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# Mesangial sclerotic change with persistent proteinuria in rats after two consecutive injections of monoclonal antibody 1-22-3

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## SUMMARY

Irreversible mesangial changes with persistent proteinuria were induced in rats given two consecutive injections 2 weeks apart of a MoAb 1-22-3 to rat mesangial cell. The characteristics of the resulting lesions were investigated and compared with those of the reversible change induced by a single injection. At 24 h after the second injection, mesangiolytic changes similar to those after a single injection were evident. The accumulation of macrophage-like cells in glomeruli observed at 1 week after the first injection was not evident during the experimental period after the second injection. Hypercellularity with the characteristics of intrinsic mesangial cell and increased mesangial matrix were already present 1 week after the second injection. And mesangial sclerotic change progressed up to 6 months. Deposition of collagen type I and type III and accumulation of collagen fibril at the ultrastructural level were evident in rats 6 months after the second injection. Proteinuria started immediately and continued for more than 6 months after the second injection. The mesangial sclerotic change with persistent proteinuria described here is considered to be a better model for investigating the mechanism of chronic progression of human mesangial proliferative glomerulonephritis.

**Keywords** MoAb proteinuria mesangial sclerosis macrophage

## INTRODUCTION

We have already reported a MoAb 1-22-3 (IgG3) [1], which was produced in mice immunized with rat glomeruli. The MoAb 1-22-3-recognized epitope may be present in the Thy 1.1 molecule, since the reactivity of the MoAb toward thymus, brain and intestine in addition to the kidney was observed and the MoAb was observed to bind a major band with an apparent mol. wt of about 25 kD. MoAb 1-22-3 is capable of inducing transient proteinuria as well as morphological changes similar to those induced by anti-thymocyte serum (ATS) [2-4]. MoAb 1-22-3 binds to the limited area of the mesangial cell surface that faces endothelial cells, and no MoAb 1-22-3 reactivity is detected on endothelial cells, epithelial cells or the glomerular basement membrane (GBM). Since MoAb ER4 reported by Bagchus *et al.* [5] does react with GBM, the antigenic determinant of MoAb 1-22-3 is thought to be a new epitope. Thus MoAb 1-22-3 is the first MoAb reported to show reactivity with the mesangial cell surface alone, resulting in significant proteinuria [1].

The mesangial lesion induced by ATS is reversible and not always accompanied by remarkable proteinuria. The model induced by the single injection of MoAb 1-22-3 is also reversible. Thus, no satisfactory experimental model to investigate the mechanism of chronic progression of human mesangial proliferative glomerulonephritis has yet been established.

We succeeded in inducing irreversible mesangial changes with persistent proteinuria by a second injection of MoAb 1-22-3 at 2 weeks after the first injection. In this study we describe the characteristics of this novel model and compare it with the reversible model induced by a single injection.

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## MATERIALS AND METHODS

All experiments were performed using female Wistar rats weighing 150–200 g and purchased from Charles River Japan Inc. (Atsugi, Japan).

*Preparation of MoAb*

MoAbs were prepared as described previously [1,6,7]. TS-11m used as a control is a murine IgG3 MoAb against rotavirus and is not reactive toward rat kidney [1].

*Experimental design*

*Experiment 1.* Eight rats were intravenously injected with 1·0 ml of saline containing 500 $\mu$g of MoAb 1-22-3 twice with an interval of 2 weeks. As a control, five rats were treated similarly with TS-11m. Urinary protein from these rats was measured

every week for 6 months by the biuret method using bovine serum albumin (BSA) as a standard [8]. Qualitative analysis of urinary protein was conducted by SDS–PAGE as described by Laemmli [9]. The rats were killed at 6 months (27 weeks) after the second injection. Blood samples and kidneys were obtained from each rat. The kidney materials were examined by light microscopy (LM). To quantify the mesangial matrix, 30 full-sized glomeruli (80–100 $\mu$m in diameter) per rat were examined by an observer who was unaware of the experimental protocol. A semiquantitative scoring system was developed to evaluate the degree of damage. The degree of glomerular matrix expansion was expressed as 0 to 4 according to the percentage of each glomerulus occupied by mesangial matrix, using a method described by Raij *et al.* [10]. This matrix score was indicated as the mean value of the degree of damage. Serum creatinine and blood urea nitrogen (BUN) were measured.

