Specific Cellular Features of Atheroma Associated With Development of Neointima After Carotid Endarterectomy

The Carotid Atherosclerosis and Restenosis Study

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**Background**—The purpose of this study was to investigate whether some cellular and molecular features of tissue retrieved at carotid endarterectomy are associated with the extent of neointima formation at ultrasound follow-up.

**Methods and Results**—One hundred fifty patients were studied. Endarterectomy specimens were tested by immunocytochemistry with the use of (1) monoclonal antibodies that identify smooth muscle cells (SMCs) and fetal-type SMCs on the basis of smooth muscle and nonmuscle myosin content, (2) the anti-macrophage HAM 56, and (3) the anti-lymphocyte CD45RO. The maximum intima-media thickness (M-IMT) of the revascularized vessel was assessed by the use of B-mode ultrasonography 6 months after surgery. The M-IMT values were related positively to the number of SMCs ($r=0.534$, $P<0.0005$) and negatively to that of macrophages and lymphocytes ($r=-0.428$, $P<0.0005$, and $-0.538$, $P=0.001$, respectively). Patients were classified as class 1 (M-IMT $\leq$1.0 mm), class 2 (1.0<M-IMT≤1.3 mm), and class 3 (M-IMT $>1.3$ mm). An abundance of SMCs, mostly of fetal type, was found in the plaque of class 3 patients, whereas lesions from class 1 patients were rich in macrophages and lymphocytes. In the multivariate analysis, factors related to M-IMT were the number of SMCs and the percentage of fetal-type SMCs present in the plaque.

**Conclusions**—Although the classic risk factors did not play a role, an abundance of SMCs and a scarcity of macrophages characterized the primary lesion of patients in whom neointima developed after surgery. In patients in whom neointima did not develop, lesions were rich in macrophages and lymphocytes. This approach can be useful in defining patients at risk of restenosis. (*Circulation*. 2000;102:771-778.)

**Key Words:** atherosclerosis ■ restenosis ■ carotid arteries ■ muscle, smooth ■ lymphocytes

Revascularization procedures are hampered by the occurrence of restenosis. The incidence of restenosis varies in different arterial segments, depending on the vessel size and the revascularization procedure itself. A peculiar aspect of restenosis is that risk factors for (primary) atherosclerotic lesions do not play a relevant role. Moreover, both in clinical and experimental conditions, intimal, medial, or adventitial remodeling may occur in response to revascularization procedures. For these reasons, among the factors potentially involved in the restenosis process, emphasis has been placed on the local, wall-related factors such as infection, inflammation, expression of growth factors and cytokines, recruitment of macrophages and lymphocytes, and activation of vascular smooth muscle cells (SMCs). However, another potentially relevant local factor, namely the cellular and molecular characteristics of the primary atheroma, has not been extensively evaluated in this respect. In particular, the expression of nonmuscle (NM) myosin in activated SMCs has been shown to be of importance in human and experimental atherogenesis as well as in restenosis.

The in vivo follow-up of neointima development by high-resolution ultrasound technique, combined with the study of cell composition of the endarterectomy tissue retrieved at carotid surgery, can furnish new information on the importance of primary atherosclerotic lesion to be predictive of restenosis. Although hemodynamically significant narrowing caused by neointima is not often observed after carotid endarterectomy, all patients undergo long-term ultrasound follow-up to rule out this possibility. The identification of those prone to the development of neointima after surgery could therefore be useful in avoiding unnecessary follow-up procedures and in concentrating on patients at risk. Moreover,
this approach may contribute to a better understanding of the process itself.

The aim of the Carotid Atherosclerosis and Restenosis Study (CARS) was the following: (1) to establish the degree of carotid intima-media thickness (IMT) developed after carotid endarterectomy in patients who had undergone this procedure for significant carotid narrowing, (2) to evaluate the cell composition and the SMC phenotype in the primary lesion of patients who have a different degree of IMT at follow-up, and (3) to compare the characteristics of the primary lesion with those of the restenosis lesion.

Methods

Subjects and Endarterectomy Procedure

One hundred fifty consecutive patients undergoing carotid endarterectomy of the internal carotid artery were included in the study. At angiography, all patients had carotid artery narrowing of ≥70%. Most patients had previous signs or symptoms of cerebrovascular disease. Before surgery, anthropometric and anamnestic data, including those from standard biochemical blood analyses, were collected. Plasma levels of Lp(a), homocysteine, minimally modified LDL, and MDA-LDL were also evaluated according to previously published methods.11–13

As an established procedure, the endarterectomy was coupled with a PTFE patch. The specimens of primary lesion retrieved at surgery were immediately placed in an OCT compound, frozen in liquid nitrogen, and stored at −80°C. The study was approved by the local ethics committee. All patients gave informed consent.

