Interleukin-1β (IL-1β) and TNF-α are known to contribute to the pathogenesis of cardiovascular diseases. We have previously shown that both cytokines can increase interleukin-1 receptor antagonist (IL-1Ra) expression in human aortic smooth muscle cells (HASMC) and that this effect is mediated by NF-κB and the mitogen-activated protein kinase (MAPK) signaling pathways.

In the present study, we investigated the effects of IL-1β and TNF-α on IL-1Ra expression in HASMC in the presence of statins. We observed that both cytokines increased IL-1Ra expression in HASMC, and this effect was attenuated by the addition of statins. The attenuation of IL-1Ra expression by statins was associated with a decrease in NF-κB and MAPK activation, suggesting that the anti-inflammatory effects of statins may be mediated, at least in part, through the inhibition of cytokine-induced IL-1Ra expression.
The red blood cells were removed by centrifugation at 4000 × g for 10 minutes at 4°C. The plasma was then collected and analyzed for TG and TC levels with the enzymatic assay kits (TG with kits from Worthington or Gilford Diagnostics: Worthington Diagnostics, Freehold, New Jersey; Gilford Diagnostics, Cleveland, Ohio); TC with kits from Worthington or BMC (Biodynamics/BMC, Indianapolis, Indiana) using a Gilford 3500 analyzer. Cord plasma having levels of either TG or TC exceeding 100 mg/100 ml were excluded, and the remaining cord plasmas were either pooled according to sex or used individually in an effort to examine only "normal" lipoprotein species. Cord blood samples having either TG or TC levels above 100 mg/dl were excluded, as this exceeded the average obtained from the literature by more than two standard deviations.

The lipoproteins were isolated from the pooled or individual plasma by ultracentrifugation according to Lindgren,12 except that the separations were all performed at 4°C; all solutions contained 0.1% EDTA and 0.02% sodium azide to minimize degradation. The VLDL were removed by centrifugation at 100,000 × g for 24 hours at d = 1.006 g/ml. The density of the subnatant was then adjusted to d = 1.063 g/ml and centrifuged for 24 hours at 100,000 × g to isolate the total LDL fraction. The total LDL (d = 1.006 to 1.063 g/ml) was separated into IDL and LDL by adjusting the density to 1.019 by dialysis followed by centrifugation at 100,000 × g for 24 hours. The IDL, the upper 3 ml, and the LDL, the lower 3 ml, were then pipetted into screw-cap vials with Teflon caps and stored at 4°C until used for analysis.

Analytical Procedures

The protein content of the lipoprotein fractions was determined by the modified Lowry protein determination of Markwell et al.13 using bovine serum albumin (BSA) (Miles, Elkhart, Indiana) as a protein standard. Phospholipid, as inorganic phosphate, was determined by ashing, followed by a phosphate analysis as detailed by Ames.14 Free and total cholesterol were determined by the enzymatic method of Allain et al.15 and the esterified cholesterol level was obtained as a difference between the total and free cholesterol. Triglyceride was determined using either the chemical method of Biggs et al.16 or the enzymatic method using the triglyceride reagent kit from Worthington. Values for triglyceride, cholesterol, esterified cholesterol, and phospholipid were calculated using 850, 387, 650, and 775 as the respective molecular weights. Mean values and standard error (SEM) were calculated by standard techniques. Data comparisons were analyzed for statistical significance by the Student’s t test.

