Binding of β-VLDL to Heparan Sulfate Proteoglycans Requires Lipoprotein Lipase, Whereas ApoE Only Modulates Binding Affinity

Femke de Beer, Wendy L. Hendriks, Leonie C. van Vark, Sylvia W.A. Kamerling, Ko Willems van Dijk, Marten H. Hofker, Augustinus H.M. Smelt, Louis M. Havekes

Abstract—The binding of β-VLDL to heparan sulfate proteoglycans (HSPG) has been reported to be stimulated by both apoE and lipoprotein lipase (LPL). In the present study we investigated the effect of the isoform and the amount of apoE per particle, as well as the role of LPL on the binding of β-VLDL to HSPG. Therefore, we isolated β-VLDL from transgenic mice, expressing either APOE*2(Arg158→Cys) or APOE*3-Leiden (E2-VLDL and E3Leiden-VLDL, respectively), as well as from apoE-deficient mice containing no apoE at all (Enull-VLDL). In the absence of LPL, the binding affinity and maximal binding capacity of all β-VLDL samples for HSPG-coated microtiter plates was very low. Addition of LPL to this cell-free system resulted in a 12- to 55-fold increase in the binding affinity and a 7- to 15-fold increase in the maximal binding capacity (B_max). In the presence of LPL, the association constant (K_a) tended to decrease in the order Enull-VLDL<E2-VLDL<E3Leiden-VLDL, whereas B_max increased in the reverse order: E3Leiden-VLDL=E2-VLDL<Enull-VLDL. Addition of LPL resulted in a marked stimulation of both K_a and B_max for binding of β-VLDL samples to J774 cells similar to that found for the binding to HSPG-LPL complexes. Our results indicate that both K_a and B_max for binding of β-VLDL to HSPG are increased more than 1 order of magnitude on addition of LPL. In addition, for the binding of β-VLDL to HSPG-LPL complexes, the presence of apoE is not a prerequisite, but results in an increased binding affinity, depending on the apoE isoform used. (Arterioscler Thromb Vasc Biol. 1999;19:633-637.)

Key Words: heparan sulfate proteoglycans ■ lipoprotein lipase ■ apoE ■ β-VLDL

Heparan sulfate proteoglycans (HSPG) are negatively charged polysaccharides consisting of a core protein and the glycosaminoglycan (GAG) heparan sulfate. In addition to their role in cell adhesion and cell growth, HSPG are suggested to be involved in the metabolism of VLDL. The interaction of VLDL with HSPG has been reported to be mediated in at least 2 ways. The first way is through apoE, which contains heparin-binding sites. The importance of apoE for this interaction has been described in several reports. Mahley et al. have postulated that HSPG may mediate the clearance of VLDL and chylomicron remnants in a so-called secretion-recapture process. After entering the space of Disse, remnant particles become enriched in apoE excreted by the hepatocytes. The increased amounts of apoE enhance the binding of the remnants to HSPG on the surface of hepatocytes, followed by internalization of the particles through either the LDL receptor (LDLR) or the LDL receptor-related protein (LRP). Ji et al. showed that the addition of apoE enhances the binding of rabbit β-VLDL to HSPG present on various cell types. Furthermore, it was shown that different variants of apoE display a variable affinity for HSPG: rabbit β-VLDL enriched in apoE2(Arg158→Cys) bound to HSPG with increased affinity, whereas addition of the dominant apoE3-Leiden variant did not lead to increased binding to HSPG.

In addition to apoE, there is strong evidence that the binding of VLDL to HSPG is mediated by lipoprotein lipase (LPL, EC 3.1.1.34). In 1977 it was suggested that LPL, which is anchored to HSPG on the luminal surface of endothelial cells, functions as a bridge between VLDL and HSPG during lipolysis of VLDL-triglycerides. More recently, it was demonstrated by several investigators (for a review, see ), including our own group, that LPL can enhance the binding and uptake of several classes of lipoproteins by different cell types through bridging between the lipoproteins and HSPG. The hydrolytic activity of LPL is not required for this bridging function.

Recently, we developed a cell-free system in which HSPG is coated to microtiter plates to investigate the direct interaction of lipoproteins with HSPG. In the present study, the
binding of mouse β-VLDL was studied to further elucidate the effect of the amount of apoE per particle and the apoE isoform, as well as the role of LPL on the interaction of β-VLDL with HSPG. We found that both the binding affinity and maximal binding of β-VLDL directly to HSPG was very low and that addition of LPL resulted in a marked stimulation. Furthermore, it appeared that for the binding of β-VLDL to HSPG-LPL complexes, the presence of apoE is not a prerequisite, but results in an increased binding affinity.