*Experiment 2.* Female Wistar rats were divided into two groups and given an i.v. injection of 1·0 ml of saline containing 500 $\mu$g of MoAb 1-22-3 once (group A) or twice with an interval of 2 weeks (group B). Rat kidneys were studied by killing groups of three rats at 30 min, 2 h and 24 h, 1, 2 and 6 weeks and 6 months after the last injection. The kidney material from each rat was examined by LM, electron microscopy (EM) and direct immunofluorescence (IF) using FITC-conjugated anti-mouse immunoglobulin (Dakopatts a/s, Glostrup, Denmark), FITC-conjugated anti-rat IgG (Nordic, Tilburk, The Netherlands) or FITC-conjugated anti-rat C3 (Nordic). The matrix score for these rats was determined in a similar way to experiment 1. The reactivity of anti-collagen type I antibody (Advance, Tokyo, Japan) and anti-collagen type III antibody (Chemicon International, Los Angeles, CA) with the kidney sections was also examined. To identify the nature of the accumulated cells, the kidney sections were incubated with anti-monocyte MoAb, ED-1 (MAB 1435) (Chemicon). ED-1 was reported to have the reactivity toward monocyte and most macrophages [11] and was confirmed not to be reactive toward mesangial cell in normal glomeruli. Numbers of MoAb-positive cells per full-sized glomerulus were counted in 50 glomeruli/rat from two rats using indirect IF sections stained with FITC-conjugated anti-mouse IgG1 (Zymed, CA). The secondary antibody (anti-mouse IgG1) used in this study was confirmed to have no cross-reactivity with IgG3.

*Morphological and immunohistological studies*

LM, EM and IF were performed as described previously [1].

*Statistics analysis*

The results are expressed as the mean $\pm$ 1 s.d. Data from the different group of rats were compared by Student's *t*-test. $P < 0{\cdot}01$ was considered significant. The Pearson *r* correlation coefficient between the amount of proteinuria and matrix score was calculated using the individual data.

## RESULTS

*Experiment 1*

The kinetics of proteinuria in individual rats and the mean value of proteinuria of five control rats are illustrated in Fig. 1. All samples from control rats showed a normal range of urinary protein (< 20 mg/24 h) during the experimental period. The mean values of proteinuria of both groups were as follows

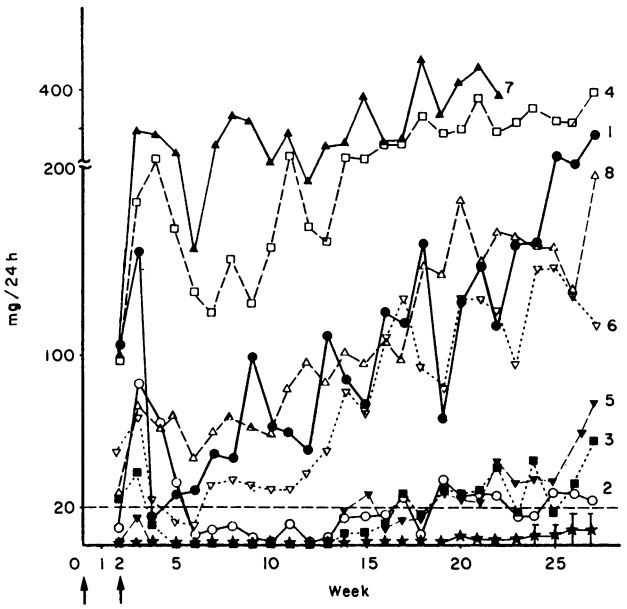


**Fig. 1.** The kinetics of proteinuria in individual rats injected with 500 $\mu$g of MoAb 1-22-3 twice with an interval of 2 weeks (mg/24 h). The numbers 1 to 8 corresponds to rat numbers in Table 1. ★, the mean value of proteinuria of five rats injected with TS-11m twice ($\pm$s.d.); ↑, injection.