Sodium dodecylsulfate (SDS) gel electrophoresis on 10% polyacrylamide gels was performed by the method of Weber and Osborn17 using mercaptoethanol as the reductant. SDS gel electrophoresis using 3% polyacrylamide gels was performed as described by Kane et al.18 except that ovalbumin was added as an internal standard and the Rf was measured relative to the ovalbumin band. Gradient gel electrophoresis (GGE) on 2% to 16% gels (Pharmacia, Piscataway, New Jersey) was performed as described by the manufacturer. The gels were fixed and stained as detailed by Blanche et al.19 Stained gels were scanned with an RFT densitometer (Transidyne Corporation, Ann Arbor, Michigan) at a wavelength of 630 nm. The molecular weight and diameter standards employed were thyroglobulin (Schwert/Mann, Orange, New Jersey), cross-linked BSA (Sigma, St. Louis, Missouri), the high molecular weight standard kit (Pharmacia, Piscataway, New Jersey), and carboxylated latex beads (Dow Diagnostics, Indianapolis, Indiana). For electron microscopy, the samples were dialyzed against 1% ammonium acetate, pH 7.4, and then negatively stained with 2% sodium phosphotungstate, as previously described.20 The size and size distribution were determined using the computerized particle analysis system described previously.20

Results

The cord blood plasma TG and TC levels were determined on 273 female and 309 male neonates. The TG values were 41.9 ± 17.6 SEM mg/100 ml and 40.4 ± 16.7 mg/100 ml for females and males, respectively, with no statistically significant difference between the two values. The TC level for female neonates was 67.5 ± 16.3 mg/100 ml, and for male neonates, 62.3 ± 14.6 mg/100 ml.
Table 1. Total Composition (mg/100 ml) of IDL and LDL from Human Cord Blood Plasma

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Phospholipid</th>
<th>Free cholesterol</th>
<th>Esterified cholesterol</th>
<th>Triglyceride</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>0.86 ± 0.26</td>
<td>1.63 ± 0.51</td>
<td>0.69 ± 0.18</td>
<td>1.75 ± 0.70</td>
<td>1.63 ± 0.28</td>
<td>6.57 ± 0.96</td>
</tr>
<tr>
<td>male</td>
<td>0.95 ± 0.29</td>
<td>1.63 ± 0.54</td>
<td>0.69 ± 0.13</td>
<td>1.63 ± 0.53</td>
<td>1.92 ± 0.48</td>
<td>6.82 ± 0.72</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>16.17 ± 1.58</td>
<td>18.83 ± 4.81</td>
<td>7.31 ± 0.50</td>
<td>26.13 ± 2.99</td>
<td>8.42 ± 1.00</td>
<td>76.86 ± 5.99</td>
</tr>
<tr>
<td>male</td>
<td>15.75 ± 2.10</td>
<td>17.44 ± 4.96</td>
<td>7.20 ± 0.70</td>
<td>24.77 ± 3.90</td>
<td>9.25 ± 1.10</td>
<td>74.41 ± 6.78</td>
</tr>
</tbody>
</table>

IDL = intermediate density lipoproteins; LDL = low density lipoproteins.
Values are means ± standard error, from six female and six male neonates.

Table 2. Percent Composition of IDL and LDL in Human Cord Blood Plasma

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Phospholipid</th>
<th>Free cholesterol</th>
<th>Esterified cholesterol</th>
<th>Triglyceride</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>13.1 ± 4.4</td>
<td>24.8 ± 5.6</td>
<td>10.5 ± 3.1</td>
<td>26.6 ± 11.3</td>
<td>24.8 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>13.9 ± 4.5</td>
<td>23.9 ± 8.3</td>
<td>10.1 ± 2.2</td>
<td>23.9 ± 8.2</td>
<td>26.2 ± 10.9</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>21.0 ± 2.6</td>
<td>24.5 ± 6.5</td>
<td>9.5 ± 1.0</td>
<td>34.0 ± 4.7</td>
<td>11.0 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>21.2 ± 3.4</td>
<td>23.4 ± 7.0</td>
<td>9.7 ± 1.3</td>
<td>33.3 ± 6.1</td>
<td>12.4 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

IDL = intermediate density lipoproteins; LDL = low density lipoproteins.
Values are means ± standard error, from six female and six male neonates.

The composition and concentration of the neonatal IDL and LDL of both sexes are presented in table 1. It is immediately apparent that the diminished lipid levels of the neonate cord blood are mirrored in the greatly reduced concentrations of the IDL (6.57 ± 0.96 mg/100 ml in females; 6.82 ± 0.72 mg/100 ml in males) and the LDL (76.86 ± 5.99 mg/100 ml in females; 74.41 ± 6.78 mg/100 ml in males).