Methods

Cells

Murine macrophage-like J774 cells were cultured in 75-cm² flasks in DMEM supplemented with 10% (vol/vol) FCS, 0.85 g/L NaHCO₃, 4.76 g/L HEPES, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 2 mM/L glutamine. The cells were incubated at 37°C in an atmosphere containing 5% CO₂ in air. For each experiment, cells were plated in 24-well plates. The cells were fed every 3 days and used for experiments within 7 days of plating.

Animals

APOE*3-Leiden and APOE*2 transgenic mice nineteen-twenty were cross-bred with apoe⁻/⁻ mice twenty-one-twenty-three to obtain mice that produce VLDL that contains apoE3-Leiden or apoE2 without the endogenous mouse apoE protein. Subsequently, these APOE*3-Leiden·apoE⁻/⁻ and APOE*2·apoE⁻/⁻ mice were cross-bred with LDLR-deficient (Ldlr⁻/⁻) mice (Jackson Laboratory, Bar Harbor, ME) to obtain mice that accumulate β-VLDL in high amounts. Mice were housed under standard conditions with free access to water and food. All mice were fed a standard rat mouse-A diet (Hope Farms).

Lipoproteins

After a 4-hour fasting period, blood was collected from 10 to 25 mice by orbital puncture. Serum was separated from the blood cells by centrifugation at 1500 g for 15 minutes at room temperature. Pooled sera were ultracentrifuged to isolate β-VLDL (d<1.006 g/mL).

Protein content of the β-VLDL samples was determined by the method of Lowry et al. Twenty-four hours before each experiment, cells were washed with DMEM containing 5% (vol/vol) of lipoprotein-deficient serum. Twenty-four hours before each experiment, cells were washed with DMEM containing 5% (vol/vol) of lipoprotein-deficient serum.

LPL was purified from fresh bovine milk as described previously. Eighteen Briefly, cells were plated in 24-well plates. The cells were fed every 3 days and used for experiments within 7 days of plating.

Results

β-VLDL Composition

To obtain β-VLDL particles containing high amounts of apoE2(Arg158→Cys), apoE3-Leiden, or no apoE at all, β-VLDL was isolated from APOE*2·apoE⁻/⁻·Ldlr⁻/⁻ mice (E2-VLDL), APOE*3-Leiden·apoE⁻/⁻·Ldlr⁻/⁻ mice (E3-Leiden-VLDL) and apoE⁻/⁻ mice (Enull-VLDL), respectively. As shown in Table 1, β-VLDL isolated from these transgenic mice were all cholesterol ester-rich particles. Particle size measurements showed that both E2-VLDL and E3-Leiden-VLDL were enlarged compared with Enull-VLDL. In addition, these particles were very rich in apoE: 48 and 66 apoE molecules per particle, respectively, versus 2 apoE molecules in human apoE3/3 VLDL.

Binding of β-VLDL to HSPG in the Absence or Presence of LPL

To investigate the effect of the amount of apoE and the apoE isoform on the interaction of β-VLDL with HSPG, we determined the binding of β-VLDL containing no apoE at all (Enull-VLDL) and of β-VLDL rich in apoE (E2-VLDL and E3-Leiden-VLDL) to microtiter plates coated with HSPG. In the absence of LPL, the binding to HSPG of all β-VLDL particles was very low (Figure 1A). Nevertheless, we performed Scatchard analysis, revealing that both the association constant (Kₐ)
and the maximal binding ($B_{\text{max}}$) increased in the order E2-VLDL-Enull-VLDL-E3Leiden-VLDL (Table 2).

Addition of LPL to this cell-free system resulted in a marked increase in the binding of all β-VLDL particles to HSPG, as compared with that in the absence of LPL (compare Figure 1B with 1A). The values of the $K_d$ for the binding of the particles in the presence of LPL were 12- to 55-fold higher than in the absence of LPL and tended to increase in the order Enull-VLDL-E2-VLDL-E3Leiden-VLDL (Table 2). The values of the $B_{\text{max}}$ were also increased compared with those in the absence of LPL (7- to 15-fold). $B_{\text{max}}$ values in the presence of LPL increased in the reverse order: E3Leiden-VLDL-E2-VLDL-Enull-VLDL, probably because of the size of the particles (Table 1).

These results indicate that, even despite very high amounts of apoE2 on the surface of the β-VLDL particle, the $K_d$ and $B_{\text{max}}$ of β-VLDL to HSPG coated to microtiter plates were very low compared with that in the presence of LPL. Strikingly, in the cell-free system, the $K_d$ and $B_{\text{max}}$ of Enull-VLDL to HSPG was in the same order of magnitude as that of the 2 apoE-containing particles, both in the absence and in the presence of LPL.

