Therapeutic Effect of Combined Treatment with Monoclonal Antibodies against Intercellular Adhesion Molecule 1 and Lymphocyte-Function-Associated Antigen 1 in Masugi Nephritis of WKY Rats

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Dear Sir,

In our previous study [1], administration of a dose of nephrotoxic serum (NTS) sub-nephritogenic to other strains was found to induce severe proliferative and necrotizing glomerulonephritis with crescent formation and glomerular accumulation of CD8-positive cells and monocytes/macrophages in WKY rats. Depletion of CD8-positive cells by treatment with anti-CD8 monoclonal antibody (mAb) prevented the NTS-induced proteinuria and glomerular lesions [1]. In this model, upregulated expression of intercellular adhesion molecule 1 (ICAM-1) was observed exclusively in the glomerular endothelial cells in association with the influx of CD8-positive cells and monocytes/macrophages on and after day 1 [2]. These cells expressed lymphocyte-function-associated antigen 1 (LFA-1). Repeated prophylactic injection of mAb against rat ICAM-1 or LFA-1 dose-dependently prevented the development of proteinuria, the influx of these cells and subsequent crescent formation in this NTS nephritis model [2]. In addition, simultaneous administration of both anti-ICAM-1 and anti-LFA-1 mAbs exerted additive protective effects against urinary protein excretion and crescentic glomerulonephritis [2]. However, the role of these adhesion molecule interactions after the onset of glomerulonephritis has not been examined. For therapeutic purposes, further information on the blocking action of mAbs on these adhesion molecules after the induction of disease is necessary, since clinically, in human glomerulonephritis, medical treatment is initiated only after disease onset. In the present study, the involvement of ICAM-1 and LFA-1 in the pathogenesis of glomerulonephritis in this model was investigated. For this, we examined not only the prophylactic effect but also the therapeutic effect of in vivo administration of mAbs directed against ICAM-1 and LFA-1. Thirty WKY rats were divided into five groups of 6 animals. These groups were treated with NTS plus both anti-ICAM-1 mAb, 1A29 [2] (1 mg/kg body weight) and anti-LFA-1 mAb, WT.1 [2] (1 mg/kg body weight) from days -1 to 13 (group I), from days 1 to 13 (group II), from days 3 to 13 (group III) and from days 9 to 13 (group IV); control rats (group V) were

treated with NTS plus normal mouse immunoglobulins (2 mg/kg body weight) from days -1 to 13. NTS was given intravenously at a dose of 0.25 ml/kg body weight on day 0, this optimal dose having been predetermined as described previously [1]. The administration doses for the mAbs were determined in accordance with the previous study [2]; anti-ICAM-1 and anti-LFA-1 mAbs were each given at a dose of 1.0 mg/kg body weight i.p. on alternate days from day -1 (or 1, 3, 9) to day 13. Under ether anesthesia, uninephrectomy was performed on day 3, and all the rats were killed on day 14. The amount of protein excreted into urine every 24 h was determined using a protein assay kit. At the time of uninephrectomy and sacrifice, each kidney specimen was divided into two parts; one part was for light-microscopic examination and the other part for an immunofluorescence study. The number of crescentic glomeruli per 100-150 glomeruli in each rat was examined by light microscopy, and the incidence of crescent formation was expressed as a percentage (mean $\pm$ 1 SD). Cryostat sections were stained with mAb MRC-OX8 (PHLS Centre for Applied Microbiology & Research, Wiltshire, UK) against the rat CD8 molecule, mAb ED1 (Dainippon Seiyaku Co., Japan) against rat monocytes/macrophages and mAb WT. 1 in combination with FITC-conjugated rabbit antimouse IgG, containing 2% normal rat serum, as a second antibody. After a single intravenous injection of antiserum against the glomerular basement membrane, proteinuria exceeding 150 mg/day was observed on day 8 and thereafter in control rats (group V; fig. 1). In rats treated with both anti-ICAM-1 and anti-LFA-1 mAbs from days -1 to 13 (group I), urinary protein excretion was completely prevented. In groups II and III, proteinuria was suppressed to 13 and 33% on day 8, and to 11 and 4% on day 14, respectively, in comparison with the levels in group V (fig. 1). Interestingly, treatment with the mAbs from days 9 to 13 showed a tendency to decrease proteinuria on days 11 and 14. Thus, the treatment with both anti-ICAM-1 and anti-LFA-1 mAbs was efficient in preventing and reducing proteinuria. In group V, glomerular