Ultrasound Follow-Up

An ultrasound examination of the supra-aortic trunks was instituted by 6 months after surgery with the use of the Biosound 2000/II/SA (Esaote Biomedica), equipped with an 8-MHz annular array mechanical transducer. This system provides an axial resolution of 0.10 mm.14 All subjects were examined in the same room, in dim light, lying comfortably in a supine position. Once an optimal longitudinal image was obtained, it was stored on half-inch super-VHS videotape. Images were analyzed by an independent reader, using a high-resolution video recorder (Panasonic AG-7355) coupled with a mouse-driven image analysis system (AMS VIDS V). IMT, defined as the distance between the lumen-intima and the media-adventitia interfaces, was measured at end-diastole in the far wall, as previously described.15 The maximum IMT (M-IMT) was assessed in the carotid artery segment subjected to endarterectomy.

According to current ultrasonographic criteria,16,17 patients were classified at follow-up into 3 different classes as normal (M-IMT ≤1.0 mm, class 1), intermediate (1.0 mm < M-IMT ≤1.3 mm, class 2), and plaque (M-IMT >1.3 mm, class 3).

Immunocytochemistry and Image Analysis

Seventy-four optimally preserved specimens without extensive fusing or disruption of the plaque and the underlying media, where plaque was analyzed. The cell composition analysis was carried out in a randomly chosen block of tissue. From each block, 8 seriate sections taken at 8-μm intervals were analyzed. The following monoclonal antibodies were used: SM-E7 anti–smooth muscle (SM)--myosin heavy chains (MyHC), NM-F6 anti–NM-MyHC, HAM 56 anti–monocyte-macrophage (gift of R. Ross and E. Reines), and CD45RO anti-lymphocyte (Dako, Dakopatts). The SM-E7 reacts with SM-type MyHC (both SM1 and SM2) exclusively.18 The NM-F6 is able to identify a specific antigenic epitope localized in the platelet-type MyHC isoforms MyHC-αplat and reacts neither with B-type NM-MyHC nor SM-MyHC.19 The combined use of SM-E7 and NM-F6 antibodies allows the identification of fetal-type SMCs, whereas SM-E7 alone identifies the whole SMC population. Primary antibodies (except for CD45RO) were applied to the freshly cut unfixed cryosection (8 μm), as previously described.20 Nuclei were revealed with the use of the bis-benzimide stain (Hoechst 33258). Sections were examined with a Zeiss Axioplan microscope equipped with a Hamamatsu CCD high-resolution camera with a ×40 Planapo objective lens. The assessment of the different cell types was carried out by the same observer, who was unaware of the outcome of the ultrasound follow-up.

For cell count, we considered 3 standard areas (70 μm²) of the plaque: the basal, the shoulder, and the cap regions. In 65% of the specimens, it was possible to clearly distinguish and analyze some of the media layer underneath the plaque. In these cases, 3 random fields (70 μm²) were considered. We evaluated the size of each cell population by assessing on seriate cryosections the number of cells per area unit positive to SM-E7, HAM 56, or CD45RO, respectively. Moreover, the relative prevalence of each cell type was calculated as the percentage of positive cells to the total number of nuclei per area. Since macrophages and lymphocytes in the plaque contain A-type NM-MyHC, the assessment of fetal-type SMCs (expressing MyHC-Apla) along with SM-MyHC was carried out in areas where SMCs were largely predominant with the use of seriate cryosections stained by the NM-F6 and the SM-E7 antibodies. Because macrophages and/or lymphocytes were not found in the media layer underneath the plaque, cells identified by the NM-F6 correspond to fetal-type SMCs inasmuch as all cells in this layer are labeled with the SM-E7. The prevalence of fetal-type SMCs was expressed as a percentage of total SMCs.

