Involvement of MCP-1 and M-CSF in glomerular foam cell formation in ExHC rats

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Involvement of MCP-1 and M-CSF in hypercholesterolemia-induced glomerular foam cell formation in ExHC rats.

Background. An increase in glomerular macrophages (MØ) is considered a potential effector mechanism by which hypercholesterolemia exacerbates glomerular injury. To investigate the mechanism underlying recruitment of MØ into glomeruli, the expression of glomerular monocyte chemoattractant protein-1 (MCP-1) and macrophage colony-stimulating factor (M-CSF) mRNA were examined using a lipid-induced glomerular injury rat model.

Methods. Eight-week-old male ExHC rats, a strain susceptible to hyperlipidemia, were divided into the following 4 groups: a control group (C), a high cholesterol diet group (HH), a high cholesterol diet/standard diet group (HN), which were fed a high cholesterol diet for the first 4 weeks and a standard diet for the following 4 weeks, and a probucol-treatment group (PT). Both MCP-1 and M-CSF mRNA expression in glomeruli were analyzed using the RT-PCR method. An additional experimental group (M) fed a high cholesterol diet was administered M-CSF daily for 4 weeks.

Results. The expression of MCP-1 mRNA in glomeruli increased accompanied by an increased total serum cholesterol level in HH and HN. However, M-CSF mRNA expression was significantly suppressed at 1 or 2 weeks and gradually increased to almost basal levels. In the PT group, MCP-1 mRNA expression was suppressed. The early suppression of M-CSF mRNA expression was inhibited in PT. Renal histology showed a significant increase in foam cells in glomeruli in HH and HN rats at 4 weeks. HH rats showed increased and expanded foam cells at 8 weeks. In HN rats, however, foam cells decreased significantly after the transfer to a standard diet from a high cholesterol diet. The MCP-1 mRNA expression was suppressed after the transfer. In the PT group, foam cell formation was also suppressed. Foam cells were identified as MØ. M-CSF treatment significantly suppressed foam cell formation in glomeruli when compared with the untreated group levels.

Conclusion. These findings suggest that hypercholesterolemia stimulated the expression of MCP-1 in glomeruli and attracted the MØ into glomeruli. They also suggest that the reduction of hypercholesterolemia after the change in diet or treatment with probucol suppressed glomerular injury by suppressing the glomerular MCP-1 expression. M-CSF may suppress the recruitment of MØ into glomeruli and foam cell formation at an early stage of hypercholesterolemia-induced glomerular injury.

Hypercholesterolemia is an aggravating factor in the progression of glomerular injury. An increase of glomerular macrophages (MØ) is considered a potential mechanism by which hypercholesterolemia exacerbates glomerular injury [1]. To investigate the mechanism underlying recruitment of MØ into glomeruli, the expression of glomerular monocyte chemoattractant protein-1 (MCP-1) and macrophage colony-stimulating factor (M-CSF) mRNA were examined using a lipid-induced glomerular injury rat model.

METHODS

Animals

Exogenous hypercholesterolemia (ExHC) rats, a strain highly susceptible to dietary hypercholesterolemia and considered an appropriate model for lipid-induced glomerular injury, were used for these experiments [2, 3].

Diets

CE-2 (CLEA Japan Inc.) was used as a standard diet. A high cholesterol diet was prepared with 3% cholesterol, 0.6% sodium cholate and 10% olive oil supplementation to CE-2.

Effects of probucol and dietary treatment on glomerular foam cell formation

Eighty 8-week-old male rats were divided into the following four groups: control group (C, N = 20) fed with a standard diet for 8 weeks; high cholesterol diet group (HH, N = 20) fed with a high cholesterol diet for 8 weeks; high cholesterol diet/standard diet group (HN, N = 20), fed with a high cholesterol diet for the first 4 weeks and then fed with a standard diet for the following 4 weeks; and probucol treatment group (PT, N = 20) which were fed with a high cholesterol diet supplemented

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S-174
Fig. 1. Expression of glomerular MCP-1 mRNA. (A) Reverse transcribed-polymerase chain reaction (RT-PCR) analyzes of MCP-1 expression in isolated glomeruli from each treated rat. These data are representative of five separate experiments. Abbreviations are: C, control group; HH, high cholesterol diet group; HN, high cholesterol diet/standard diet group; PT, probucol-treatment group. (B) Relative signal intensities are shown graphically for MCP-1 for five separate sets of experiments and are presented as mean ± SD. The intensities of cDNA bands for MCP-1 were normalized to b-actin band intensities. Symbols are: (●) Control group; (□) high cholesterol diet group; (△) high cholesterol diet/standard diet group; (■) probucol-treatment group. *P < 0.005, **P < 0.001 vs. high cholesterol diet group.

with 5% probucol for 8 weeks. Kidneys were removed and histologically examined at 0, 1, 2, 4, 6 and 8 weeks. Glomerular mRNA expressions of MCP-I, M-CSF and b-actin were analyzed at 0, 1, 2, 4, 6 and 8 weeks.

Effects of M-CSF-treatment on foam cell formation in glomeruli

Six 12-week-old male ExHC rats were divided into the following two groups; M-CSF treatment group (M, N = 3) fed with a high cholesterol diet and administered a subcutaneous injection of M-CSF (150 × 10⁴ U/kg BW) daily for 4 weeks, and the control group (S, N = 3) fed with a high cholesterol diet and administered a subcutaneous injection of saline daily for 4 weeks. After 4 weeks, the rats were sacrificed and the kidneys were removed and examined histologically.