Control
11 13 15
40 60
Crescent (%)
100

Fig. 1. Urinary protein excretion in mAb-treated WKY rats with NTS nephritis. Urinary protein excretion in WKY rats treated with NTS plus anti-ICAM-1 and anti-LFA-1 is shown. The curves represent: treatment with mAbs from days -1 to 13 (•), from days 1 to 13 (○), from days 3 to 13 (■) and from days 9 to 13 (□), and treatment with NTS plus normal mouse immunoglobulin (A, control), respectively. Data are mean values $\pm$ 1 SD for 6 rats. * $p < 0.05$, * $p < 0.01$, significantly different from control group (Student's t test).

Fig. 2. Quantitative study of crescent formation in kidney sections. The data are expressed as percentage of crescentic glomeruli. NTS plus anti-ICAM-1 (1A29) and anti-LFA-1 (WT.1) treatment produced a significant time-dependent reduction of crescent formation. The bars show treatment with mAbs from days -1 to 13 (group I), from days 1 to 13 (group II), from days 3 to 13 (group III) and from days 9 to 13 (group IV), and treatment with NTS plus

normal mouse immunoglobulin (group V; control). * $p < 0.01$, ** $p < 0.001$, significantly different from the NTS-treated control group (Student's *t* test).

hypercellularity was observed in the animals with nephritis on day 3, and severe necrotizing lesions and crescent formation were evident on day 14. These lesions were ameliorated by treatment with both anti-ICAM-1 and anti-LFA-1 mAbs, depending on the duration of the mAb treatment. A quantitative study revealed that the frequency of crescentic glomeruli on day 14 was markedly decreased in group I (10.4 ± 7.4 vs. $96.0 \pm 1.1\%$ in group V; $p < 0.001$). In groups II, III and IV, the number of crescentic glomeruli was reduced according to the duration of treatment with the mAbs (fig. 2). Immuno-fluorescence microscopy revealed rabbit IgG along the capillary walls in a linear pattern in all groups throughout the experimental period. No changes in the intensity of deposited rabbit IgG were observed in either group. The numbers of CD8-positive cells, ED1-positive monocytes/macrophages and LFA-1-positive cells in glomerular cross-sections were prominently increased 3 days after NTS injection in group V. However, very few CD8-positive cells, ED1-positive monocytes/macrophages and LFA-1-positive cells were detected in the glomeruli of groups I, II and III. In group IV, a lower but significant number of ED1-positive monocytes/macrophages had infiltrated into the glomeruli on days 3 and 14.

Therapeutic administration of mAbs directed against ICAM-1 and LFA-1 effectively blocked these mechanisms and prevented the development of glomerulonephritis, according to the duration of the mAb treatment. Therefore, these mAbs have a beneficial effect on rat crescentic nephritis, even when the treatment is started 1 or 3 days after induction of the disease. Interestingly, prominent proteinuria was observed on day 8, and the mAb treatment decreased the proteinuria in group IV on days 11 and 14. This means that the treatment with mAbs directed against LFA-1 and ICAM-1 blocked the interaction of inflammatory cells that had infiltrated successively into the glomeruli. The number of crescentic glomeruli in group IV was lower than that in group V, but nevertheless still high. These data show that once irreversible change has occurred, recovery may not take place readily. Furthermore, the mechanism of proteinuria may be different from that of crescent formation. The present findings suggest that the LFA-1/ICAM-1 pathway plays a role mainly in increasing the permeability of the glomerular capillary walls to serum protein, rather than in the proliferation of epithelial cells in Bowman's capsule.

In conclusion, both anti-LFA-1 and anti-ICAM-1 mAbs interrupt the binding of infiltrating cells to endothelial cells in the glomeruli and the subsequent glomerular injury. This approach, whereby inflammatory cell function can be altered by interference with the intercellular adhesion pathway, may provide an important therapeutic tool for control of human glomerulonephritis.

References

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102

Kawasaki/Yaoita/Yamamoto/Kihara

Therapeutic Effect of Anti-ICAM-1 and Anti-LFA-1 Antibodies