The distribution of lipid and protein moieties in both IDL and LDL is shown in table 2. The IDL of both male and female neonates was characterized by an enrichment of the TC and phospholipid. The LDL of the neonate showed a TG elevation; approximately 12% of the mass is TG compared to approximately 5% in the adult LDL (d = 1.019 to 1.063 g/ml). Cholesteryl ester, however, is the major core lipid of LDL particles, as indicated by the EC/TG ratio of 3.75 seen in table 3. IDL, in contrast, has a relatively larger portion of the core composed of TG, as shown by the EC/TG ratio of 1.24. The molar ratio of free cholesterol to phospholipid (FC/PL) is constant for both fractions, although the esterified cholesterol to free cholesterol (EC/FC) is decreased in both the IDL and LDL fractions (1.45 and 2.09, respectively) compared to the adult values (2.04 and 2.38 for adult IDL and LDL, respectively).

The size distributions of the neonatal IDL and LDL, as determined by densitometric scanning of the 2% to 16% gradient gels after staining for protein, are presented in figure 1 along with the size distribution for the total LDL fraction (d = 1.006 to 1.063 g/ml). The IDL, when analyzed by GGE, showed a single somewhat broad peak with a maximum corresponding to a calculated diameter of 300 Å, which is in good agreement with no statistically significant difference between the two values. Approximately 3% of the cord blood plasma analyzed had levels of either TG or TC exceeding the 100 mg/100 ml cutoff point, and thus were excluded.

Table 3. Molar Ratios of Components of IDL and LDL in Human Cord Blood Plasma

<table>
<thead>
<tr>
<th></th>
<th>EC/FC</th>
<th>FC/PL</th>
<th>EC/TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>1.51 ± 0.72</td>
<td>0.85 ± 0.34</td>
<td>1.40 ± 0.61</td>
</tr>
<tr>
<td>male</td>
<td>1.41 ± 0.53</td>
<td>0.85 ± 0.32</td>
<td>1.11 ± 0.46</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>2.13 ± 0.28</td>
<td>0.78 ± 0.21</td>
<td>4.06 ± 0.67</td>
</tr>
<tr>
<td>male</td>
<td>2.05 ± 0.38</td>
<td>0.83 ± 0.25</td>
<td>3.50 ± 0.69</td>
</tr>
</tbody>
</table>

EC = esterified cholesterol; FC = free cholesterol; PL = phospholipid; TG = triglyceride; IDL = intermediate density lipoproteins; LDL = low density lipoproteins.
Values are means ± standard error, from six female and six male neonates.
with the 310 Å obtained by electron microscopy (data not shown). The neonatal LDL on GGE had a large major peak at 257 Å and a minor peak component at 240 Å; by comparison, the EM value for LDL was 257 Å. The GGE patterns of particle size were similar for both sexes and for individual and pooled IDL and LDL fractions. LDL peak positions were remarkably reproducible, with peak sizes of 255 ± 3 Å and 242 ± 3 Å for individual samples analyzed (n = 15), and 256 ± 4 Å and 244 ± 6 Å for the pooled samples (n = 6).

The apoprotein content of the IDL and LDL fractions from neonates are shown in figures 2 and 3. Figure 2 presents the results of SDS gel electrophoresis of LDL on 10% polyacrylamide; apo B, which remained near the top of the gel, was the major apolipoprotein, but in addition, small quantities of apo A-I and E were also present. The identification of apo A-I and apo E was confirmed by Ouchterlony immunodiffusion kindly provided by Dr. P.N. Herbert (personal communication). Again, there was no difference between male and female neonates with regard to the presence of apo A-I and apo E. Figure 3 shows the analysis of the neonatal IDL and LDL by SDS gel electrophoresis on 3% polyacrylamide gels. The IDL displayed only one high molecular weight form of apo B, with a calculated molecular weight of 540,000 daltons. The LDL from the neonate contained, in addition to the high molecular apo B, two smaller molecular weight forms of apo B, with calculated molecular weights of 480,000 and 180,000 daltons, respectively. The neonate IDL and LDL showed a consistent pattern for both pooled and individual samples of high molecular weight apo B in both IDL and LDL, while LDL fractions had, in addition, the low molecular weight forms of apo B. Adult LDL treated in the same way (data not shown) consistently revealed the presence of the high molecular weight apo B, but the presence of the lower molecular weight forms was variable, being dependent on the individual sample.

