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以小鼠模式研究 TH1 與 TH2 型腎絲球腎炎之致病機轉與治療

***Key words*: Glomerulonephritis; lipopolysaccharde; interleukin-4; interleukin-12; ICR mice;**

Abstract

Renal injury in glomerulonephritis (GN) is characterized by injurious immune responses to self or foreign antigen. These create often an unrecovered renal injury in human. Therefore, many GN-prone and lipopolysaccharde (LPS)-induced mice model were established for the research of GN. The different mice model showed also the different effect of Th cell subsets. The induction of a chronic state of polyclonal activation of lymphocytes by LPS triggers a lupus-like mesangial proliferative GN. According the reported data, however, it normally took at least 2 or more weeks to induce the glomerular disease, which showed predominantly mesangial and/or endothelial cell proliferation. In our data, we found that consecutive 7 daily i.v. low doses of LPS induced acute proliferative and exudative glomerular lesions in ICR mice. These mice also showed intermittent hematuria and slight proteinuria. This study investigated the pathogenesis of acute glomerular damage and evaluated the effect of IL-4 and IL-12 expressing plasmid on this type of glomerular injury in ICR mice. The results of preventive and post treatment showed potential therapeutic effect, But we still need further study to confirm the conclusion.

INTRODUCTION

Experimental studies have emphasized the potential pathogenic role of polyclonal activation of lymphocytes (PAL) in the development of immune-mediated nephritis [Lambert *et al.,* 1980; Goldman *et al.,* 1988] PAL is a common feature of MRL/1pr, NZB/W and DXSB mouse lupus [Izui *et al.,* 1978; Theofilopoulos *et al.,* 1985]. The induction of a chronic state of PAL by lipopolysaccharide (LPS) triggers a lupus-type glomerulonephritis [Fournie *et al.,* 1980; Ramos-Niembro *et al.,* 1982]. In outbred mice, the spontaneous development of immune complex-type glomerular lesions is dependent on environmental factors that can induce PAL [Lule *et al.,* 1989]. In man, acute and chronic nephritis are often associated with granular deposits of immunoglobulin and complement in glomeruli, that are thought to represent immune complexes. The development and maintenance of cognate immune responses generally involve the activation of T lymphocytes, which direct antigen-specific, cell-mediated effector mechanisms and promote antibody production and

antibody-mediated effector mechanisms [Holdsworth *et al.,* 1999].

The available evidence suggests that some types of human glomerulonephritis, including crescentic glomerulonephritis and membranoproliferative glomerulonephritis, are directed by Th1-predominant nephritogenic immune responses. The strong evidences come from the renal biopsy demonstration [Pankewycz *et al.,* 1994; Rosen *et al.,* 1994; Niemir *et al.,* 1998] and patients treated with IFN-ã for rheumatoid arthritis developed SLE with proliferative and/or crescentic GN [Graninger *et al.,* 1991; Machold and Smolen, 1990]. On the other hand, some reports have show that IgG4 (Th2-type subclass antibody) predominates in renal biopsies. This patterns observed in both idiopathic- and lupus-associated membranous GN [Haas, 1994; Roberts *et al.,* 1983; Iskandar *et al.,* 1992; Imai *et al.,* 1997]. These data would suggest that the initiating glomerular antigen in membranous GN initiate Th2-type predominantly humoral immune responses in membranous nephropathy.

Materials and Methods

Animals

Experiments are performed on female six-week-old ICR mice weighing 20 - 25 g (purchased from the National Laboratory Animal Breeding and Research Center, Taipei, Taiwan, ROC).

therapeutic agents

The eukarytic expressing plasmid was used for IL-12 or IL-4 gene therapy in LPS-induced glomerulonephritis mouse model.