(MoAb 1-22-3-injected group *versus* TS-11m-injected group (mean $\pm$ s.d.)): 5 weeks after the first injection, 67·5 $\pm$ 79·1 *versus* 0·0 $\pm$ 0·0; 10 weeks, 64·3 $\pm$ 71·3 *versus* 0·0 $\pm$ 0·0; 15 weeks, 113·5 $\pm$ 122·9 *versus* 1·0 $\pm$ 1·6; 20 weeks, 155·0 $\pm$ 135·0 *versus* 5·1 $\pm$ 5·2; 25 weeks, 133·7 $\pm$ 104·0 *versus* 3·0 $\pm$ 3·8. Many bands, some corresponding to albumin or $\gamma$-globulin, were observed in the urine in the early phase 1 week after the first injection by SDS–PAGE under non-reducing conditions. Such bands were also observed in urine samples obtained in late phase (6 months after the second injection). The amount of proteinuria, the matrix score and serum creatinine and BUN level for each rat are summarized in Table 1. The correlation between the amount of proteinuria and the severity of sclerotic changes was observed ($r = 0{\cdot}860$, $P < 0{\cdot}01$).

*Experiment 2*

*Group A.* The deposition of mouse IgG and rat C3 was observed in the mesangium of rats killed 30 min and 2 h after injection of MoAb 1-22-3. The intensity of fluorescence for mouse IgG and rat C3 was decreased at 24 h. After infiltration of polymorphonuclear leucocytes (PMN) (30 min, 2 h) and mesangiolytic changes defined by Morita *et al.* [12] characterized by large capillary dilatation and ballooning of lumens filled with plasma proteins, erythrocytes and leucocytes (24 h), prominent accumulation of macrophage-like cells was observed in almost all glomeruli (1 week). At the ultrastructural level, these cells were characterized by large cell bodies possessing numerous cytoplasmic process. These cells were not surrounded by the matrix. In a more advanced phase (2 weeks), cell proliferation in the mesangial area was still evident. Ultrastructurally these cells showed the characteristics of intrinsic mesangial cells such as irregular shaped cell bodies and little cytoplasm. Cells were accompanied with an increased amount of mesangial matrix. Such morphological alterations were no

**Table 1.** Matrix score, proteinuria and renal function in each rat

| Rat | MS | PU (mg/24 h) | Cre (mg/dl) | BUN (mg/dl) |
|---|---|---|---|---|
| 1 | 1·53 | 244 | 0·58 | 20·8 |
| 2 | 1·67 | 22 | 0·54 | 19·2 |
| 3 | 1·20 | 55 | 0·66 | 25·6 |
| 4 | 2·60 | 380 | 0·70 | 21·3 |
| 5 | 1·47 | 75 | 0·71 | 24·1 |
| 6 | 0·97 | 115 | 0·67 | 21·3 |
| 7 | End-stage | 461 | 3·83 | 246·1 |
| 8 | 1·83 | 194 | 0·74 | 30·3 |
| Control | 0·1 ± 0·3* | 6·4 ± 7·7* | 0·64 ± 0·03* | 20·6 ± 2·7* |

Rats 1–6, 8, 25 weeks after the second injection of MoAb 1-22-3 with an interval of 2 weeks (27 weeks after the first injection); rat 7, 21 weeks after the second injection of MoAb 1-22-3.

* Mean value (± s.d.) of five rats 25 weeks after the second injection of TS-11m with an interval of 2 weeks.

MS, Matrix score; PU, proteinuria; Cre, creatinine; BUN, blood urea nitrogen.