**Binding of β-VLDL to J774 Macrophages in the Absence or Presence of LPL**

To determine whether similar results could be obtained in a more physiological system, we performed binding experiments using J774 macrophages instead of HSPG coated to microtiter plates. Like other macrophages, J774 cells express an extracellular matrix containing HSPG, although they do not secrete apoE.31,32 As expected, binding of all β-VLDL samples to J774 cells in the absence of LPL was higher compared with that in the cell-free system (Figure 2A). This is because of the presence of lipoprotein receptors on the cell membrane. As expected from previous results, Enull-VLDL and E2-VLDL both displayed a very low affinity to these receptors compared with E3Leiden-VLDL (Table 3).

Addition of LPL resulted in a 6- to 11-fold stimulation of the maximal binding to J774 cells of all β-VLDL particles tested (Figure 2 and Table 3), similar to that found for the HSPG-LPL complexes coated to microtiter plates (Figure 1 and Table 2). Furthermore, Table 3 shows that the $K_d$ for the binding of β-VLDL to J774 cells in the presence of LPL tended to increase in the same order as found for the binding of β-VLDL to HSPG-LPL in microtiter plates: Enull-VLDL-E2-VLDL-E3Leiden-VLDL. Again, as for the cell-free system, the values for the $B_{\text{max}}$ increased in the reverse order.

These results show that the data obtained using the HSPG-coated microtiter plates were similar to those obtained using a cell culture system, supporting the suitability of this cell-free system for testing the binding affinities of lipoproteins to HSPG.

**Discussion**

It has previously been shown for all lipoproteins, except rabbit β-VLDL, that the binding to HSPG is very low,
TABLE 2. Binding Characteristics of 125I-Labeled β-VLDL to HSPG in the Absence or Presence of LPL

<table>
<thead>
<tr>
<th>β-VLDL</th>
<th>K_a (mL/μg)</th>
<th>B_max (ng/well)</th>
<th>K_a (mL/μg)</th>
<th>B_max (ng/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enull-VLDL</td>
<td>0.12±0.03</td>
<td>1.0±0.1</td>
<td>2.6±0.5</td>
<td>14.6±0.2</td>
</tr>
<tr>
<td>E2-VLDL</td>
<td>0.06±0.02</td>
<td>0.7±0.2</td>
<td>3.3±1.2</td>
<td>9.7±1.2</td>
</tr>
<tr>
<td>E3-Leiden-VLDL</td>
<td>0.39±0.11</td>
<td>1.2±0.2</td>
<td>4.5±1.6</td>
<td>8.4±0.4</td>
</tr>
</tbody>
</table>

The specific binding of 125I-labeled β-VLDL to microtiter plates coated with HSPG was determined as described in the Methods section. Association constants were calculated by Scatchard analysis based on the amount of apoB protein, which reflects particle number. The values represent the mean±SD of 3 measurements.

whereas addition of LPL increases this binding several fold.33 In addition to LPL, an excess of apoE results in a marked enhancement of the binding of rabbit β-VLDL to HSPG.4 Furthermore, different isoforms of apoE display variable interactions with HSPG: addition of apoE2(Arg158→Cys) results in a marked stimulation of the binding of rabbit β-VLDL to HSPG present on hepatocytes and fibroblasts, whereas addition of apoE3-Leiden does not stimulate this binding.6

In the present study we investigated the influence of LPL as well as the effect of high amounts of apoE isoforms on the binding of mouse β-VLDL to HSPG compared with the binding of β-VLDL containing no apoE at all. For this purpose, binding experiments and Scatchard analyses were performed using both HSPG coated to microtiter plates and HSPG present on J774 cells, either in the absence or presence of LPL. We found that in the absence of LPL, the K_a and B_max for the binding of all β-VLDL particles to HSPG coated to microtiter plates were in the same order of magnitude, but very low. These results indicate that in the presently used system, apoE is not an essential factor for the binding of β-VLDL to HSPG.