Doses of the employed compounds

IL-12 expressing plasmid: 20-100 μ g, im

IL-4 expressing plasmid: 20-100 μ g, im

Experimental protocol

1. Establishment of mouse model of LPS-induced glomerulonephritis:

   The mice 12/group) are daily injected i.v. with 2 mg/Kg b.w. of LPS (*E. coli,* 0127:B8) for consective 6 doses. These mice are then sacrificed one day after the last injection and subjected to clinical, laboratory and pathological studies to characterize their glomerular lesions.

2. Evaluation of preventive and post-treatment effects of IL-12-plasmid, IL-4-plasmid on this mouse model:

   Each group of 12 mice are subjeced to the following experiments:

gp 1. Saline control

gp 2. LPS + IL-4 plasmid (given 7days before 1st LPS injection).

gp 3. LPS + IL-12 plasmid (given 7days before 1st LPS injection).
gp 4. LPS + IL-4 plasmid (given 24 h after 1st LPS injection).
gp 5. LPS + IL-12 plasmid (given 24 h after 1st LPS injection).
gp 6.. LPS

Evaluation of Proteinuria and Hematuria

Proteinuria is measured as the ratio of urinary albumin (μg/ml) to creatinine (Cr) (mg/dl) in urine samples collected daily as previously described [Ginsberg et al, 1983; Montinaro et al, 1992]. Urinary albumin is quantitatively determined in an enzyme-linked immunosorbent assay, for which purified mouse albumin (Cappel, Organon Teknika, Durham, North Carolina) at concentrations ranging 5 - 50 μg/ml is used as a reference standard. Urine samples are also examined for hematuria by means of a Hema-Combistix strip (Ames Division, Miles Laboratories, Inc., Elkhart, Indiana) according to the manufacturer's instructions. Grading is done from 0 to 3+.

Results

Consecutive 7 daily iv doses of LPS induced acute proliferative and exudative glomerular lesions in ICR mice. These mice also showed intermittent hematuria and slight proteinuria. Under the light microscopy, the glomerulus showed an acute proliferative change in the tufts. In the dip-stick test, all of the urine samples showed 3-4 positive in occult blood test.

In the LPS-induced lupus-like syndrome in C57B2/6J mice treated with either IL-4 or IL-12 expression plasmid. The results of urine albumin. Creatinine and protein/creatinine ratio showed in the following tables and figures.

Tab. 1: Albumin (mg/L) of urine in the mouse model treated with IL-4 and IL-10 before and after injection of LPS

Day	GP1	GP2	GP3	GP4	Gp5	GP6
0	250	245	200	400	135	800
6	1100	500	498	1150	250	750
11	750	600	500	600	980	1250
15	745	1500	1600	1250	1250	2400
22	800	600	1000	900	750	1450
28	750	1000	1200	900	1350	1600

Tab.2: Creatinine (mg/dL) of urine in the mouse model treated with IL-4 and IL-10 before and after injection of LPS

Day	GP1	GP2	GP3	GP4	Gp5	GP6
0	2	1.9	1.8	1.9	3.9	1.5
6	2	1.2	1	1.4	1.8	0.9
11	1.4	2.8	1	2.2	2.8	2
15	7.5	3	5	5.6	3	4.4
22	2.1	0.7	2.9	4	4	1.8
28	1.1	2	3	3	2	2.2

Tab. 3: Protein/Creatinine ratio of urine in the mouse model treated with IL-4 and IL-10 before and after injection of LPS

Day	GP1	GP2	GP3	GP4	Gp5	GP6
0	125	129	111	211	35	533
6	550	417	498	821	139	833
11	536	214	500	273	350	625
15	99	500	320	223	417	545
22	381	857	345	225	188	806
28	682	500	400	300	675	727

