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Zymosan phagocytosis by mouse peritoneal macrophages is increased by apoHDL- and not by intact HDL-covered particles

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Abstract

The uptake of lipids and lipoprotein particles by macrophages undergoes phagocytic activation and the formation of foam cells are key events in atherosclerosis. In this study we determined how intact high density lipoproteins (HDL) and apolipoproteins-HDL (removal of the lipid component from HDL, i.e., apoHDL) influence the phagocytosis of zymosan by mouse peritoneal macrophages. Zymosan particles preincubated together with lipoproteins or alone (control) were incubated with the macrophages. Phagocytosis activity was reported as the percent of macrophages that internalized three or more zymosan particles. HDL co-incubated with zymosan did not influence the overall uptake of zymosan particles compared to apoHDL, which greatly enhanced the ability of the particle to be phagocytized ($P<0.001$). Part of this effect might be related to a greater binding of apoHDL to the particles compared to that of HDL ($P<0.05$). We conclude that this can be a useful method to study the ability of lipoproteins, including modified lipoproteins obtained from subjects with genetic forms of hyperlipidemia, to opsonize particles such as red blood cells and thus to investigate the processes that control the formation of foam cells and the mechanisms of atherogenesis.

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Macrophages play a central role in the recognition, internalization, and degradation of cell ghosts (1). The phagocytic process in monocytes-macrophages represents a fundamental biological mechanism whereby lipoprotein molecules from biological tissue and fluids are rapidly internalized (2,3). In most cases this is beneficial for the organism but in some cases may contribute to the pathogenesis of an inflammatory disease. In this regard, mononuclear phagocytes play a major role in atherogenesis, where monocyte-derived macrophages and macrophage-derived foam cells in the arterial tissue undergo phagocytic activation (4).

A key event in atherosclerosis is the formation of lipid-laden foam cells resulting from the uptake of lipoproteins by resident macrophages at the arterial intima level (2), although not all the mechanisms involved

are fully understood. For instance, several studies suggest that high density lipoproteins (HDL) have anti-atherogenic properties which very probably involve their capacity to remove cell cholesterol (5). The role of these lipoproteins in phagocytosis has been investigated to a lesser extent (6). To study the phagocytic process we have used an assay that measures the uptake of inert zymosan particles by mouse peritoneal macrophages and demonstrates how this is modified by intact HDL and by apolipoproteins-HDL (apoHDL).

The uptake of zymosan by macrophages has been classified as nonspecific phagocytosis, and this characteristic has been used to distinguish it from receptor-mediated uptake of ligands such as immunoglobulin G (IgG) or complement (7,8). The interaction of coated zymosan particles with cells also involves a number of important biological functions such as superoxide generation, which may oxidize low density lipoproteins (LDL) and HDL, the release of a variety of enzymes, and phagocytosis (9,10).

HDL (d = 1.063-1.21 g/ml) from plasma of healthy volunteer blood donors was isolated by sequential preparatory ultracentrifugation (11). ApoHDL was prepared by extracting the HDL preparation with pre-cooled (-20°C) diethyl ether-ethanol (3:2 v/v) and further processed as described in Ref. 12. This procedure resulted in a 96% reduction of the lipid content.

Resident mouse peritoneal exudates were used as source of macrophages. The cells were harvested from mice in phosphate-buffered saline (PBS), pH 7.4, and washed twice in PBS, and their concentration was adjusted with RPMI 1640 medium containing antibiotics (13).

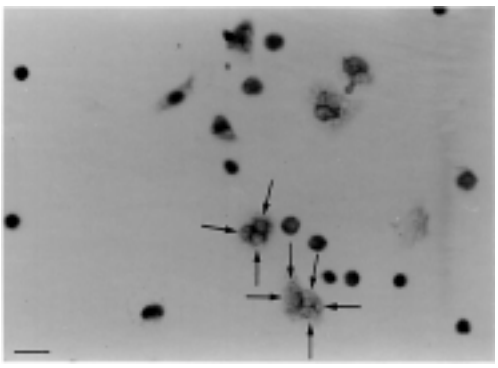

Figure 1 - Phagocytosis assay. Phagocytic macrophages were identified as cells that internalized three or more zymosan particles. The identification of phagocytosis in a typical experiment is indicated by arrows. Macrophages were stained with Leishman solution (13). Magnification, 400X; bar represents 10 µm.