To ascertain the reproducibility of the assessment of the various cell types, 3 standard sites were selected in 8 randomly chosen endarterectomy specimens: (1) distal (cranially), (2) intermediate, and (3) distal (caudally). The length of specimens ranged between 0.5 and 2.6 cm. In the 3 sites, seriate cryosections were cut at 8-μm intervals and processed for immunocytochemistry for SMCs, macrophages, and lymphocytes, respectively. Variability in cell counts obtained from the 3 sites was assessed for each cell type by the use of (1) ANOVA for repeated measurements within subjects (ie, specimens) and (2) the coefficient of variation of the mean difference of repeated measurements. No difference between repeated assessments of SMCs (F=2.482, P=0.120), macrophages (F=0.225, P=0.801), and lymphocytes (F=1.298, P=0.304) was found by ANOVA. The coefficient of variation was 5.3% for SMCs, 4.8% for macrophage, and 3.4% for lymphocyte measurements.

Statistical Analysis

Continuous variables were averaged, expressed as mean±SD, and compared by means of ANOVA and Bonferroni’s correction. Prevalences of categoric variables were evaluated with 2-way contingency tables, expressed as percentage rate, and compared by means of the Pearson χ² test. The univariate correlation between M-IMT and other continuous variables was evaluated by Pearson correlation coefficient with Bonferroni’s adjusted probabilities. M-IMT was also played into a multiple regression analysis, obtaining 1 multiple correlation coefficient (r). Class of IMT was played as a categoric variable into a logistic regression analysis. The SYSTAT package was used for this purpose.

Results

Ultrasound Follow-Up, Demographic Data of Patients, and Risk Factor Profile

The M-IMT values recorded by 6 months after surgery and the number of patients ascribed to each class are reported in Table 1. The incidence of major perioperative events (stroke, death) was 1.3% (2 of 150 patients). Values of blood pressure, plasma lipids, and the prevalence of risk factors for atherosclerosis were similar in the 3 classes. Moreover, in the whole population, a relation between M-IMT and the variables listed in Table 1 was found neither in the univariate nor in the multivariate analysis (not shown).
In the subset of patients in which minimally modified LDL and MDA-LDL were tested, the distribution of MDA-LDL did not change according to the class. Conversely, higher values of minimally modified LDL (expressed as % of total LDL) were found in class 1 compared with class 3 patients (6.32±1.76, n=13, versus 4.58±2.23, n=20, P=0.024), and an inverse relation between levels of these lipoproteins and M-IMT was present in the whole population (r=−0.397, P=0.018).

### Cell Composition of Primary Lesion and Restenosis Tissue

As shown in Figure 1, the primary lesions retrieved at surgery from patients who subsequently had an M-IMT value >1.3 mm at ultrasound follow-up (class 3) displayed an abundance of SMCs, mostly of the fetal type (Figure 1, B and D), compared with lesions from patients who had the lower level of M-IMT at follow-up (class 1, Figure 1, A and C). Moreover, in lesions from class 3 patients, the media layer underneath the plaque appeared to be poorer in SMCs than in lesions from class 1 patients (Figure 1, E and F). In either case, the labeling of medial SMCs with the SM-E7 and the NM-F6 was present, indicating coexpression of SM-MyHC and NM-MyHC-Apla1, which characterizes the fetal-type SMCs. Another relevant feature was that macrophages and lymphocytes were less abundant in primary lesions from class 3 patients compared with class 1 (Figure 2).

In 4 class 3 patients (2.6% of the series), hemodynamically significant carotid artery restenosis occurred within the

### TABLE 1. Demographic Data and Risk Factors According to Level of Maximum IMT at Ultrasound Follow-Up

<table>
<thead>
<tr>
<th>Variables</th>
<th>Class 1: M-IMT≤1.0 mm (n=35)</th>
<th>Class 2: 1.0&lt;M-IMT≤1.3 mm (n=41)</th>
<th>Class 3: M-IMT&gt;1.3 mm (n=74)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, %</td>
<td>77</td>
<td>80</td>
<td>78</td>
<td>0.936</td>
</tr>
<tr>
<td>Age, y</td>
<td>68.3±8.6</td>
<td>66.6±7.8</td>
<td>68.9±8.2</td>
<td>0.318</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5±2.6</td>
<td>25.7±3.0</td>
<td>25.0±2.9</td>
<td>0.362</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>144±14</td>
<td>152±16</td>
<td>147±19</td>
<td>0.084</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>84±8</td>
<td>84±9</td>
<td>82±10</td>
<td>0.268</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>112±47</td>
<td>116±43</td>
<td>121±51</td>
<td>0.653</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>221±37</td>
<td>237±50</td>
<td>231±41</td>
<td>0.268</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>141±66</td>
<td>161±85</td>
<td>149±71</td>
<td>0.489</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>50±13</td>
<td>49±14</td>
<td>52±14</td>
<td>0.530</td>
</tr>
<tr>
<td>Cholesterol/HDL-cholesterol</td>
<td>4.7±1.4</td>
<td>5.2±1.9</td>
<td>4.8±1.4</td>
<td>0.401</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>22±25</td>
<td>24±24</td>
<td>22±23</td>
<td>0.871</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>300±100</td>
<td>310±70</td>
<td>319±69</td>
<td>0.592</td>
</tr>
<tr>
<td>Log homocysteine, μmol/L</td>
<td>1.14±0.16</td>
<td>1.15±0.13</td>
<td>1.22±0.19</td>
<td>0.170</td>
</tr>
<tr>
<td>M-IMT, mm</td>
<td>0.90±0.08</td>
<td>1.16±0.09</td>
<td>1.99±0.67</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