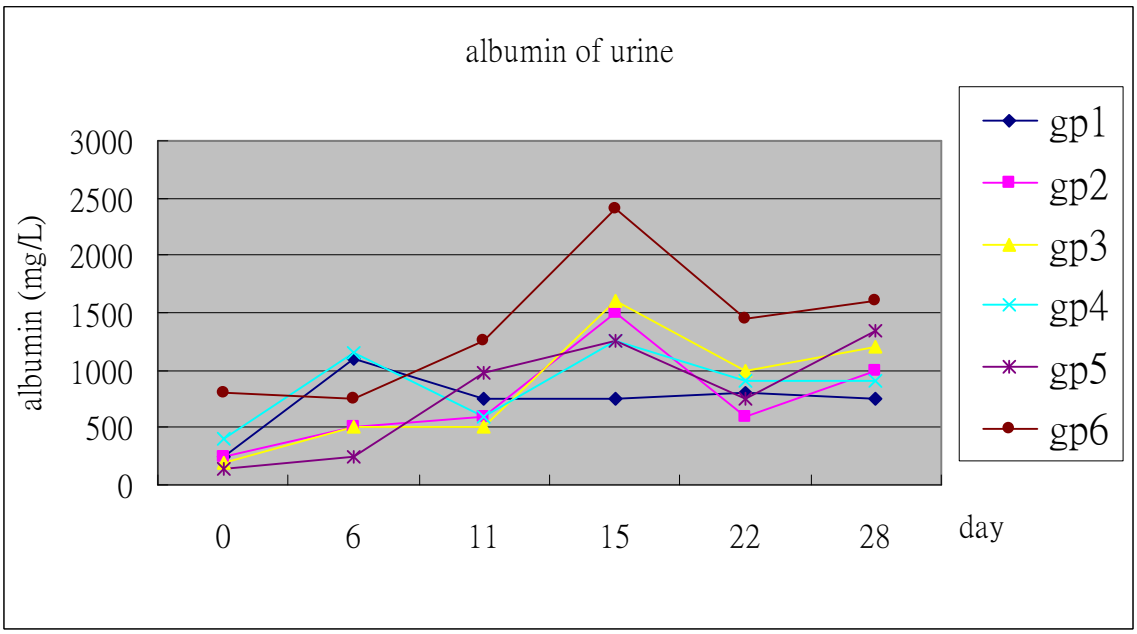

Fig. 1 Albumin (mg/L) of urine in the mouse model treated with IL-4 and IL-10 before and after injection of LPS