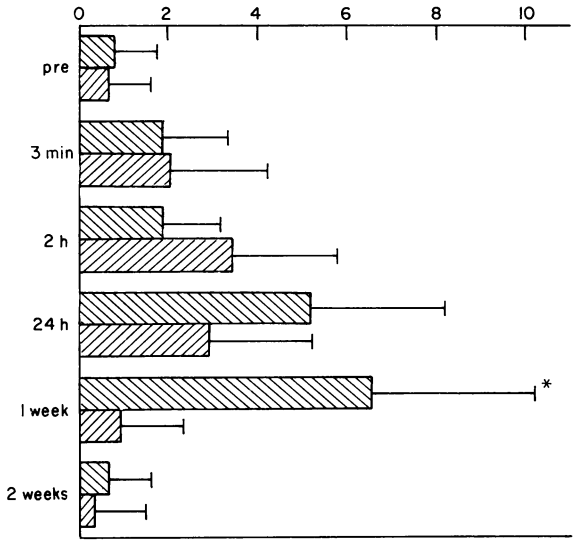


**Fig. 2.** The kinetics of the number of ED-1 (anti-monocyte)-positive cells after the last injection of MoAB 1-22-3. ▧, Number of ED-1-positive cells after the first injection of MoAb 1-22-3 (group A); ■, Number of ED-1-positive cells after the second injection (group B). All data are expressed as mean ± s.d. of the number of MoAb-positive cells per glomerulus. *$P < 0·001$, compared with the data of 2 h, 24 h, 1 week after the second injection.

longer observed in glomeruli from rats 6 months after a single injection of MoAb 1-22-3 (matrix score: 0·1 ± 0·4). The numbers of ED-1-positive cells are shown in Fig. 2.

*Group B.* Deposition of mouse immunoglobulin and rat C3 with the MoAb 1-22-3-specific mesangial pattern was observed in rats killed at 30 min and 2 h after the second injection of MoAb. The intensity of fluorescence for mouse immunoglobulin and rat C3 was already decreased at 24 h. Deposition of

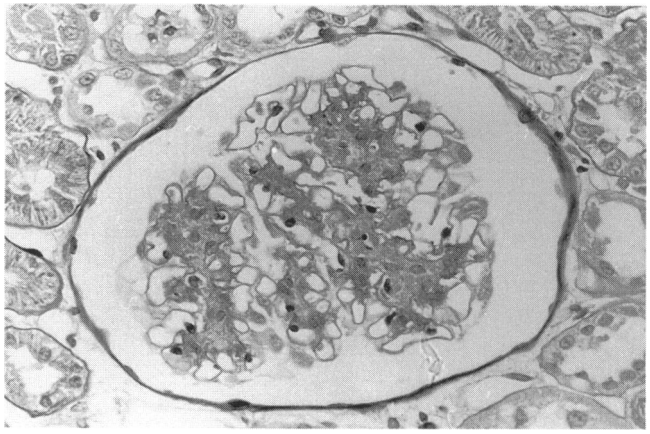

**Fig. 3.** Light micrograph (periodic acid-Schiff × 400) showing glomeruli 6 months after the second injection of MoAb 1-22-3. Cell proliferation with an increase in the mesangial matrix is evident.

mouse IgG or rat C3 was no longer observed at 1 week. Weak deposition of rat IgG, with a pattern unlike the MoAb 1-22-3-specific pattern, was also detected in glomeruli at 1 week after the second injection. Infiltration of PMN and subsequent mesangiolytic changes were detectable at 30 min to 24 h after the second injection. Hypercellularity with the characteristics of intrinsic mesangial cell and increased mesangial matrix were already evident at 1 week after the second injection. Matrix scores for rates at 1, 2 and 6 weeks and 6 months after the second injection are 1·2 ± 0·9, 0·4 ± 0·5, 0·8 ± 0·6, 1·9 ± 0·9, respectively. Sclerotic changes observed at 2 or 6 weeks were significantly milder in comparison with those observed at 1 week ($P < 0·01$). Significantly severer change was observed at 6 months than at 1 week ($P < 0·01$). LM and EM data for rats 6 months after the second injection are shown in Figs 3 and 4 respectively. Cell proliferation with an increase in the mesangial matrix is evident, and some glomeruli (6·0–9·7%) show crescentic change. At the ultrastructural level, accumulation of collagen fibril is evident. Broad deposition of collagen type I in mesangium area and relatively weak deposition of collagen type III limited to around the mesangial cell were detected by indirect IF (Fig. 5). Such IF findings were observed in all rats at 6 months. The thickening of GBM and the detachment of foot processes of podocyte were partially observed. Foot process fusion was frequently observed. The numbers of ED-1-positive cells are shown in Fig. 2.