Discussion

The TG and TC levels for neonatal cord blood plasma examined here are comparable to those reported by earlier workers.1-8 The values show a bias toward the higher values, similar to those reported by Hardell.1 However, the level of total cholesterol in the female vs male neonate, while somewhat higher, is not significantly different, in

![Figure 1](image1.png)

Figure 1. Representative densitometric scans of Coomassie blue R-250 stained 2% to 16% polyacrylamide gradient gel. A. Total low density lipoprotein levels (LDL) from cord blood plasma (d = 1.006 to 1.063 g/ml). B. LDL (d = 1.019 to 1.063 g/ml). C. Intermediate density lipoprotein levels (IDL) (d = 1.006 to 1.019 g/ml).

![Figure 2](image2.png)

Figure 2. Pooled cord blood plasma low density lipoprotein content (LDL), reduced, upon sodium dodecyl-sulfate (SDS) polyacrylamide electrophoresis on 10% gels. Gel 1 contains the standards: top band (> 100,000 daltons); bovine serum albumin (BSA, 67,000 daltons); catalase (CAT, 60,000 daltons); lactate dehydrogenase (LDH, 36,000 daltons); and ferritin (FER, 18,500 daltons). The remaining gels represent increasing amounts of LDL: Gel 2 (32 μg protein); Gel 3 (48 μg protein); and Gel 4 (64 μg protein).
contrast to the results of Hardell and Carlson.\textsuperscript{2} Perhaps the heterogeneity of the population in our study (60% black, 30% Caucasian, 7% Hispanic, and 3% Oriental), in contrast to the population studied by Hardell in Scandinavia, or the exclusion of cord bloods having elevated TG or TC may account for the difference in results.

The neonatal lipoprotein concentrations are considerably reduced in relation to the adult levels. The LDL level for the neonate is not sex dependent and is approximately 75 mg/100 ml, only 17\% to 19\% of the adult levels of 380 mg/100 ml for the male population (30 to 49 years old) and 330 mg/100 ml for the female population (30 to 49 years old).\textsuperscript{21} The IDL levels are similarly reduced in comparison to the adult levels of 57 mg/100 ml for the male (30 to 49 years old) and 41 mg/100 ml for the female (30 to 49 years old).\textsuperscript{21} While the IDL and LDL are decreased in the neonate, the decrease is not merely an overall decrease but also a shift, as the mass of the neonatal IDL represents only 8\% to 9\% of the total d = 1.006 to 1.063 mass, while in the adult, the IDL represents 11\% to 13\% of the total LDL. The total d = 1.006 to 1.063 g/ml concentration of 80 mg/100 ml reported here is in general agreement with that reported by several investigators,\textsuperscript{1,4} but is higher than the approximately 60 mg/100 ml reported by both Winkler et al.\textsuperscript{9} and Wilie and Phillips.\textsuperscript{10} The reasons for this discrepancy between our values along with the values of other workers when compared to the values reported by Winkler et al. and Wilie and Phillips are unknown, but the population studied and the methodology employed may be factors.

The composition data obtained for our total d = 1.006 to 1.063 g/ml fraction agree with those of Morris et al.\textsuperscript{5} but differ in certain respects from those reported by Patsch et al.\textsuperscript{6} In particular, the percentage of cholesteryl ester as reported by Patsch et al. is much lower than that obtained in our study (19\% vs 32\%) or the value (25\% to 31\%) that can be calculated from the data of Morris et al.\textsuperscript{5} The values for percent TG and percent protein reported by Patsch et al.\textsuperscript{6} are also elevated compared to our present study (TG, 17\% vs 13\%; protein, 26\% vs 21\%).