Surprisingly, the K_a for the binding to HSPG of E2-VLDL, containing high amounts of apoE2, was even decreased compared with E3-Leiden-VLDL or Enull-VLDL. This is in contrast to the data of Ji et al.5,15 and Mann et al.,7 who showed that rabbit β-VLDL enriched in apoE variants associated with a dominant mode of inheritance of type III hyperlipoproteinemia (ie, apoE3-Leiden) bound less efficiently to HepG2 cells, McA-RH7777 cells, and isolated HSPG, compared with rabbit β-VLDL enriched in apoE variants associated with the recessive form [ie, apoE2(Arg158→Cys)]. This discrepancy can be explained by several reasons. First, in our study isolated mouse β-VLDL that contained only the respective apoE variants was used, whereas in the experiments of Ji et al.,5 cells were incubated with rabbit β-VLDL that contained endogenous apoE in addition to the respective exogenously added apoE variants. Second, Ji and coworkers7 also used McA-RH7777 cells transfected with human apoE isoforms to test the effect of apoE secretion on the binding of rabbit β-VLDL. They found that in comparison with the nontransfected cells, the apoE2-secreting cells displayed a 2-fold enhancement in the binding of β-VLDL, whereas there was no enhancement of the binding of β-VLDL to the apoE3-Leiden-secreting cells. Therefore, we hypothesize that the presence of free apoE during the binding experiments and the method used to enrich β-VLDL with apoE is of importance for the outcome of the experiments. This hypothesis is supported by the data of Mann et al.,7 who found that the presence of free apoE enhances the binding of apoE-enriched β-VLDL to HSPG to a larger extent, compared with apoE-enriched β-VLDL that has been reisolated before the binding experiment. Third, Ji et al.5 performed a dot-blot assay, using HSPG coated to nitrocellulose membranes, to test direct binding of β-VLDL to HSPG. This difference in methodology may also (partly) explain the difference between their and our results.

Rabbit β-VLDL has been shown to bind directly to HSPG,4,33 whereas in the present study the binding of mouse β-VLDL was low. Whether this discrepancy could be explained by the presence of LPL on rabbit β-VLDL particles, whereas mouse β-VLDL does not contain LPL, is at present only subject to speculation. In this respect, it is important to note that several studies have indicated that in human preheparin and postheparin plasma, LPL is attached to LDL-like particles34–36 and VLDL.37

In accordance with Lookene et al.31 we showed that addition of LPL markedly enhanced K_a and B_max values of all β-VLDL particles, including that of apoE-deficient VLDL, to both HSPG coated to microtiter plates and HSPG present on the plasma membrane of J774 cells. Furthermore, the presence of apoE was not a prerequisite, but resulted in an increased K_a. For both systems, in the presence of LPL, the values for the K_a tended to increase in the order Enull-VLDL<E2-VLDL<E3-Leiden-VLDL, whereas the values for the B_max decreased in this order. The latter can be explained by differences in steric hindrance, caused by a different size of the particles (Table 1). Calculations revealed that, at B_max≈10^6 E3-Leiden-VLDL particles were bound to HSPG-LPL complexes coated to the surface of one well versus 3.5×10^5 Enull-VLDL particles. This is in accordance with the observation that E3-Leiden VLDL was approximately 2-fold larger compared with Enull-VLDL (Table 1). Another explanation for a lower K_a of LPL for E3-Leiden particles may be the high number of apoE molecules per VLDL particle.

There are several reports suggesting that in the arterial wall, LPL is involved in the atherosclerotic process by enhancing the uptake of lipoproteins by macrophages and smooth muscle cells, probably in a process mediated by HSPG.15,16,38 The fact that macrophages in atherosclerotic plaques synthesize both LPL39 and HSPG40,41 further supports the proatherogenic role of LPL in the intima of the vessel wall. It has also been shown that

TABLE 3. Binding Characteristics of 125I-Labeled β-VLDL to J774 Macrophages in the Absence or Presence of LPL

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<tr>
<th>β-VLDL</th>
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<th>B_max (ng/mg)</th>
<th>K_a (mL/μg)</th>
<th>B_max (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enull-VLDL</td>
<td>0.6±0.1</td>
<td>56.8±7.3</td>
<td>0.9±0.1</td>
<td>604±33</td>
</tr>
<tr>
<td>E2-VLDL</td>
<td>0.3±0.1</td>
<td>53.2±7.7</td>
<td>1.2±0.3</td>
<td>314±9</td>
</tr>
<tr>
<td>E3-Leiden-VLDL</td>
<td>4.0±2.1</td>
<td>39.8±6.8</td>
<td>4.0±1.3</td>
<td>274±21</td>
</tr>
</tbody>
</table>

The specific binding of 125I-labeled β-VLDL to J774 macrophages in the absence or presence of LPL was determined as described in the Methods section. Association constants were calculated by Scatchard analysis based on the amount of apoB protein, which reflects particle number. The values represent the mean±SD of 3 measurements.
cholesterolen-loading of macrophages enhances the apoE secretion by these cells, indicating that apoE is abundantly present in atherosclerotic lesions. These findings, together with the present finding that apoE further enhances the binding affinity of β-VLDL for HSPG-LPL complexes, indicate the importance of the combined roles of LPL, apoE, and HSPG in the atherosclerotic process.

Acknowledgments

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References

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