## DISCUSSION

In preliminary experiments, we confirmed that the MoAb 1-22-3-recognized epitope, which was decreased after MoAb 1-22-3 injection, recovered at 2 weeks. This is why we gave the second injection at this time.

As shown in Fig. 1, proteinuria in five of eight rats started immediately and then increased with time for more than 6 months until severe proteinuria (> 100 mg/24 h) occurred and the other three rats showed mild proteinuria (> 20 mg/24 h). The rats with severe proteinuria showed severe sclerotic changes. One of these rats showed progression to chronic renal failure (CRF) at 21 weeks after the second injection, with a BUN level of 246·1 mg/dl and a creatinine level of 3·83 mg/dl. Even

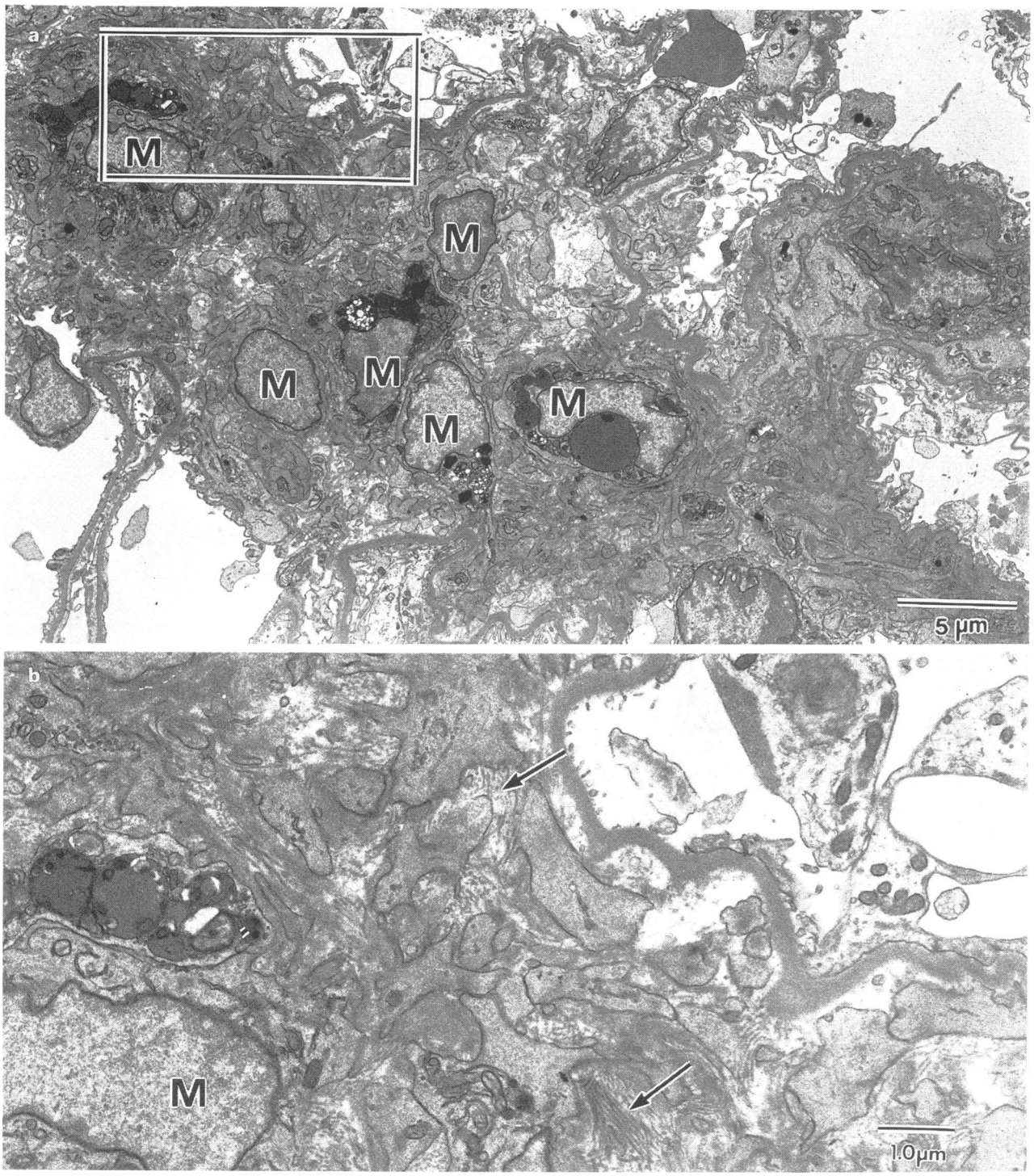


**Fig. 4.** Electron micrograph showing glomeruli 6 months after the second injection of MoAb 1-22-3. Accumulation of collagen fibril is evident (arrows). (b) is a higher magnification of (a) (a) × 4100, (b) × 13000).

rats with mild proteinuria showed the sclerotic change (Table 1). In experiment 2, all rats killed at 6 months after the second injection showed severe sclerotic change (Figs 3, 4 and 5). Thus, sclerotic change with abnormal proteinuria was sucessfully induced in all rats. Although there are several types of experimental glomerulonephritis model showing mesangial alterations [2, 13–17], no satisfactory model has been established. Though the mesangial lesions induced by ATS have been used in many laboratories to investigate the mechanism of mesangial alterations [2–5, 18–20], this model differs from human chronic glomerulonephritis in that (i) the mesangial morphological changes induced by ATS are reversible, and (ii) these alterations are not always accompanied by remarkable proteinuria. Therefore the mesangial sclerotic change with

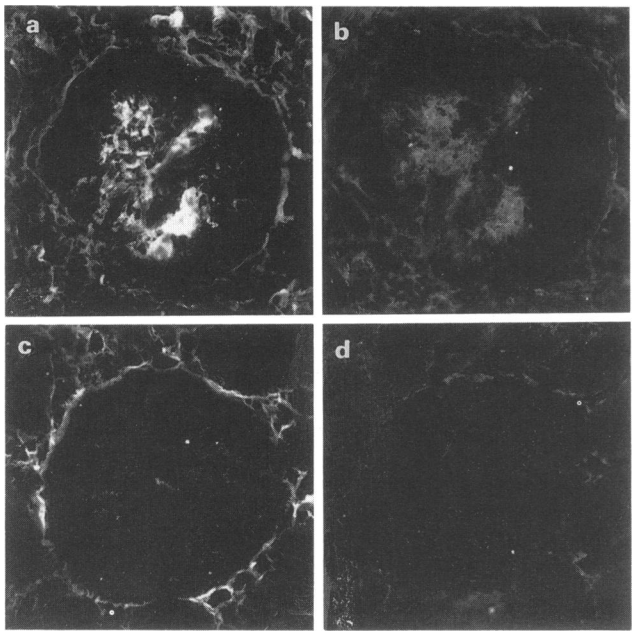


**Fig. 5.** Indirect IF findings showing the deposition of collagen type I (a) and type III (b) in the glomerulus from rat 6 months after the second injection of MoAb 1-22-3. Neither collagen type I nor collagen type III is demonstrated in normal glomeruli (c, d) ( × 400).

persistent proteinuria described here is considered to be a better model for investigating the mechanism of the chronic progression of human mesangial proliferative glomerulonephritis. In this study individual differences in the amounts of proteinuria in experiment 1 were observed. The differences might come from the individual diversity of the level of recovery of MoAb 1-22-3 epitope at 2 weeks after the first injection. Also, Wistar rats used in this study are not an inbred strain, which might contribute to the occurrence of the individual difference.

