IN VITRO INCUBATION OF LOW-DENSITY LIPOPROTEINS WITH INFLAMMATORY CELLS CAUSES ENHANCED DEGRADATION BY MACROPHAGES IN CULTURE

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Abstract—Utilizing the polyvinyl sponge-implant model, we have reported in vivo modification of low-density lipoproteins (LDL) isolated from interstitial inflammatory fluid (IF) of the rabbit. Further studies on the metabolism of IF-LDL by resident mouse peritoneal macrophages (MPM), demonstrated enhanced uptake and degradation of these modified lipoproteins by scavenger mechanisms. Based upon these studies, we attempted to examine the mechanisms of these observed in vivo modifications in IF-LDL by in vitro incubation of plasma LDL with inflammatory fluid subfraetions. Incubation of LDL with inflammatory cells at 37°C resulted in an increased anodal electrophoretic mobility and alteration in apolipoprotein (APO) composition. Subsequent incubation of this modified plasma LDL with MPM resulted in a significant increase in cell surface binding and an increase in the appearance of degradation products in the medium. The formation of lipid peroxides, measured as thiobarbituric acid-reacting substances (T Bars), increased with the time of LDL incubation with inflammatory cells. Conversely, incubation of LDL with cell-free, lipoprotein-deficient IF (LPDIF, d > 1.210 g/ml) significantly inhibited LDL degradation by MPM. LPDIF did not alter the electrophoretic mobility of LDL or result in the appearance of T Bars in the medium. These results implicate peroxidative reactions associated with an inflammatory response as mediators of the in vivo modifications in IF-LDL which facilitates enhanced uptake via the scavenger receptor in MPM.

INTRODUCTION

Enhanced uptake of lipoproteins by scavenger cells at sites of focal injury in the arterial wall is thought to play a central role in the early development of the atherosclerotic lesion (1). In an attempt to model this mechanism, several laboratories have demonstrated accelerated macrophage uptake of lipoproteins
modified by reaction with acetylating agents and malondialdehyde or by incubation with aortic endothelial cells in culture (2–5). In concert with these in vitro findings, we have reported in vivo modification of low-density lipoproteins (LDL) isolated from interstitial inflammatory fluid (IF) of the rabbit (6). IF-LDL was found to have an altered chemical composition and electrophoretic mobility when compared to LDL isolated from homologous plasma. Further studies examining the metabolism of IF-LDL by resident murine peritoneal macrophages (MPM) in culture reported that IF-LDL is degraded substantially faster by MPM and results in a significant increase in cellular cholesteryl ester when compared with that of macrophages incubated with plasma LDL (7). In addition, competition studies demonstrated primary macrophage uptake of IF-LDL to occur by the scavenger receptor pathway, similar to that described for chemically modified LDL (2, 3). Based upon these findings, we attempted to examine the mechanisms which facilitate modification of IF-LDL by in vitro incubation of plasma LDL with constituents of interstitial inflammatory fluid.

MATERIALS AND METHODS

Adult, male New Zealand white rabbits were utilized for these studies. All animals were housed individually on a 12-h light–dark schedule, and fed standard laboratory rabbit chow (Wayne, Chicago, Illinois) and water ad libitum. Interstitial inflammatory fluid was prepared by aseptic subcutaneous implantation of polyvinyl sponges (Ivalon, Unipoint, High Point, North Carolina) as previously described (6). At the time of sponge explantation, the animals were reanesthetized and the sponges removed via the original incision.

Lipoprotein Preparation. Blood was obtained from anesthetized New Zealand white rabbits by cardiac puncture and transferred to tubes containing disodium diamine ethylene tetraacetate (Na₂EDTA, 1 mg/ml). Low-density lipoproteins (LDL, d = 1.019–1.063 g/ml) were prepared by sequential density ultracentrifugation as described by Havel et al. (8). Each fraction was subjected to a wash spin at maximum density and exhaustively dialyzed against 0.15 M NaCl, 1 mM Na₂EDTA, pH 8.6. Lipoproteins were iodinated with [¹²⁵I]NaI (New England Nuclear, Boston, Massachusetts) in the presence of Iodo-gen (Pierce Laboratories, Rockford, Illinois) as previously described (9). Lipoprotein-deficient inflammatory fluid (LPDIF) was prepared by adjustment of cell-free, inflammatory fluid to d = 1.210 g/ml with solid KBr, and ultracentrifugation at 172,000g for 48 h at 10°C. The infranatant fraction was removed by tube slicing and extensively dialyzed prior to use. Lipoprotein-deficient plasma (LPDP) was prepared in similar fashion.

Cell Culture. Inflammatory cells were obtained by centrifugation of interstitial inflammatory fluid at 300g, utilizing sterile plastic tubes (Falcon). Cells were washed 3 × with sterile phosphate-buffered saline (0.15 M NaCl/0.01 M sodium phosphate; PBS) at pH 7.4, and resuspended in Ham’s F-10 media (Gibco, Grand Island, New York). Resident peritoneal macrophages were obtained by lavage from female Swiss Webster mice and plated onto Linbro Multiwell plates (Flow, McLean, Virginia) as previously described (7).

Cellular Assays. In a typical experiment, 2–3 mg of 0.45 μm filtered [¹²⁵I]LDL was added to suspensions of inflammatory cells (1 × 10⁶ cells/μg LDL protein) in Ham’s F-10 media with penicillin/streptomycin. Lipoproteins were incubated at 37°C in a humidified air (5% CO₂) incubator for up to 48 h. Additional dishes contained identical amounts of LDL in F-10 incubated at 37°C in the absence of cells. Equivalent amounts of LDL in F-10 maintained at 4°C were utilized