Mechanisms of glomerular macrophage infiltration in lipid-induced renal injury

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Mechanisms of glomerular macrophage infiltration in lipid-induced renal injury.

Background. A number of studies have demonstrated an important role for macrophages (Mφ) in lipid-induced glomerular injury; however, little is known of the mechanisms that facilitate Mφ infiltration in this disease. This study examined the expression of Mφ chemotactic molecules Mφ colony-stimulating factor (M-CSF) and Mφ migration inhibitory factor (MIF) and leukocyte adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) during the induction of glomerular Mφ infiltration in ExHC rats, a strain that is susceptible to lipid-induced glomerular injury.

Methods. Groups of five ExHC rats were fed a high-cholesterol diet (HCD) containing 3% cholesterol, 0.6% sodium cholate, and 15% olive oil and were killed after three days or one, two, or six weeks. Control animals were killed on day 0 or after six weeks on a normal diet.

Results. ExHC rats fed an HCD showed marked hypercholesterolemia in the absence of any increase in plasma triglyceride levels from day 3 and developed mild proteinuria and segmental glomerular lesions at week 6. Immunoperoxidase staining identified a significant increase in glomerular ED1+ Mφ at week 1, which was further increased at week 6, when Mφ-derived foam cells were seen in almost all glomeruli. Many of the infiltrating glomerular Mφ expressed lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4), which are ligands for ICAM-1 and VCAM-1, respectively. Coincident with the induction of hypercholesterolemia on day 3 and prior to significant Mφ infiltration, combined in situ hybridization and immunohistochemistry staining demonstrated a marked up-regulation of M-CSF and MIF mRNA expression by glomerular mesangial cells and podocytes. There was also a significant increase in ICAM-1 and VCAM-1 mRNA expression by intrinsic glomerular cells, including endothelial cells, on day 3 of the HCD.

Conclusion. These results suggest that hypercholesterolemia can induce a classic proinflammatory response within the kidney glomerulus, involving production of well-described Mφ chemotactic and adhesion molecules, which results in Mφ recruitment and the development of glomerular injury.

Key words: lipids, hypercholesterolemia, leukocyte adhesion molecule, MIF, M-CSF.

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Hyperlipidemia has been shown to accelerate the induction and progression of renal injury leading to glomerular sclerosis. Lipid-induced glomerular injury is thought to be mediated in part by macrophages (Mφ), on the basis that glomerular Mφ accumulation is a feature of animal models of endogenous and diet-induced hyperlipidemia, and Mφ depletion inhibits lipid-induced renal injury [1]. However, the mechanisms by which Mφ accumulate in the glomerulus during the hyperlipidemic state are poorly understood.

Given the similarities between the development of atherosclerotic lesions and cellular immune responses [2], we examined the expression of Mφ chemotactic molecules Mφ colony-stimulating factor (M-CSF) and Mφ migration inhibitory factor (MIF) and leukocyte adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) during the induction of glomerular Mφ infiltration in exogenous hypercholesterolemic (ExHC) rats, a strain that is susceptible to lipid-induced glomerular injury [3, 4].

METHODS

ExHC rats are derived from the Sprague-Dawley strain and are highly susceptible to dietary hypercholesterolemic stimuli. Groups of five ExHC rats were fed a high-cholesterol diet (HCD) containing 3% cholesterol, 0.6% sodium cholate, and 15% olive oil and were killed on day 3 or weeks 1, 2, and 6 after the start of the HCD. Control animals were killed on day 0 or after six weeks on a normal diet.

RESULTS AND DISCUSSION

Clinicopathological features of ExHC rats

ExHC rats fed an HCD rapidly developed significant hypercholesterolemia (41 ± 3, 190 ± 14, 226 ± 25, 423 ± 36, and 362 ± 27 mg/dl on days 0 and 3 and weeks 1, 2,
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Fig. 1. Representative glomeruli from ExHC rats fed a cholesterol-supplemented diet for two (a) and six weeks (b, c). (a) Some foam cells are indicated by arrows (PAS ×200). (b) The marked accumulation of numerous lipid-filled foam cells is visible within a glomerulus. In addition, a segmental cluster of foam cells associated with a capsular adhesion and destruction of glomerular tufts can be seen (PAM ×200). (c) ED1-positive cells correspond to foam cells within the glomerulus.

and 6, respectively; all P < 0.01 vs. day 0) and mild proteinuria at week 6 (21.9 ± 2.7 vs. 4.3 ± 0.4 mg/24 hr on day 0, P < 0.01) but maintained normal levels of plasma triglycerides. ExHC rats that were fed a normal diet showed no changes in plasma lipid levels or urinary protein excretion.

