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Altered Functional Characteristics of Rat Macrophages During Nephrosis

Synergistic Effects of Hypercholesterolemia

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The effects of alimentary hypercholesterolemia and nephrotic hyperlipidemia, alone and in combination, on rat peritoneal macrophage phagocytosis, basal eicosanoid production, and glomerular macrophage number during peak PA nephrosis were evaluated in rats fed four different diets: 1) normal/standard chow; 2) PA/standard chow; 3) normal/cholesterol-supplemented diet; and 4) PA/cholesterol-supplemented diet. Both PA/standard chow and normal/cholesterol-supplemented rodent groups manifested significantly greater peritoneal macrophage phagocytosis and glomerular macrophage number when compared with normal/standard chow animals. However, the combination of the nephrotic state with superimposed alimentary hypercholesterolemia (PA/cholesterol-supplemented group) produced the greatest rise in these parameters, a rise that was significantly greater than was produced in the three other groups. Regarding basal eicosanoid production by macrophages, there was a numerical trend toward increased production of thromboxane B_2 in the PA/standard chow animals and normal/cholesterol-supplemented rats when compared with normal/standard chow. Again, the combination of nephrosis and alimentary hypercholesterolemia in the PA/cholesterol-supplemented group was associated with a significantly greater amount of thromboxane B_2 generated when compared with the other three groups. Regarding PGE_2 production, there were no significant differences among the groups, despite marked differences in fasting serum lipid levels. This data suggest that there is a synergistic effect between alimentary hypercholesterolemia and the secondary hyperlipidemia of nephrosis in producing these macrophage functional alterations. Because fasting triglyceride values between the two nephrotic groups were indifferent, one can further speculate that it is the elevation of the serum cholesterol value that predominantly evokes these changes in macrophage function. (Am J Pathol 1989, 135:711–718)

A putative role for the secondary hyperlipidemia of nephrosis as a mediator in the progression of initial glomerular injury to glomerulosclerosis was demonstrated in a number of experimental models of glomerular disease.[1–3] Hypolipidemic therapy with agents such as mevinolin,[4] clofibric acid,[4] and cholestyramine,[5] has conferred functional and histologic protection, whereas the superimposition of alimentary hypercholesterolemia, with a 4% cholesterol/1% cholic acid dietary supplement during the course of chronic aminonucleoside nephrosis, aggravated the disorder.[6]

Schreiner et al[7] showed that, in both acute nephrotoxic serum nephritis and acute aminonucleoside nephrosis, there is an increase in the number of glomerular macrophages accompanying peak protein excretion. The role of the glomerular macrophage in either the acute or chronic phases of aminonucleoside nephrosis, a nonimmune disorder, remains unclear. The pathologic features of glomerulosclerosis are analogous to those of the atherosclerotic process,[1] most notably regarding mesangial *versus* vascular smooth muscle cell (VSMC) proliferation and ma-

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trix overproduction. The entry of bone marrow-derived monocytes into the glomerular mesangium could be viewed as analogous to the developing fatty streak and the functional role of intimal monocyte/macrophages in the vessel wall. Members of this laboratory demonstrated that alimentary hypercholesterolemia in normal rats augmented several macrophage functions including adhesion to endothelium and secretion of chemotactic and mitogenic substances.[8] These authors and others (reviewed in reference 8) postulated that dietary cholesterol may accelerate atherosclerosis by inducing specific changes in the properties of circulating monocytes and intimal macrophages,[8] such as those mentioned above, as well as the production of proinflammatory factors. These observations are intriguing and raise the possibility that the hyperlipidemia of nephrosis may also alter rat macrophage functions with additional effects being compounded by the superimposition of alimentary hypercholesterolemia. In this study, we examined particularly the effects of alimentary hypercholesterolemia and nephrotic hyperlipidemia, alone and in combination, on rat peritoneal macrophage phagocytosis, basal eicosanoid production, and glomerular macrophage number during peak acute aminonucleoside nephrosis.