The percentage of phagocytized particles was determined by the method of Miller (14). Briefly, 10^6 cells in 1 ml RPMI 1640 were placed on coverslips in Leighton tubes and preincubated at 37°C for 30 min in a humidified 5% CO_2 atmosphere. Non-bound cells were removed by washing. Meanwhile, 10^7 zymosan particles in 1 ml RPMI 1640 were preincubated at 37°C for 30 min with either 10 mg protein/ml of intact HDL or apoHDL. Zymosan particles preincubated together with lipoproteins or zymosan particles alone (control) were added to the cells that adhered to the coverslips and incubated for an additional 30 min at 37°C in a humid 5% CO_2 atmosphere. The coverslips were then washed with pre-warmed RPMI 1640 medium to remove excess particles, stained with Leishman solution (15) and read under the light microscope. The ANOVA test was used for statistical comparison of the percentage of macrophages that phagocytized zymosan particles; a 0.05-confidence level was considered significant.

The phagocytosis of zymosan targeted for removal by macrophages is a two-step process initiated by its recognition and binding, followed by internalization (16). Due to this sequence the number of phagocytic macrophages was defined as the number of cells that internalized three or more zymosan particles. The results were then reported as percent phagocytic macrophages. At least 100 cells were counted on each coverslip. The visual identification of phagocytosis by 2 macrophages in a typical experiment is indicated by arrows in Figure 1.

Zymosan particles (10^8) were incubated with HDL or apoHDL (10 mg/ml) or with no addition (control) at 37°C for 30 min. After incubation the particles were washed extensively with RPMI 1640, and the HDL or

apoHDL protein content was determined by the method of Lowry et al. (17). The results are reported as µg protein coated/10^8 zymosan particles. The coated amounts of apoHDL and of HDL were compared by the Student *t*-test; a 0.05-confidence level was considered significant.

The pooled data from 5 independent experiments using different preparations of HDL and apoHDL are shown in Table 1. It can be seen that when apoHDL was used there was a significantly higher percent of macrophages presenting phagocytosis. This was seemingly due to the fact that 80% more protein is available in zymosan as apoHDL than as intact HDL. In this regard, as compared to intact LDL, intact HDL_3 has relatively more protein and is more efficient in stimulating erythrocyte phagocytosis through the Fc receptor (18).

By using zymosan particles preincubated with lipoproteins we could demonstrate the different biological effects of HDL and of apoHDL on the capacity of macrophages to promote phagocytosis. Furthermore, this method could be used to determine how these lipoproteins, including modified lipoproteins such as oxidized LDL, interfere with the phagocytosis of bacterial and viral pathogens, damaged or senescent cells (19), and cell ghosts (16).

Table 1 - Effect of preincubated zymosan (Zy) particles with intact HDL and apoHDL on their phagocytosis by mouse peritoneal macrophages.

Data are reported as means ± SD for 5 experiments in which N = 5 coverslips were used in each group, and for which two different preparations of intact HDL and apoHDL were used. Phagocytic activity is reported as the percent of macrophages that internalized three or more Zy particles (see Figure 1). *P<0.001 for apoHDL compared to zymosan alone or to HDL (ANOVA). The mean concentrations of HDL and apoHDL protein-coated Zy particles were obtained by the method of Lowry et al. (17). Results are reported as µg protein coated/10^8 Zy. $^+$P<0.05 for apoHDL compared to HDL (Student *t*-test).

Groups	Phagocytic activity (%)	µg Protein coated/ 10^8 particles
Untreated zymosan	23.6 ± 1.9	–
HDL	23.6 ± 4.6	10.1 ± 2.2
ApoHDL	40.6 ± 1.0*	18.7 ± 3.6$^+$

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