Risk factors, n (%)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Class 1: M-IMT≤1.0 mm (n=35)</th>
<th>Class 2: 1.0&lt;M-IMT≤1.3 mm (n=41)</th>
<th>Class 3: M-IMT&gt;1.3 mm (n=74)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Family history</td>
<td>9 (60)</td>
<td>27 (59)</td>
<td>28 (50)</td>
<td>0.617</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>29 (83)</td>
<td>37 (90)</td>
<td>68 (92)</td>
<td>0.353</td>
</tr>
<tr>
<td>Hyperglycemia*</td>
<td>20 (57)</td>
<td>26 (63)</td>
<td>45 (61)</td>
<td>0.855</td>
</tr>
<tr>
<td>Hypercholesterolemia*</td>
<td>27 (77)</td>
<td>36 (88)</td>
<td>56 (76)</td>
<td>0.286</td>
</tr>
<tr>
<td>Hypertriglyceridemia*</td>
<td>14 (40)</td>
<td>21 (51)</td>
<td>31 (42)</td>
<td>0.541</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>20 (57)</td>
<td>24 (59)</td>
<td>53 (72)</td>
<td>0.211</td>
</tr>
<tr>
<td>BMI ≥25 kg/m²</td>
<td>19 (56)</td>
<td>25 (61)</td>
<td>33 (45)</td>
<td>0.207</td>
</tr>
<tr>
<td>Fibrinogen ≥300 mg/dL</td>
<td>12 (41)</td>
<td>21 (58)</td>
<td>29 (55)</td>
<td>0.342</td>
</tr>
<tr>
<td>Lp(a) ≥25 mg/dL</td>
<td>9 (30)</td>
<td>13 (36)</td>
<td>16 (31)</td>
<td>0.832</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure; M-IMT, maximum IMT by 6 months after endarterectomy.

*Anamnestic data and/or high levels on measurements: blood pressure (systolic BP ≥140 and/or diastolic BP ≥90 mm Hg), glycemia (≥100 mg/dL), cholesterol (≥200 mg/dL), triglycerides (≥150 mg/dL).

Pearson χ² for categorical variables and ANOVA for continuous variables. Mean±SD values are reported for continuous variables. n=150 patients.
6-month follow-up period. When a new endarterectomy was performed, the restenosis tissue displayed a cell composition very close to that of the primary lesion of class 3 patients, namely, an abundance of fetal-type SMCs and scattered macrophages and lymphocytes (Figure 3).

Relation Between M-IMT at Follow-Up and Cell Composition of the Primary Lesion

The immunocytochemistry-based assessment of cells present in both medial and intimal layers of the different classes is reported in Table 2.

A higher number of SMCs was found in the plaque of class 3 patients compared with the other classes, class 1 in particular. Most SMCs were of the fetal type (also see Figure 1). Conversely, lesions from class 1 patients displayed a strikingly higher number of macrophages and lymphocytes than those from class 2 patients, and even more so when compared with class 3 patients.

In the tunica media underneath the plaque, a reduced number of SMCs was found in class 3 and 2 patients compared with class 1. A significantly higher percentage of
fetal-type medial SMCs was also found in specimens from class 3 compared with class 1.