Materials and Methods

Experimental Animal Groups

Male Sprague-Dawley rats (Charles River, Wilmington, MA; CD Strain), weighing 150 to 200 g, were divided into four groups. The PA/Standard chow group comprised the nephrotic controls that were given a single jugular intravenous bolus injection of puromycin aminonucleoside (PA, Sigma Chemical Co., St. Louis, MO) at a dose of 5 mg/100 g body weight (BW) and were maintained on Purina standard laboratory rodent chow. PA/HICHOL animals were made similarly nephrotic but were fed a 4% cholesterol/1% cholic acid supplement (Teklad Diets, Madison, WI) that began at the time of PA injection, thus superimposing alimentary hypercholesterolemia. The normal/standard chow group was normal saline-injected rats on standard rodent chow, whereas the normal/HICHOL group was also injected with saline but fed the 4% cholesterol/1% cholic acid dietary supplement.

Cell Isolation

Elicited rat macrophages were obtained from the peritoneal cavity 96 hours after 5 mL of sterile 4% thioglycollate broth (DIFCO Laboratories, Detroit, MI) was injected. Cells were washed twice in Hanks balanced salt solution buffered with 15 mM HEPES, pH 7.4 (Hanks-HEPES). More than 90% of these cells were found to be macrophages by cytochemical demonstration of alphanaphthyl esterase in the cytoplasm[9] and by immunocytochemical staining with ED 1 (Serotec Limited, Oxford, United Kingdom), which is a mouse monoclonal IgG antibody obtained from ascitic fluid that recognizes cytoplasmic antigen in monocytes and macrophages, free and fixed[10] (Figure 1).

Peritoneal Macrophage Thromboxane B_2(TXB_2) and Prostaglandin E_2(PGE_2) Levels

Macrophages were plated at a concentration of 2×10^6 cells/mL in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). Cells were allowed to adhere for 2 hours at 37 C. Nonadherent cells were removed with two Hanks-HEPES rinses. Adherent cell monolayers remained in Hanks-HEPES for an additional 1 hour at 37 C. After this incubation, the buffer was removed and centrifuged at 1000 rpm to pellet cell debris, and the supernate was stored immediately at −70 C for subsequent TXB_2 and PGE_2 determination using ^{125}I-TXB_2 and ^{125}I-PGE_2 obtained from DuPont-New England Nuclear (Boston, MA). Remaining cell monolayers were solubilized in 1N NaOH for 12 to 18 hours and stored at −20 C for protein determination by the Lowry method,[11] with eicosanoid values expressed as picomoles/mg cellular protein.

Peritoneal Macrophage Phagocytic Activity

Macrophages were plated at a concentration of 2×10^6/mL in DMEM plus 10% FCS in 16 mm wells on a 24-well plate. Cells were allowed to adhere to the plastic surface for 3 hours at 37 C. Adherent cell monolayers were then washed twice with sterile Hanks-HEPES to remove unattached cells and serum. The adherent cell layers were then covered with 1 ml of sterile Hanks-HEPES and 30 μl of a 1:100 dilution of latex beads (LB-11, Sigma), giving a final concentration of 4.2×10^7 beads per well.[12] The beads and macrophages were incubated for 1 hour at 37 C. Cells were washed twice with sterile Hanks-HEPES to remove unbound beads. Cells were fixed in 2% paraformaldehyde for 1 hour and examined under inverted phase contrast light microscopy. One hundred cells were viewed, with those interiorizing three or more particles being scored as positive (Figure 2).