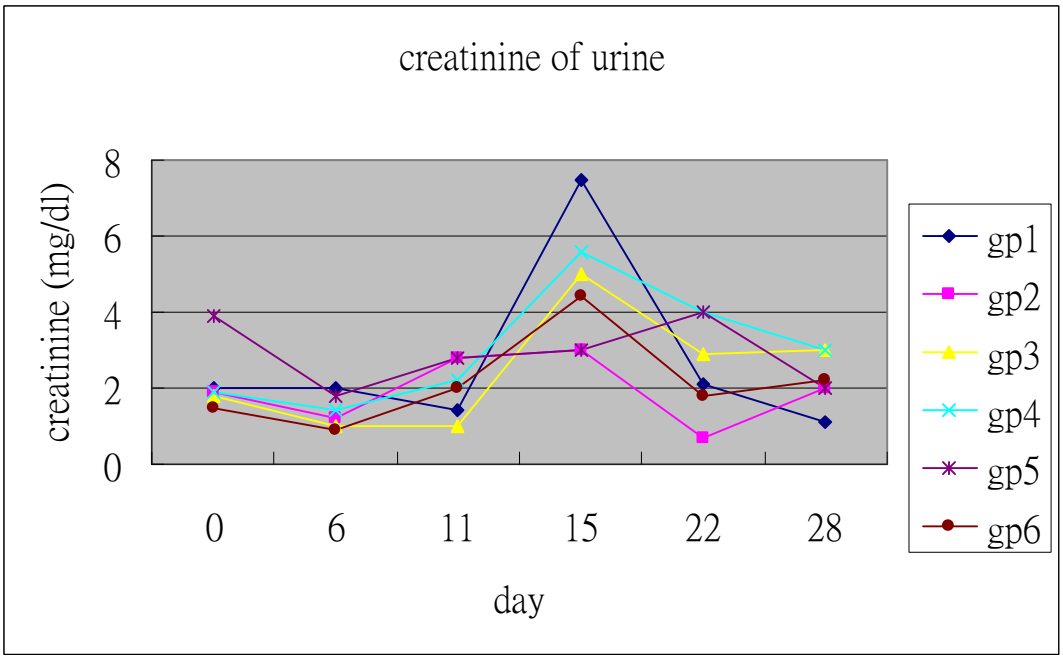

Fig 2: Creatinine (mg/dL) of urine in the mouse model treated with IL-4 and IL-10 before and after injection of LPS

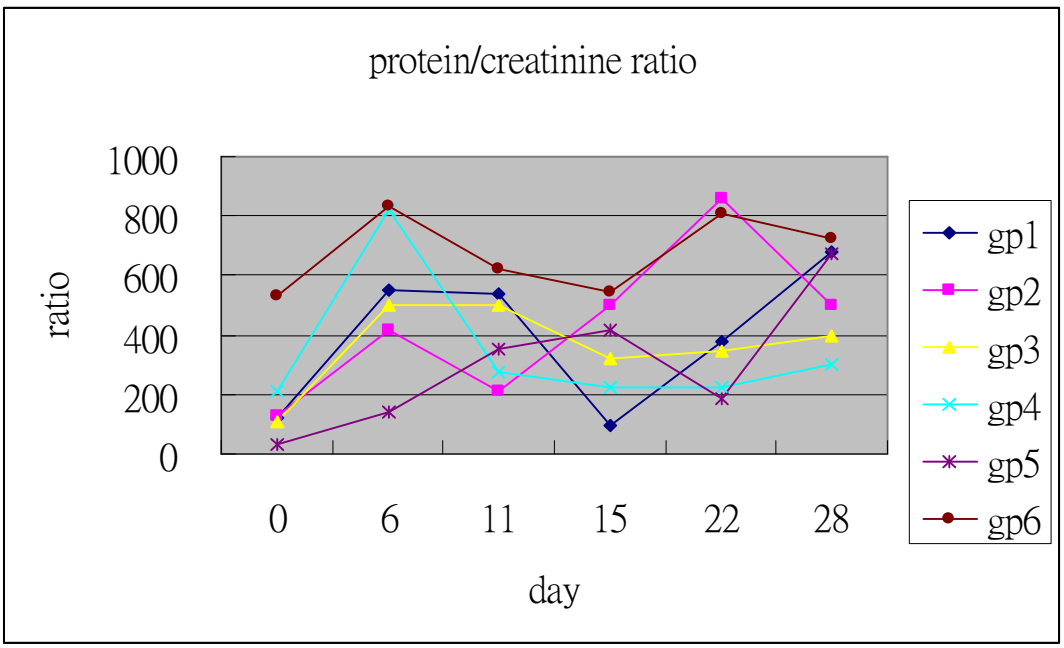

Fig 3: Protein/Creatinine ratio of urine in the mouse model treated with IL-4 and IL-10 before and after injection of LPS

Discussion

In our data, we found that consecutive 7 daily iv doses of LPS induced acute

proliferative and exudative glomerular lesions in ICR mice. These mice also showed intermittent hematuria and slight proteinuria. It's generally accepted that glomerulonephritis results from cognate immune responses. Glomerulonephritis exhibits a variety of histopathological subtypes with different outcomes. These subtypes have variable deposition and accumulation of Th1 and Th2 immune effectors.

We also have treated LPS-induced lupus-like syndrome in C57B2/6J mice with either IL-4 or IL-12 expression plasmid. Our results demonstrated that mice injected with IL-4 plasmid before or after injection of LPS seems to express lower protein/creatinine ratio in urine than mice injected with IL-12 plasmid. Therefore, IL-12 and IL-4 may play the pivotal roles for variable Th1/Th2 predominance in immune responses leading to glomerulonephritis.