In addition to the alterations in absolute composition, neonatal IDL and LDL display differences in the ratios of various components. The neonatal particles have an elevated amount of free cholesterol relative to esterified cholesterol, as reflected by the ratio of esterified to free cholesterol (EC/FC) of 1.45 for IDL and 2.09 for LDL; adult ratios are 2.04 for IDL and 2.38 for LDL.\textsuperscript{22,23} The ratio of esterified to free cholesterol for the total d = 1.006 to 1.063 g/ml fraction (combined IDL and LDL) of 2.01 is similar to that obtained by both Morris et al.\textsuperscript{5} and Winkler et al.\textsuperscript{9} but differs significantly from the value of Klimov et al.,\textsuperscript{8} who reported a ratio of 0.9. The increased free cholesterol relative to esterified cholesterol may be the result of a lowered activity for neonatal lecithin-cholesterol acyltransferase (LCAT). This possibility has been raised by Klimov et al.,\textsuperscript{5} who reported that cord blood LCAT has only one-third the activity of the adult. However, Lacko et al.\textsuperscript{24} in an earlier study related the LCAT activity to the level of free cholesterol in the cord blood and concluded that cord blood can esterify cholesterol at a rate comparable to, or greater than, that of the adult blood.

While there is an excess of free cholesterol relative to esterified cholesterol, the LDL of the neonate shows an overall deficit of total cholesterol per particle as evidenced by the phospholipid to total cholesterol mole ratio (PL/TC). The ratio for LDL in the neonate is 0.43, while in the adult it is 0.30.\textsuperscript{22} Since the neonatal particles have similar particle sizes to the adult particles (see below), the implication is that some other component is

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) from pooled male and female cord blood plasma, reduced (+ ME, mercaptoethanol), and unreduced, upon sodium dodecylsulfate (SDS) polyacrylamide electrophoresis on 3\% gels. The LDL gels were loaded with approximately 50 \(\mu\)g of protein, and the IDL gels with approximately 25 \(\mu\)g of protein.
maintaining the size of the core. In the case of the neonatal LDL, this component appears to be TG since the particles are enriched with TG compared to that of the adult particle (12% vs 5% for adult LDL). The IDL, however, do not show such a pattern of TG enrichment; rather they are enriched in total cholesterol (35%) and phospholipid (24%) compared to the adult IDL cholesterol (29%) and phospholipid (17%).[21] Enrichment of TG in the LDL may reflect the fact that the fetal energy needs are apparently met mostly by carbohydrate. Such reliance on carbohydrate may result in an alteration of the lipoprotein composition similar to that observed in studies of hepatic VLDL during high carbohydrate regimens.[25]

The apolipoprotein compositions of the neonatal IDL and LDL are different from those of the comparable adult fractions. Cord blood IDL is unusual in that apo B is the sole apolipoprotein present; in contrast, adult IDL contain, in addition to apo B, the smaller molecular weight apo E and C.[26] The absence of apo C from the IDL is consistent with the finding of McConathy and Lane[11] that lipoproteins containing apo B, in combination with either apo C or apo E or both, are markedly diminished or absent in cord blood relative to adult levels. Cord blood LDL contain, in addition to apo B, appreciable amounts of apo E and apo A-I. The presence of apo A-I and apo E does not appear to be due to any contamination of the LDL fraction, as the fraction did not show any VLDL- or HDL-like particles by either GGE or electron microscopy. Although the presence of apo A-I and apo E is established, it is unclear whether apo A-I and/or apo E exist on the same particle along with apo B, or whether they constitute a separate population of particles. The relatively high cord blood plasma apo C levels reported by McConathy and Lane[11] combined with our finding of only trace amounts of apo C in the LDL fractions of neonates suggest that other lipoprotein density classes must be enriched in apo C.