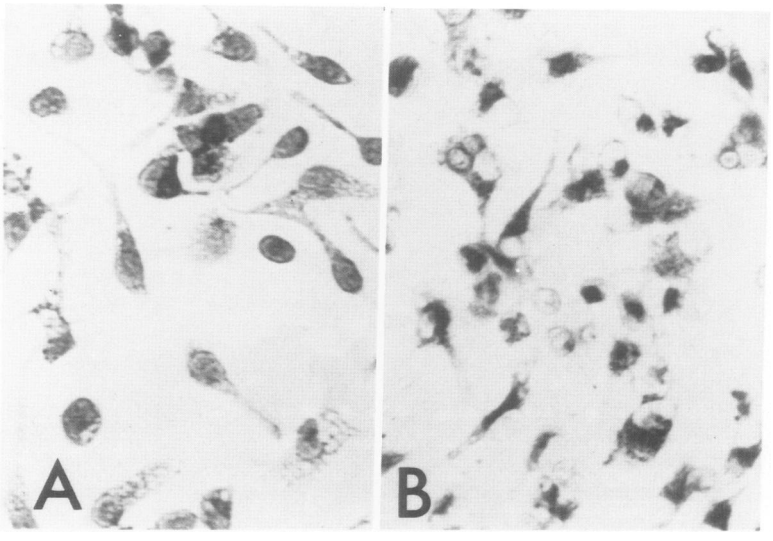

Figure 1. *Elicited rat peritoneal macrophages after they were adhered to plastic for 2 hours and stained with alphanaphthylesterase* **(A)** *and ED 1* **(B).** *More than 90% of peritoneal cells were identified as of monocyte/macrophage origin after thioglycollate elicitation (original magnification ×450).*

Glomerular Macrophage (GMϕ) Number

Methanol-Carnoy's-fixed, paraffin-embedded, midcoronal kidney sections (4-μm thick) were serially deparaffinized with xylene and then rehydrated with ethanol. After quenching endogenous peroxidase activity with a 4:1 methanol/H_2O_2 solution, sections were incubated in a 1:20 dilution of normal horse serum (Vector Laboratories, Burlingame, CA) for 20 minutes at 25 C. Using an avidin-biotin complex immunoperoxidase system,[13] ED 1, was added as the primary antibody at a dilution of 1:2000 for a 1 hour incubation at 25 C. With intervening washes in phosphate buffer solution (PBS), the following steps were performed: biotinylated anti-mouse, rat adsorbed antibody (Vector, 1:150 dilution in PBS) for 1 hour at 25 C; avidin-biotinylated horseradish peroxidase complex (Vector) in PBS, pH 7.2, for 1 hour at 25 C; and a 0.1 M solution of 3,3′-diaminobenzidine (DAB, Sigma) in 0.05 M Tris buffer, pH 7.6 (10 minutes), to which had been added 0.75 mL of a 3% H_2O_2. Sections were then immersed in a 1% methyl green solution for 10 minutes for nuclear counterstaining. To quantify the number of glomerular macrophages within a 4-μm thick midcoronal section, 100 randomly chosen glomeruli were scored for ED 1-positive cells observed within the mesangium (Figure 3). This number is referred to throughout this article as "glomerular macrophage number (GMϕ number)".

All *in vitro* peritoneal macrophage studies and *in vivo* quantification of GMϕ number were performed 14 days after PA or saline injection, which temporally coincides with peak protein excretion in the two nephrotic groups.

Analytical

Fasting total cholesterol (FTC) and triglycerides (FTG) were measured spectrophotometrically. Urinary protein