In the whole population, individual values of carotid M-IMT at follow-up were played against the cell types found in the plaque and media of the endarterectomy specimen by univariate (Table 3) or multivariate (Table 4) analysis. In the plaque, M-IMT was related positively to SMCs (irrespective of the differentiation pattern) and negatively to macrophages and lymphocytes. In the media, values of M-IMT were related negatively to SMCs and positively to fetal-type SMCs (Table 3). However, when all data from the population (including risk factors and anthropometric data) were played in the multivariate regression analysis, the only factors related to M-IMT were the number of SMCs and the percentage of fetal-type SMCs in the plaque (Table 4). The role of these factors was confirmed at the logistic regression analysis (not shown). Moreover, in this latter analysis, the number of macrophages present in the plaque (class 3 versus class 1 as reference) was a negative predictor of M-IMT at follow-up ($P<0.0005$, OR 0.210, 95% CI 0.500 to 0.089).

**Discussion**

By stratifying patients at ultrasound follow-up according to the levels of IMT adopted in previous studies, we found that the increase in carotid artery IMT after the endarterectomy procedure was more pronounced than the physiological 0.010 mm/y reported for internal carotid artery. By 6 months after surgery, ~50% of patients had $>$1.3-mm M-IMT and only 23% had a value $\leq$1.0 mm. Hence, a relevant increase in IMT had taken place in the majority of patients, although the incidence of hemodynamically significant restenosis was only 2.6% and surgery was optimally carried out. As expected from previous reports, a significant relation between risk factors and the increase in neointima was not observed in our sample. This was true even for "new" risk factors such as homocysteine, Lp(a), minimally modified LDL, and MDA-LDL, which were not tested before in this setting. Instead, the analysis of the primary lesions retrieved at surgery, in particular those from class 3 and 1 patients, offered some insights into the process of neointima formation.
Atherosclerotic plaques from patients of class 3 displayed an abundance of SMCs compared with lesions from patients of class 1. Moreover, the media layer underneath the plaque showed a reduced number of SMCs in specimens from class 3 patients compared with those from class 1. Another relevant feature was that macrophages and lymphocytes were less numerous in primary lesions from class 3 patients compared with class 1 patients. When the restenosis tissue was analyzed, it displayed a cell composition very close to that of primary lesion of class 3 patients, namely, abundance of fetal-type SMCs and a few scattered macrophages and lymphocytes. The reliability of cell counts was endorsed by the survey specifically done, for example, the low coefficients of variation and the nonsignificant difference in measurements with the use of repeated-measures analysis. For each cell type, the difference in cell number among the 3 classes was too large to be explained by intrinsic variability within a specimen or by counting bias.

The individual value of carotid M-IMT at follow-up was then played against the cell types found in the plaque and media of the endarterectomy specimen. In the plaque, the M-IMT value of the whole population was related positively to SMCs (mostly of the fetal type), whereas there was a negative correlation with macrophages and lymphocytes. In the media, the M-IMT value was related negatively to the SMC number and positively to the percentage of fetal-type SMCs. The relation between M-IMT and these cell types was also confirmed by multivariate and logistic regression analyses.

The decreased number of SMCs observed in the media underneath the atheroma of class 3 patients, along with the prevalence of the fetal phenotype, suggests that these cells have the ability to migrate and accumulate in the intima after revascularization. This hypothesis is strongly supported by the finding that (1) both medial and intimal SMCs displayed a fetal-type pattern of differentiation, and (2) fetal-type SMCs are prevalent in the primary atheroma, well before the process leading to cell accumulation in the intima has started. The acquisition of this cell phenotype is the hallmark of atherosclerosis and neointima formation in experimental models as

Figure 3. Seriate cryosections from representative restenosis lesion. Immunocytochemistry with SM-E7 anti–SM-type MyHC (A), NM-F6 anti–NM-MyHC-Apla1 (B), HAM 56 anti-macrophage (C), and CD45RO anti-lymphocyte (D) antibodies. Restenosis tissue displayed cell composition very close to that of primary lesion of class 3 patients, namely, abundance of fetal-type SMCs (labeling of cells with SM-E7 and NM-F6) and scattered macrophages and T-lymphocytes. Scale bar: 100 μm.
well as in human pathology.\textsuperscript{9,10,18–22} In these circumstances, NM-MyHC-\textit{Apla1} expression in SMCs (ie, a marker of fetal-type SMCs) is upregulated, resembling the pattern found at the early stages of development.\textsuperscript{9,18,19} It is of interest that class 3 primary and secondary lesions shared the same content of NM-MyHC-\textit{Apla1} in SMCs, in agreement with Nikol et al.\textsuperscript{9} Other studies have postulated that B-type NM-MyHC expression in SMCs from the primary lesion of coronary arteries is a predictive marker of restenosis after the revascularization procedure.\textsuperscript{7,8} In humans, however, this NM myosin isoform is not developmentally regulated as occurs, instead, with NM-MyHC-\textit{Apla1}\textsuperscript{19} and with the SM2 isoform of SM-MyHC,\textsuperscript{10} which is downregulated in both the atherosclerotic plaque and the restenosis tissue.