ExHC rats that were fed a normal diet for six weeks had a normal kidney histology. In contrast, ExHC rats fed an HCD developed characteristic glomerular lesions, as illustrated in Figure 1 and as described in detail previously [3–5]. Briefly, foam cells and/or mesangial cells containing lipid vacuoles were seen in 4.6 ± 1.8% of glomeruli on day 3. There was a progressive increase in the number and size of foam cells, and by week 6, virtually all glomeruli (97.6 ± 1.6%) contained foam cells, and segmental clusters of foam cells associated with capsular adhesions and destruction of the glomerular tuft were seen in 5.1 ± 1.0% of glomeruli (Fig. 1a, b). Most foam cells within the glomeruli were identified as Mφ by the use of a monoclonal antibody specific for rat Mφ (ED1; Fig. 1c). There was a significant increase in glomerular ED1⁺ Mφ at week 1 (2.0 ± 0.2 vs. 1.0 ± 0.1 ED1⁺ Mφ per glomerular cross section in day 0, P < 0.01), which was further increased at week 6 (6.9 ± 0.4 ED1⁺ Mφ per glomerular cross section; Fig. 2A).

Thus, ExHC rats may be well suited for use in investigations of the mechanisms that facilitate Mφ infiltration and accumulation during the development of lipid-induced glomerular injury.

Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1

In situ hybridization demonstrated a significant increase in glomerular ICAM-1 and VCAM-1 mRNA expression after just three days on the HCD (Fig. 2B) [5]. This preceded the initiation of glomerular Mφ infiltration at week 1. The expression of Mφ ligands for ICAM-1 and VCAM-1 was examined by immunoperoxidase staining. The infiltrating Mφ expressed lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4), which are ligands for ICAM-1 and VCAM-1, respectively; however, there was no change in Mac-1 expression, which is another ligand for ICAM-1. This is consistent with studies in rat anti-glomerular basement membrane nephritis in which glomerular polymorphonuclear leukocyte accumulation is mediated through the ICAM-1/Mac-1 inter-
action, whereas an influx of mononuclear leukocytes is mediated through the ICAM-1/LFA-1 interaction. The potential role for the ICAM-1/LFA-1 and VCAM-1/VLA-4 interactions in Mφ infiltration in lipid-induced lesions is consistent with studies of atherosclerotic lesions in human and rabbits [6]. Moreover, in vitro study has demonstrated that lysophosphatidylcholine (Lyso-PC) selectively increases cell surface expression of ICAM-1 and VCAM-1 in cultured rabbit and human arterial endothelial cells [7]. Because Lyso-PC is a component of oxidized low-density lipoprotein (LDL) and β–very low-density lipoprotein (VLDL), and there is a progressive accumulation of β-VLDL in the plasma of ExHC rats fed an HCD [4], components of lipoproteins such as Lyso-PC may directly trigger endothelial cell adhesion molecule expression and thereby initiate monocyte recruitment in this model.

**Macrophage colony-stimulating factor**

Macrophage colony-stimulating factor is the main growth factor for monocyte production in bone marrow, is a potent chemotactic molecule for monocytes, and can prime or directly stimulate a range of monocyte/Mφ functions. Local production of M-CSF has been described in vascular atherosclerotic lesions, and M-CSF may contribute to foam cell formation by up-regulating monocyte expression of the LDL receptor and lipid metabolism. In addition, LDL has been shown to induce M-CSF synthesis and secretion by cultured mesangial cells [8].

Combined in situ hybridization and immunohistochemistry staining showed a significant increase in the number of glomerular M-CSF+ cells coincident with the onset of hypercholesterolemia on day 3, which further increased with time (Fig. 2C) [9]. Up-regulation of glomerular M-CSF expression preceded glomerular Mφ accumulation. Although some ED1+ Mφ-derived foam cells showed M-CSF mRNA expression, most M-CSF+ cells were intrinsic glomerular cells (mesangial cells and podocytes).

**Macrophage migration inhibitory factor**

Macrophage migration inhibitory factor is a potent proinflammatory mediator that is produced by many cell types, including Mφ and resident kidney cells. It is required for Mφ accumulation in the skin delayed-type hypersensitivity response and is a counter-regulator of glucocorticoid action. We have recently shown a dramatic up-regulation of renal MIF expression, which mediates Mφ infiltration and the development of glomerular lesions in a rat model of crescentic glomerulonephritis [10]. However, the potential role for MIF to contribute to Mφ recruitment in the development of lipid-induced renal injury is unknown.

We found a dramatic increase in glomerular MIF
mRNA expression on day 3 after the start of the HCD, which further increased over the six-week period (Fig. 2C) [9]. The up-regulation of glomerular MIF expression preceded Mφ infiltration. Although double staining identified MIF expression by 32% of ED1+ Mφ, the main source of the increased glomerular MIF production was intrinsic glomerular cells, particularly podocytes.

Currently, we do not know whether the up-regulation of glomerular MIF expression is a direct or indirect effect of hypercholesterolemia. Given the ability of MIF to activate Mφ and inhibit migration of Mφ [10], this cytokine may play a similar key role in promoting Mφ accumulation and the subsequent development of lipid-induced glomerular injury.

SUMMARY

These results suggest that hypercholesterolemia can induce a classic proinflammatory response within the kidney glomerulus, involving production of well-described Mφ chemotactic and adhesion molecules, which results in Mφ recruitment and the development of glomerular injury.

ACKNOWLEDGMENTS

A portion of this work was supported by a Grant-in-Aid for General Scientific Research (C) (08671306 and 08671307) from the Japanese Ministry of Education, Science and Culture, and by Dr. Itoe Okamoto International Exchange Fund (Tokyo Women’s Medical University, School of Medicine, Tokyo, Japan).

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