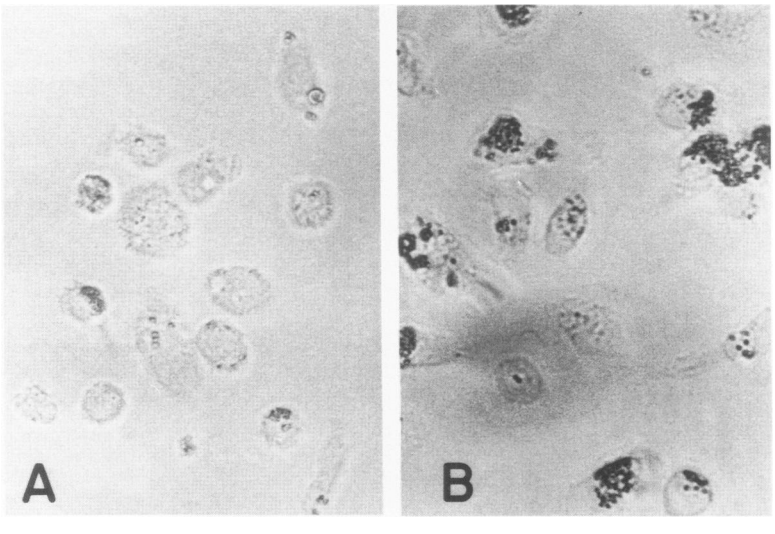

Figure 2. *Comparative photomicrograph of elicited rat peritoneal macrophages 1 hour after addition of latex beads to the culture medium.* **A:** *From a normal animal on standard rat chow.* **B:** *From a nephrotic animal on the 4% cholesterol/1% cholic acid diet supplement (PA/HICHOL). Original magnification ×500, unstained.*

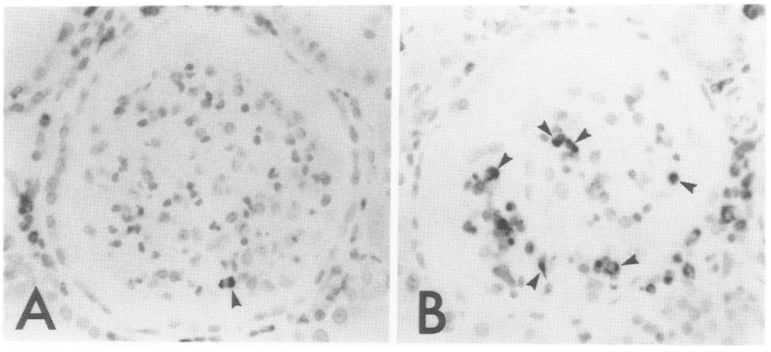

Figure 3. *Comparative photomicrographs of rat glomeruli stained with ED 1 to detect macrophages within the glomerular mesangium.* **A:** *A representative glomerulus from a normal animal on a standard diet (normal/standard chow).* **B:** *A glomerulus from a nephrotic animal on the dietary cholesterol supplement (PA/HICHOL) 14 days after puromycin aminonucleoside administration. The ED 1-positive cells are the darkly stained cells within the mesangium and are denoted by the black arrowheads (original magnification ×300).*

excretion was quantified using the sulfosalicylic acid (SSA) assay. Values were expressed as the mean ± 1 SEM. Statistically significant differences among groups were determined by one-way analysis of variance (ANOVA).

Results

Serum Cholesterol and Triglyceride Analysis

As shown in Table 1, consistent major differences in fasting lipid levels existed. The nephrotic state (PA/standard chow) was accompanied by both significantly elevated FTC and FTG values when compared with normal rats (normal/standard chow). Normal/HICHOL animals had significant alimentary hypercholesterolemia, of approximately a five-fold increment, 2 weeks after initiation of the 4% cholesterol/1% cholic acid dietary supplement when compared with normal/standard chow rats; however, there was only a modest increment in the FTG levels (81 ± 5 *vs.* 41 ± 6 mg/dl; $P < 0.001$). The degree of alimentary hypercholesterolemia produced in the normal/HICHOL group was equivalent to the secondary hypercholesterolemia seen in the PA/Standard chow group, but the nephrotic state was also associated with a fivefold to six-fold increase in FTG levels when compared with the normal/HICHOL group ($P < 0.001$). The combination of the nephrotic state and dietary cholesterol supplementation was accompanied by the most profound alterations in FTC values, which were significantly greater than the levels found in any of the other three groups. FTG levels in PA/HICHOL rats were significantly greater than those produced in either of the two normal rat groups; however, the difference observed between the two nephrotic groups was not significant (466 ± 28 *vs.* 611 ± 171 mg/dl).

