in human membranous glomerulonephritis. J Nephrol 2005;18:328-333.
31. Remuzzi G, Chiurchiu C, Abbate M, Brusegan V, Bontempelli M, Ruggenenti P. Rituximab for idiopathic membranous nephropathy. Lancet 2002;360:923-924.
32. Ruggenenti P, Chiurchiu C, Brusegan V, Abbate M, Perna A, Filippi C, Remuzzi G. Rituximab in idiopathic membranous nephropathy: a one-year prospective study. J Am Soc Nephrol 2003;14:1851-1857.
33. Lakkis FG, Baddoura FK, Cruet EN, Parekh KR, Fukunaga M, Munger KA. Anti-inflammatory lymphokine mRNA expression in antibody-induced glomerulonephritis. Kidney Int 1996;49:117-126.
34. Niemir ZI, Ondracek M, Dworacki G, Stein H, Waldherr R, Ritz E, Otto HF. In situ upregulation of IL-10 reflects the activity of human glomerulonephritides. Am J Kidney Dis 1998;32:80-92.
35. Niemir ZI, Stein H, Dworacki G, Mundel P, Koehl N, Koch B, Autschbach F, Andrassy K, Ritz E, Waldherr R, Otto HF. Podocytes are the major source of IL-1 alpha and IL-1 beta in human glomerulonephritides. Kidney Int 1997;52:393-403.
36. Tesch GH, Yang N, Yu H, Lan HY, Foti R, Chadban SJ, Atkins RC, Nikolic-Paterson DJ. Intrinsic renal cells are the major source of interleukin-1 beta synthesis in normal and diseased rat kidney. Nephrol Dial Transplant 1997;12:1109-1115.
37. Spicer ST, Ha H, Boyd RA, He XY, Carter N, Tran G, Penny MJ, Hodgkinson SJ, Hall BM. Il-4 therapy prevents the development of proteinuria in active Heymann nephritis by inhibition of Tc1 cells. J Immunol 2001;167:3725-3733.
38. Bryan SA, O'Connor BJ, Matti S, Leckie MJ, Kanabar V, Khan J, Warrington SJ, Renzetti L, Rames A, Bock JA, Boyce MJ, Hansel TT, Holgate ST, Barnes PJ. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000;356:2149-2153.
39. Kroegel C, Bergmann N, Foerster M. Workalemahu G, Machnik A, Mock B, Reissig A. Interferon-alpha-con-1 treatment of three patients with severe glucocorticoid-dependent asthma. Effect on disease control and systemic glucocorticosteroid dose. Respiration 2006;73:566-570.
40. Simon HU, Seelbach H, Ehmann R, Schmitz M. Clinical and immunological effects of low-dose IFN-alpha treatment in patients with corticosteroid-resistant asthma. Allergy 2003;58:1250-1255.
41. Colovic M, Jurisic V, Jankovic G, Jovanovic D, Nikolic LJ, Dimitrijevic J. Interferon alpha sensitisation induced fatal renal insufficiency in a patient with chronic myeloid leukaemia: case report and review of literatu re. J Clin Pathol 2006;59:879-881.
42. Markowitz GS, Nasr SH, Stokes MB, D'Agati VD. Treatment with IFN-{alpha}, -{beta}, or -{gamma} is associated with collapsing focal segmental glomerulosclerosis. Clin J Am Soc Nephrol 2010;5:607-615.