Histology

Glomerular MØ was stained using an immuno-enzymatic method with mouse monoclonal antibody against rat monocytes/macrophages (ED-1; Chemicon International Inc., Tamecula, CA). Foam cell formation in glomeruli was assessed by comparing the ratio of foam cells occupying area/whole glomerular area (measuring 100 glomeruli from each specimen).

RNA extraction and RT-PCR analysis

RNA samples were prepared using the acid guanidinium thiocyanate-phenol-chloroform extraction method.
from isolated glomeruli. RNA samples were subjected to a reverse transcription-polymerase chain reaction (RT-PCR) analysis with primers for MCP-I, M-CSF and β-actin. The PCR products were electrophoresed in 1.5% agarose gel and identified by staining with ethidium bromide. The gel was photographed with Polaroid film under UV light at the same exposure and same developing time for all samples. The bands on the films were scanned using an image scanner and the band densities were determined using an image analysis software package, NIH Image.

Statistical analysis

All data are expressed as mean ± SD. Comparisons between groups were assessed by Student’s t-test. Differences were determined significant at a value of \( P < 0.05 \).

RESULTS

Effects of probucol and dietary treatment on glomerular foam cell formation

HH rats and HN rats fed a high cholesterol diet showed marked hypercholesterolemia (HH, 1740 ± 84 mg/dL at 4 weeks; HN, 1720 ± 68 mg/dL at 4 weeks). In HN rats, total serum cholesterol levels (TC) decreased to 120 ± 22 mg/dL at 4 weeks after transfer to a standard diet from a high cholesterol diet. In HH rats, urinary protein excretion increased accompanied by increasing serum TC levels. Renal histology showed a significant increase in foam cells in glomeruli in HH rats (HH, 12.1 ± 3.6% at 8 weeks; C, 0.0 ± 0.0 at 8 weeks, \( P < 0.001 \)). After transfer to standard diet from high cholesterol diet, foam cells in glomeruli were significantly decreased in HN rats.
(HN, 1.8 ± 1.8% at 8 weeks; HH, 12.1 ± 3.6% at 8 weeks, \( P < 0.001 \)). In PT rats, urinary protein excretion (PT, \( 41 \pm 11 \text{ mg/day at 8 weeks; HH, } \text{105} \pm 22 \text{ mg/day, } P < 0.005 \)) and foam cells (PT, 4.2 ± 3.6%; HH, 12.1 ± 3.6%, \( P < 0.001 \)) in glomeruli were significantly decreased in spite of the failure in improvement of hypercholesterolemia. These foam cells were identified as macrophages by immunoperoxidase staining of ED-1. Expression of MCP-I mRNA in glomeruli were increased gradually in HH and HN rats. After transfer to a standard diet from a high cholesterol diet, MCP-I mRNA expressions were significantly suppressed in HN rats. MCP-I mRNA expression was also significantly suppressed in PT rats (Fig. 1). Expression of M-CSF mRNA in glomeruli were significantly suppressed at an early stage (at 1, 2, 4 week) in HH and HN rats, M-CSF mRNA expression gradually increased and was almost the same as basal-level expression at 6 weeks. Probucol treatment reversed the suppression of glomerular M-CSF expression in rats fed with a high cholesterol diet (Fig. 2).

**Effects of M-CSF-treatment on foam cell formation in glomeruli**

M-CSF treatment significantly suppressed the foam cell formation in glomeruli (M, 2.9 ± 0.9%; S, 4.1 ± 1.2%, \( P < 0.001 \)) and significantly suppressed the urinary protein (M, 17 ± 5 mg/day; S, 30 ± 7 mg/day, \( P < 0.001 \)) at 4 weeks in spite of the failure in improvement of hypercholesterolemia.

**DISCUSSION**

When investigating the nephropathies caused by lipids, it is important to clearly detail the formation of foam cells in glomeruli. Experiments on the involvement of MCP-1 and M-CSF in the infiltration and differentiation of monocytes and macrophages have used cultured mesangial cells [4, 5] or vascular wall cells associated with arteriosclerosis. In contrast, the present study investigated the expression of MCP-1 and M-CSF in glomeruli using alimentary hypercholesterolemia rats (EXHC rats). In the kidney, MCP-1 was expressed in endothelial cells, mesangial cells and monocytes. MCP-1 was found to stimulate the migration of monocytes. In the present study, as total cholesterol levels increased, the expression of MCP-1 in glomeruli increased and foam cells were formed. Although no decrease in the total cholesterol level was detected when probucol was administered, MCP-1 expression and foam cell formation were found to be suppressed. These findings suggest that an increase in total cholesterol, or LDL, increased the level of oxidized LDL which in turn enhanced the expression of MCP-1 in endothelial cell, mesangial cells and monocytes, thus accelerating the infiltration of monocytes, into mesangial areas. M-CSF facilitates the differentiation of macrophages and accelerates the excretion of incorporated lipoproteins as free cholesterol from cells [6]. The results of the present study showed that the administration of M-CSF suppressed glomerular foam cell formation and urinary protein excretion, thus clarifying the antifoam cell formation effect and urinary protein suppression effect of M-CSF. Therefore, the suppression of M-CSF expression that was seen in the early stages of the present study is believed to accelerate foam cell formation and urinary protein excretion. However, the mechanism underlying the suppression of M-CSF expression has not been clarified. Although platelet derived growth factors [7] and nitric oxide [8] reportedly suppress the expression of M-CSF in vascular smooth muscle cells, this effect must be investigated further in future research.

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