Table 1. *Effect of Diet and Nephrotic State on Fasting Serum Lipid Values*

Group	FTC (mg/dl)	FTG (mg/dl)
Normal/standard chow (N = 6)	56 ± 7	41 ± 6
PA/standard chow (N = 6)	266 ± 22*†	466 ± 28*‡
Normal/HICHOL (N = 6)	279 ± 39*	81 ± 5*
PA/HICHOL (N = 6)	1548 ± 227*‡	611 ± 171*‡

* $P < 0.001$ *vs.* Normal/standard chow; † $P < 0.001$ *vs.* PA/HICHOL; ‡ $P < 0.001$ *vs.* normal/HICHOL.

Standard chow, standard rodent laboratory diet; PA, puromycin aminonucleoside; HICHOL, 4% cholesterol/1% cholic acid dietary supplement; FTC, fasting total cholesterol; FTG, fasting triglycerides.

Urine Protein Excretion

As shown on Table 2, the 4% cholesterol/1% cholic acid supplement produced no adverse effect on urine protein excretion in normal rats, and this is consistent with data previously reported for Sprague-Dawley rats maintained on this dietary supplement for 18 weeks during both acute and chronic phases of aminonucleoside nephrosis.[6] Both groups of normal animals had 24-hour proteinuria values within the normal range 2 weeks after their respective diets were instituted. Likewise, despite the superimposition of alimentary hypercholesterolemia, both nephrotic groups excreted equivalent amounts of protein, suggesting that significantly increasing FTC during acute nephrosis (2 weeks after PA) has no exacerbating effect on glo-

Table 2. *Urine Protein Excretion and GMϕ Number in Response to Hyperlipidemia*

Group	$\dot{U}V$prot (mg/dl)	GMϕ number
Normal/Standard chow (N = 5)	6 ± 2	1.6 ± 0.1
PA/standard chow (N = 14)	153 ± 22*	2.6 ± 0.2†‡
Normal/HICHOL (N = 6)	4 ± 1	3.7 ± 0.6†‡
PA/HICHOL (N = 6)	106 ± 15*	6.8 ± 0.6†

* $P < 0.001$ *vs.* normal/standard chow and normal/HICHOL; † $P < 0.02$ *vs.* normal/standard chow; ‡ $P < 0.01$ *vs.* PA/HICHOL.

GMϕ, glomerular macrophage; $\dot{U}V$prot, urine protein excretion; standard chow, standard rodent laboratory diet; PA, puromycin aminonucleoside; HICHOL, 4% cholesterol/1% cholic acid dietary supplement.

Table 3. *Peritoneal Mϕ Phagocytosis in Response to Hyperlipidemia*

Group	Cells ingesting three or more latex beads (%)
Normal/standard chow (N = 5)	24.8 ± 6.7
PA/Standard chow (N = 5)	59.2 ± 7.0*†
Normal/HICHOL (N = 5)	72.1 ± 1.9*†
PA/HICHOL (N = 5)	88.4 ± 2.4*

* $P < 0.010$ *vs.* normal/standard chow; † $P < 0.010$ *vs.* PA/HICHOL.
Mϕ, macrophage; standard chow, standard rodent laboratory diet; PA, puromycin aminonucleoside; HICHOL, 4% cholesterol/1% cholic acid dietary supplement.

merular dysfunction, as evidenced, at least, by proteinuria.