Th1 cell is characterized by the production of interferon-γ (IFN-γ), interleukin (IL)-2, and lymphotoxin-2 [tumor necrosis factor β (TNF β)]. Th1 cell responses induce macrophage and cytotoxic T-lymphocyte activation and immunoglobulin IgG subclass switching to favor complement fixation and opsonization.

Th2 cells secrete interleukin (IL)-4, IL-5, and IL-10, are important in allergy, mast cell /IgE-mediated immediate type hypersensitivity responses. In addition, cytokines produced by Th2 cells act also as regulators of the immune response. IL-4. IL-13 and IL-10 regulated Th1 responses, suppress delayed type hypersensitivity (DTH), and have inhibitory effects on macrophage, especially in the context of the activation by Th1 cytokines such as TNF-γ. [Bogdan *et al.,* 1991; Fiorentino *et al.,* 1991; Bogdan and Nathan, 1993; Zurawski and De Vries, 1994]. Th2 can promote the production of high levels of antibody, because IL-4 can stimulate B-cell growth.

IL-12 stimulates both NK and T cells and is particularly potent in its ability to induce gamma interferon (IFN-γ) production. These biological activities led to the suggestion that IL-12 may play a critical role in the development and determination of effector cell functions. Indeed, IL-12 can also induce Th1-cell differentiation and inhibit development of Th2 cells. [Schopf, 1999]

Increased expression of IL-4 mRNA in the thymus and spleen has been reported during the early stages of disease in MRI*/lpr* mice [Tsai *et al.,* 1995]. Mitogen-stimulated T cells from NEB/W mice produce higher levels of Th2 cytokines (IL-4 and IL-10) and lower levels of Th1 cytokines (IFN-γ and IL-2) than C57BL/6 mice [Lin *et al.,* 1995]. These data suggest that Th2 immune responses involve in lupus-like syndromes in NEB/W mice model. But there are a number of studies demonstrated the involvement of Th1 immune responses in lupus-like syndromes in another mice model. The ratio of IFN-γ to IL-4 secreting cells in MRL/*lpr* mice is increased in comparison with MRL+/+mice [Shirai *et al.,* 1995]. Moreover IL-12

levels are increased in sera of MRL/*lpr* mice [Huang *et al.,* 1996]. Il-12 also directed severe renal injury, crescent formation and Th 1 responses in glomerulonephritis of C57BL/6 and BALB/c mice models [Kitching *et al.,* 1999]. These observations show Th1/Th2 ratio was increased in the most lupus-like syndromes mice model.

The pattern of cytokines expression in lupus mice does not clearly identify a predominant role for either Th1 or Th2 subsets, therefore IgG3 was developed as another index to distinguish the tendency of Th1/Th2 predominant type in MLR/lpr mice. IgG3 is a Th1-type IgG subclass [Takahashi *et al.,* 1996; Lemoine *et al.,* 1992; Reininger *et al.,* 1990]. IgG3 monoclonal antibodies with cryoglobulin activity derived from lupus-prone mice induce 〝wire loop〞 lesions in glomeruli [Lemoine *et al.,* 1992] and are nephritogenic independent of their capacity to from immune complexes [Lemoine *et al.,* 1992; Reininger *et al.,* 1990]

This study evaluated the effects of cytokine- (IL-4 and IL-12) plasmids and their antagonists (anti-IL-4 mAb or anti- IL-12 mAb) administed in the therapy of a LPS-induced acute glomerulonephritis in ICR and KO mice model. Data in this research may suggest a protocol for therapeutic studies of acute proliferative glomerulonephritis, and contribute in sight to the understanding of the pathogenetic mechanism of proliferative glomerulonephritis. Compared with other mice model, ICR strain is outbred and does not have the congenital problems happening as in inbred GN-prone strains. Therefore, the responses and results of this model may be more significant for the therapy of Th1-type glomerulonephritis in human.

The results of preventive and post treatment showed potential therapeutic effect, but we still need further study to confirm the conclusion.

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