An accumulation of fewer intimal cells occurred when NM cells of the inflammatory type predominated in the primary lesion (class 1 patients). This suggests that macrophages and lipids play a role in the pathogenesis of atheroma but not in the neointima development, with its inherent SMC encroachment. In accordance with this hypothesis, in class 1 patients a higher level of minimally modified plasma LDL was found compared with class 3 patients, which was mirrored by an inverse relation between these lipoproteins and the level of M-IMT at follow-up. We speculate that the reduced cellularity in the atherosclerotic lesion of class 1 patients is due to a slowing down of the passage of medial SMCs to intima. Such a process also might be accompanied by a direct effect of macrophage-released cytokines and lipids on the SMC differentiation profile.\textsuperscript{21,22}

In conclusion, this study shows for the first time that the NM myosin isoform is not developmentally regulated as occurs, instead, with NM-MyHC-\textit{Apla1}\textsuperscript{19} and with the SM2 isoform of SM-MyHC,\textsuperscript{10} which is downregulated in both the atherosclerotic plaque and the restenosis tissue.

### TABLE 2. Cell Composition of Carotid Endarterectomy Specimens

<table>
<thead>
<tr>
<th>Atherosclerotic plaque (n=74 specimens)</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>P</th>
<th>1 vs 3</th>
<th>1 vs 2</th>
<th>2 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunica media (n=48 specimens)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMCs, n/au</td>
<td>25.0±10.1</td>
<td>14.8±3.5</td>
<td>13.2±6.2</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>0.008</td>
<td>1.000</td>
</tr>
<tr>
<td>SMCs, %</td>
<td>98.3±2.3</td>
<td>100±0.0</td>
<td>99.9±7.7</td>
<td>NS</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Fetal-type SMCs, %</td>
<td>68.2±20.6</td>
<td>75.4±21.2</td>
<td>90.4±10.5</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>0.948</td>
<td>0.073</td>
</tr>
</tbody>
</table>

SMC indicates SMCs expressing SM-MyHC only; Fetal-type SMC, SMCs coexpressing SM- and NM-MyHC-A\textit{Apla1}; MØ, macrophages recognized by HAM 56; Lympho; lymphocytes recognized by CD45RO; n/au, number of positive cells per area unit; %, percentage of positive cells to total cell number. The prevalence of fetal-type SMCs was expressed as a percentage of total SMC number.

ANOVA and Bonferroni post hoc test. Mean±SD values are reported.

### TABLE 3. Multivariate Regression Analysis for Factors Potentially Linked to Maximum IMT at Ultrasound Follow-Up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMCs of plaque, n/au</td>
<td>0.053</td>
<td>0.013</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Fetal-type SMCs of plaque, %</td>
<td>0.008</td>
<td>0.003</td>
<td>0.026</td>
</tr>
</tbody>
</table>

SMC indicates SMCs expressing SM-MyHC only; n/au, number of positive cells per area unit; Fetal-type SMC, SMCs coexpressing SM-and NM-MyHC-A\textit{Apla1}; %, percentage of positive cells to total cell number.

Multivariate general linear model. Multiple \( r^2 = 0.592, P>0.0005 \).
peculiar cellular and molecular features that might be useful for the identification of patients at risk of the development of restenosis. Of course, our data are retrospective and need to be confirmed in the multicenter, prospective part of the CARS, which will be aimed at defining the cellular threshold for high or low risk of neointima formation. This would permit the possibility of selecting patients who need to be followed up very closely over those who do not, with obvious advantages in terms of saving time and money.

Acknowledgments
This work was supported by the MURST project n° 9905157119, the Fondazione Cassa di Risparmio of Padova, and the Biomedical Association for Vascular Research of Padova.

References
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Paolo Pauletto, Massimo Puato, Elisabetta Faggin, Nicoletta Santipolo, Valeria Pagliara, Miranda Zoleo, Giovanni Paolo Deriu, Franco Grego, Mario Plebani, Saverio Sartore, Gabriele Bittolo Bon, Christophe Heymes, Jeane-Lise Samuel and Achille Cesare Pessina

_Circulation_. 2000;102:771-778
doi: 10.1161/01.CIR.102.7.771

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/7/771

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