Glomerular Macrophage (GMϕ) Number

As shown in Table 2, the GMϕ number was significantly different among the groups with respect to the different diets and the nephrotic state. Acute nephrosis (PA/standard chow) was associated with a significant increase in GMϕ number from 1.6 ± 0.1 to 2.6 ± 0.2 ($P < 0.02$). When comparing groups of non-nephrotic rats, the superimposition of alimentary hypercholesterolemia (normal/HICHOL) significantly raised this value from 1.6 ± 0.1 to 3.7 ± 0.6 and was only numerically greater than the value observed for the PA/standard group. However, the combination of the nephrotic state with superimposed alimentary hypercholesterolemia (PA/HICHOL) produced the greatest rise in GMϕ number, to 6.8 ± 0.6, a rise significantly different from that of the three other groups. Of interest, the increment in GMϕ number in both normal and nephrotic animals was not associated with an increase in protein excretion, suggesting that in this nonimmune model of glomerular injury, the presence of mesangial macrophages neither causes nor aggravates glomerular barrier dysfunction, as manifested by proteinuria.

Peritoneal Macrophage Phagocytosis

When examining peritoneal macrophage phagocytosis, as determined by the ingestion of latex beads, similar changes in this parameter were observed in relationship to hyperlipidemia produced by either diet or nephrosis. Table 3 demonstrates that the nephrotic state, with its accompanying hypercholesterolemia and hypertriglyceridemia, significantly increased phagocytosis from 24.8% ± 6.7% in normal/standard chow animals to 59.2% ± 7.0% in the PA/standard chow group ($P < 0.010$). Likewise, the superimposition of alimentary hypercholesterolemia in normal rats was associated with 72.1% ± 1.9% of peritoneal macrophages examined ingesting three or more latex beads, and this too was significantly greater than that of the normal/standard chow rats ($P < 0.010$). Despite the marked fasting hypertriglyceridemia noted in the PA/standard chow rats, in contrast to the normal/HICHOL group, there were no significant differences in the phagocytosis of latex beads by macrophages in either of these groups. Again, the combination of nephrosis and alimentary hypercholesterolemia produced phagocytosis that was significantly greater than that in any of the other three groups.

Peritoneal Macrophage Basal Thromboxane B_2 and Prostaglandin E_2 Production

Table 4 shows the basal levels of TXB_2 and PGE_2 produced by isolated peritoneal macrophages harvested from animals in the four experimental groups. There were no significant differences among normal/standard chow, PA/standard chow, and normal/HICHOL groups' basal TXB_2 generated, although a numerical trend existed. The amount of TXB_2 generated by PA/HICHOL rats was significantly greater (199 ± pmol/mg cellular protein) than that of the normal/standard chow (46 ± 15; $P < 0.02$), PA/standard chow (71 ± 31; $P < 0.02$), and normal/HICHOL (91 ± 30; $P < 0.05$) groups. Regarding PGE_2 production, there were no significant differences among the groups, despite the marked differences in fasting serum lipid levels.

Discussion

We chose to study rat peritoneal macrophages, as a potential reflection of perturbed glomerular macrophage

Table 4. *Peritoneal Mϕ Basal TXB_2 and PGE_2 Produced in Response to Hyperlipidemia*

Group	TXB_2 produced (pmol/mg cellular protein)	PGE_2 produced (pmol/mg cellular protein)
Normal/standard chow (N = 7)	46 ± 15	5.4 ± 1.5
PA/standard chow (N = 5)	71 ± 31*	4.2 ± 0.9
Normal/HICHOL (N = 6)	91 ± 30†	8.6 ± 3.3
PA/HICHOL (N = 5)	199 ± 46‡	7.9 ± 3.2

* $P < 0.02$ *vs.* PA/HICHOL; † $P < 0.05$ *vs.* PA/HICHOL; ‡ $P < 0.02$ *vs.* normal/standard chow.
Mϕ, macrophage; TXB_2, thromboxane B_2; PGE_2, prostaglandin E_2; standard chow, standard rodent laboratory diet; PA, puromycin aminonucleoside; HICHOL, 4% cholesterol/1% cholic acid dietary supplement.