The results of group A in experiment 2 reconfirmed our previous report [1]. The reversible model induced with a single injection of MoAb 1-22-3 was characterized as follows. After infiltration of PMN and mesangiolytic changes, accumulation of macrophage-like cells in glomeruli was observed by LM and EM at 1 week after a single injection of MoAb. At this time increased numbers of ED-1-positive cells and a decreased amount of epitope recognized by MoAb 1-22-3 were observed by IF. At 2 weeks after injection, hypercellularity with the characteristics of intrinsic mesangial cell was observed by LM and EM. At this stage, the increase in the number of ED-1-positive cells was no longer observed, and the immunofluorescence intensity of the MoAb 1-22-3 epitope recovered. These changes were followed by the normalization of proteinuria 2 or 3 weeks after injection. Furthermore, the morphological alterations including an increased amount of mesangial matrix were no longer observed in rats 6 months after the injection. In the irreversible model induced with two consecutive injections of MoAb 1-22-3 (group B in experiment 2), mesangiolytic changes were observed at 24 h after the second injection, similarly to the case of single injection, but subsequent changes were different. At 1 week after the second injection, hypercellularity with the characteristics of intrinsic mesangial cells and increased mesangial matrix were observed by LM. No increase in the number of ED-1-positive cells was observed in glomeruli at this point, although a mild increase was observed at 2 h or 24 h after the second injection (Fig. 2). Prominent accumulation of macrophage-like cells in glomeruli, which was observed at 1 week after the first injection [1], was not observed during the experimental period after the second injection. It was unclear why no prominent accumulation of macrophage-like cells occurred after mesangiolytic changes in the irreversible model and whether this was causally related to the induction of irreversible alterations. Many studies using experimental models have demonstrated both *in vivo* and *in vitro* that macrophages are present within glomeruli [21–24], and have suggested that macrophages have the potential to contribute to intrinsic glomerular cell hypercellularity [25–27]. In contrast, Sterzel *et al.* have reported that the prominent presence of monocyte-macrophages within mesangium may take place without major functional abnormality in glomerulus in polyvinyl alcohol-treated rats [28]. Shigematsu has reported that monocytes and macrophages show phagocytic activity against immune and inflammatory products, and that complete recovery of glomerular structure may occur after their disappearance in acute monocytic glomerulonephritis [29]. The accumulated macrophage-like cells observed in our reversible model induced by a single injection of MoAb 1-22-3 might indirectly inhibit the progression of sclerotic changes. Thus, the absence of the prominent accumulation of ED-1-positive cells after the second injection of MoAb 1-22-3 might have some etiological significance regarding the induction of irreversible changes. However, we cannot completely exclude the possibility that such an observation is nothing but a by-phenomenon without etiological meaning. The difference of the changes between once-and twice-injected groups was not considered to derive from the difference betwen total injected doses, because the glomerular injury induced by a single injection of 1 mg or 5 mg of MoAb 1-22-3 was confirmed to be reversible (data not shown). The role of host antibody reaction against injected mouse IgG in the development of the irreversible changes should also be considered. Although weak but relatively broad deposition of rat IgG in the sclerotic area in the mesangium was observed at 1 week after the second injection, the pattern was different from the specific binding pattern of MoAb 1-22-3. Such a pattern for rat IgG is considered to come from the passive deposition, since it is reported that passive deposition of host IgG in the sclerotic area is often observed , when the change is accompanied with massive proteinuria. Furthermore, in this model injected mouse IgG, the target antigen in this case, disappeared with mesangiolysis. Thus, host antibody does not seem to play an active role in the subsequent changes. We believe that a detailed examination of the parameters in the irreversible model described here and a comparison of the findings of this model with those of the reversible model will yield some clues for clarifying the mechanism of progression of glomerulonephritis.

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