Apo B of IDL and LDL was examined for the polymorphism reported for adult LDL by Kane et al.[18] In cord blood IDL, apo B consisted of a single, high molecular weight band with a calculated molecular weight of 540,000 daltons. The LDL from the neonate had, in addition to the high molecular weight band, two other bands of lower molecular weight, one with a calculated molecular weight of 480,000 daltons and the other, a molecular weight of 180,000 daltons. Under the nomenclature proposed by Kane et al. to allow comparisons between laboratories, the 540,000 dalton band is designated B100, while the 480,000 dalton band is B83, and the 180,000 dalton band is B31. The numbers obtained differ slightly from those published by Kane et al. for adult LDL, but the differences are not likely to be significant as the molecular weight calculations are sensitive to the measurement errors introduced by the easy deformability of the 3% gels.

The size determined for neonatal IDL by GGE is comparable to that found for adult IDL (300 Å; T. Forte, unpublished observation) but show some variability between pooled and individual samples. Neonatal LDL size distributions, as determined by GGE, are noteworthy for their constancy when both pooled samples and individuals are analyzed. The LDL (d = 1.019 to 1.063 g/ml) give well-defined, reproducible peaks at approximately 255 Å (major peak) and 240 Å (minor peak). Although the size of the cord blood LDL particles falls within the range of the particle sizes found in the adult (230 to 280 Å), the size and distribution of the sizes are much more homogeneous compared to those evident in the adult LDL.[29] That is, while the LDL from cord blood consistently shows the presence of two peaks with diameters of 255 and 240 Å, the adult LDL shows no consistent pattern, with a variety of particles appearing in a wide range of sizes. Examination of several adult LDL fractions by GGE reveals the presence of several distinct bands of varying size within a single LDL. This size heterogeneity occurs in addition to the density heterogeneity reported by Shen et al.[28] LDL fractions obtained by the method of Shen et al. when run on gradient gels reveal the presence of several different size particles within the same density region.[27]

The origin of the observed size homogeneity of neonatal LDL is obscure. One hypothesis is that the turnover of the neonatal LDL is so rapid that the particles do not undergo the exchanges and alterations of the components that probably produce the heterogeneity observed in the adult. That the LDL of the neonate does turn over rapidly has been inferred by Parker et al.[29] who have sought to link the fetal adrenal steroidogenic activity with the level of LDL in the fetus. They have proposed and presented data to show that the levels of LDL and DS (dihydropiandrostosterone sulfate), an indicator of fetal adrenal steroidogenesis, are inversely related. The fetal adrenal gland has been shown to preferentially use LDL as a source of cholesterol for DS biosynthesis[30] and, to that end, has been shown to possess the greatest concentration of LDL receptors of any organ tested.[31] If we assume that only 50% of the cholesterol needs of the fetal adrenal gland are met by LDL cholesterol, which is a conservative estimate (see Carr et al.), this would result in the turnover of neonatal LDL exceeding 200% of the plasma pool per day, far in excess of the turnover rate of adult LDL, approximately 44%. In total, the evidence points to a significant, if not determining, role for the fetal adrenal gland in the level and turnover of fetal LDL, and this may in turn determine the relative homogeneity of the LDL species observed. It is of interest to note that after birth, while the lipoprotein pattern is...
evolving toward that of the adult, the neonatal adrenal gland undergoes a profound change in morphology with the loss of a fetal zone of tissue found only in the developing adrenal gland.14

In summary, the IDL and LDL of neonates are significantly different in composition, size distribution, concentration, and, most probably, metabolism when compared to adult IDL and LDL. The significance of these differences is unclear but may point to differences in the metabolism of IDL and LDL which, when combined with the data derived from the study of adult IDL and LDL, may allow a better insight into the metabolism of lipoproteins and its control.

Acknowledgments

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References

Neonatal umbilical cord blood lipoproteins. Isolation and characterization of intermediate density and low density lipoproteins.
P A Davis and T M Forte

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