function, in response to serum lipid alterations for the following reasons. First, from a practical standpoint, a sufficient number of cells could be obtained from the peritoneum for phagocytosis and eicosanoid production assays, whereas the number of glomerular macrophages recovered from whole glomeruli of normal or nephrotic animals, using standard sieving and enzymatic digestion techniques,[12] was inadequate. This may, in fact, be related to the nonimmune nature of aminonucleoside nephrosis. Despite a significant rise in GMϕ number *in situ* during nephrosis, it does not compare with the large macrophage influx in glomeruli seen in actively induced proliferative glomerulonephritis.[14] Second, the technique of peritoneal macrophage isolation reproducibly yields a homogenous population of cells with more than 90% being macrophage in origin. Third, the agent used to elicit peritoneal macrophages, thioglycollate broth, activates these cells least, as assessed by superoxide anion[15] and hydrogen peroxide[16] production. Finally, direct assessment of alterations in macrophage eicosanoid production could be performed in response to lipid perturbations, whereas evaluations of isolated glomerular production of thromboxane B_2 and prostaglandin E_2 preclude identification of the glomerular cell type responsible for altered eicosanoid production because the change could be derived from one or more of the glomerular cell populations present. It was previously shown by this laboratory that peritoneal macrophages from normal rats with alimentary hypercholesterolemia demonstrate functional changes that include increased adhesion to tissue culture-grade plastic and endothelial cell monolayers and release of mitogenic and chemotactic substances for vascular smooth muscle cells (VSMCs) into the culture medium.[8] In this present investigation, we also showed that in alimentary hypercholesterolemia in normal animals, the fasting hypercholesterolemia and hypertriglyceridemia of nephrosis, and the further marked elevation in FTC and FTG values after the superimposition of the 4% cholesterol/1% cholic acid dietary supplement onto the nephrotic state, were all associated with alterations in macrophage function. These changes included an increase in glomerular macrophage number during peak nephrosis; an increase in basal TXB_2 production by rat peritoneal macrophages in PA/HICHOL animals; and an increase in peritoneal macrophage phagocytosis in the PA/standard chow, normal/HICHOL, and PA/HICHOL groups compared with normals rats on a normal diet. Furthermore, these data suggest that there is a synergistic effect between alimentary hypercholesterolemia and the secondary hypercholesterolemia of nephrosis in producing these functional macrophage alterations. Because FTG values between the two nephrotic groups were not significantly different, it can be speculated that it is elevation of the serum cholesterol value that evokes the changes in macrophage function.

There was a significant increase in TXB_2 produced by isolated rat peritoneal macrophages from PA/HICHOL animals as well as a trend toward higher basal TXB_2 levels in the PA/standard chow and normal/HICHOL groups in contrast with normal rats on a normal diet. It has been previously demonstrated that macrophages, of all leukocytes,[17] are the major producers of prostaglandins, thromboxanes, and other metabolites. Stimulation of arachidonic acid conversion may occur in response to a number of stimuli, including the Fc fragment of immunoglobulins, immune complexes, lymphokines, endotoxin, opsonized bacteria and erythrocytes, zymosan, colchicine, phorbol myristate acetate, and C3a and C3b.[17] Hartung et al[18] showed that acetylated-LDL stimulated production of TXB_2, PGE_2, and LTC_4, suggesting that uptake by the scavenger receptor stimulates both the cyclooxygenase and lipoxygenase pathways of arachidonate. Kelley et al[19] showed that the secretion of beta-glucouronidase activity by human blood monocytes, in response to LDL, was significantly decreased by inhibitors of the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism, indicating a role for these products in arachidonate metabolism in monocyte activation.[19] Another altered macrophage function found in this study was that of phagocytosis of latex beads. Clearly, the nephrotic state or alimentary hypercholesterolemia alone significantly increased phagocytosis; however, synergism between these two perturbations seemed to be apparent by the marked increase in the percentage of cells ingesting three or more latex beads in PA/HICHOL rats.

To reconcile the marked increase in TXB_2 with no significant alterations in PGE_2, it should be emphasized that the amount and relative proportion of any generated arachidonic acid-derived compound critically depend on the source of the macrophages used, their preactivation state, culture conditions, and the nature of the stimulus. Under our isolation conditions we found that, for adherent monocytes, as these rat peritoneal macrophages were, TXB_2 is the predominant arachidonic acid derivative synthesized.[20,21] Thioglycollate broth is an exogenous inducer of inflammation and is associated with a preferential increase in TXB_2 generation over PGE_2, in part by stimulating an influx of blood-born monocytes into the peritoneal cavity.[22] Tripp et al[23] demonstrated that during acute bacterial infection the production of PGI_2, PGE_2, and LTC_4 by macrophages is greatly reduced, whereas the synthesis of TXA_2 remains unchanged, probably through conservation of thromboxane synthetase activity. This occurs in the peritoneum as well as in other organs and this alteration in macrophage arachidonate metabolism appears to be due, in part, to the influx of monocytes to

the site of infection.[23] Blood monocytes produce larger quantities of TXA_2 relative to the other arachidonate metabolites due to a very active thromboxane synthetase activity.[23] Our observations are in accord with those of Cook et al[14] who found that PGE_2 is reduced with relative preservation of TXB_2 in glomerular macrophages isolated from animals with proliferative glomerulonephritis. These authors[14] noted that their findings were similar to those seen in studies of immune-activated mouse peritoneal and granuloma macrophages.[14]

This study confirms observations previously made by Schreiner et al,[7] who demonstrated an increase in glomerular macrophage number during acute aminonucleoside nephrosis. This model of experimental nephrotic syndrome has typically been regarded as a nonimmune model of glomerular barrier dysfunction, in which direct visceral epithelial cell toxicity from PA is associated with increased urine protein excretion. To date, no one has demonstrated increased flux of bone marrow-derived mononuclear cells into the glomerular mesangium in response to hypercholesterolemia and without affecting proteinuria. Our results also support this contention because a nearly threefold significant increase in glomerular macrophage number in PA/HICHOL animals, as contrasted to PA/standard chow rats, was not accompanied by an increase in proteinuria during peak acute aminonucleoside nephrosis. In the normal rat, Sterzel et al[24] showed that daily PVA injections for 4 weeks produced significant increases in mesangial macrophages without consistent elevations in protein excretion. Our present data are also in agreement with this observation because a significant increase in glomerular macrophage number in normal/HICHOL animals over the normal/standard chow group was not accompanied by heightened protein excretion. One could interpret all of these findings as suggesting that, although these bone marrow-derived mononuclear cells have a close temporal association with peak protein excretion during acute aminonucleoside nephrosis, the augmentation of glomerular macrophage number does not exacerbate the severity of the preexisting glomerular lesion, in this case resulting from epithelial cell toxicity after PA delivery. Augmentation of glomerular macrophage number also does not produce deleterious functional alterations in the glomeruli of normal animals. However, because macrophages secrete vasoactive substances, including thromboxane,[25] and because they are also mediators of cellular proliferation and fibrosis,[26] it seems plausible that the secondary hyperlipidemia of nephrosis, especially when amplified by the dietary maneuvers that raise serum cholesterol levels,[6] could unfavorably affect the process of glomerulosclerosis by recruiting these bone marrow-derived cells into the glomerular mesangium. Recently, we noted[27] that lowering the number of glomerular macrophages during the acute phase of glomerular injury with an essential fatty acid-deficient diet was associated with significant reductions in recurrent proteinuria values and a significant lessening in the percentage of glomeruli exhibiting segmental glomerulosclerosis at the conclusion of the study, ie, 18 weeks after PA administration. These data suggest that modulation of glomerular macrophage number, and thus possibly monokine factor release, during acute nephrosis can be associated with the significant functional and histologic sequellae noted during the late, recurrent phase of injury that characterizes chronic aminonucleoside nephrosis.

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