Comparison between Adventitial and Intimal Inflammation of Ruptured and Nonruptured Atherosclerotic Plaques in Human Coronary Arteries

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Objective - To verify the possible role of adventitial inflammation in atherosclerotic plaque vulnerability and coronary artery remodelling.

Methods - We compared the mean numbers of lymphocytes in the adventitia and in the plaque of ruptured thrombosed and stable equi-stenotic coronary segments of 34 patients who died due to acute myocardial infarction. We also analysed adventitial microvessels, adventitial fibrosis and the external elastic membrane.

Results - In the adventitia, the numbers of lymphocytes and microvessels/mm² were 69.5±88.3 and 60.9±32.1 in culprit lesions and 16.4±21.1 and 44.3±16.1 in stable lesions (p<0.05); within the plaques, the mean number of lymphocytes was 24±40.8 in culprit lesions and 10.9±13.2 in stable ones (p=0.17). The mean percent area of adventitial fibrosis/cross-sectional area of the vessel was significantly lower in unstable plaques (p<0.001). The confocal images showed holes in the external elastic membrane.

Conclusion - Unstable plaques exhibit chronic pan-arteritis, accompanied by enlargement, medial thinning, and less fibrosis than in stable lesions, which is compatible with vessel aneurysm. Adventitial inflammation may contribute significantly to atheroma instability.

Keywords: coronary atherosclerosis, unstable plaques, arteritis, aneurysm, adventitial inflammation

Much experimental and clinical evidence links inflammation and atherosclerosis; for example, systemic indices of inflammation may play a role in predicting risk of coronary events. Many reports have attributed to inflammation in the cap and shoulder of the atherosclerotic lesion a fundamental pathogenetic role in plaque disruption and consequent acute thrombotic complications; otherwise, microvessels in atherosclerotic plaques have been considered to favor the influx of inflammatory cells into them. Few pathological studies have addressed the involvement of inflammation in the media or adventitia in the development of atherosclerosis and arterial remodelling. We have recently demonstrated that unstable thrombosed plaques responsible for fatal acute myocardial infarction (AMI) are usually larger than equi-stenotic stable plaques in the same patient, having larger lumina due to the compensatory positive remodelling of the vessels. In the present study, we propose that the chronic adventitial inflammation and neovascularization may contribute to the thinning of the medial layer and compensatory enlargement of coronary segments with vulnerable atherosclerotic plaques.

Methods

The present work was approved by the Scientific and Ethics Committee of the Heart Institute (InCor) of São Paulo University Medical School.

Thirty-four specimens from consecutive necropsies performed at the Heart Institute (InCor) of the University of São Paulo Medical School from patients who died due to AMI from 1985 to 1986 were studied. This period was chosen because at that time procedures like angioplasty or thrombolysis were not a routine in this hospital, thus the coronary arteries were free of conditions that could produce artificial changes in plaque morphology. All but 2 specimens had been used in a previous study from our group. Cross sections from 2 groups of coronary artery seg-
ments were analyzed: Group I - 34 thrombosed coronary artery segments responsible for the AMI; and Group II - 34 nonruptured (stable) segments situated in another coronary artery branch of the same heart, with a similar grade of stenosis and matched according to the distance from the coronar
y stem.

Tissue blocks containing the culprit lesions were serially sectioned to define the exact site of plaque rupture or erosion. At each interval of 30µm, we reserved 5µm-thick sections for histological analysis. The section exhibiting the most extensive plaque rupture was used for morphometry of the vessel areas and plaque constitution. Subsequent sections were used to quantify the inflammatory cells, adventitial microvessels, and thicknesses of the medial layer, adventitia, and external elastic membrane (EEM).

Atherosclerotic plaque areas - We used the data obtained in the previous study 13, presented in table 1: a Leica Image Analysis Quantimet 500 system was used to measure the plaque area (the region encompassed by the elastic internal membrane) in Movat-stained coronary artery histological cross sections. Group I had larger plaques (9.8 ± 4.8mm²) than did Group II (4.8 ± 2.3mm²).

Microvessels present in all tangential 400x microscopic fields that surrounded the arterial cross section were counted in Movat-stained slides.

Immunohistochemistry was used for characterization of the CD8-T, CD4-T, and CD20-B cells, with Novocastra, UK (CD8) and Dako-Patts (CA, USA) antibodies. For CD8-epitope recovery, the sections were boiled in sodium citrate buffer (10 mM, pH 6.0) for 15 seconds before reactions. Immunohistochemical detection of the epitopes used the indirect horseradish peroxidase technique as previously described 14.

The lymphocytes present in all 400x microscopic fields of the adventitia were counted in each of the selected segments. The total amounts were divided by the number of fields and divided by 0.36 mm² (which is the area of each microscopic field) to obtain the mean adventitial number of lymphocytes/mm².

Lymphocytes located within the plaques were also counted; their numbers were divided by the area of the plaque (discounting the fat area, which was devoid of lymphocytes) to obtain the plaque concentration of lymphocytes/mm².

The mean number of adventitial microvessels/mm² in group I (coronary artery segments with culprit lesions) and 44.3±6.1 in group II (with stable atherosclerotic plaques) (p=0.04).

The quantitative data concerning lymphocytes are summarized in table 1; figure 1 is a graphic representation of the amounts of total lymphocytes.

In the adventitia, the mean numbers of CD20-B, CD8-T, and CD4-T, and total lymphocytes/mm² were significantly higher in the adventitial layer of Group I than in Group II (p<0.001 for all of them; table I). The CD20-B cell was the main lymphocyte present in the adventitia of Group I; in Group II, a similar proportion existed among the 3 subtypes of lymphocytes.

Within the plaques (excluding the lipidic area), the difference between the 2 groups was not significant with regard to the mean number of total lymphocytes (24±40.8 in Group I vs 10.9±13.2 in Group II, p=0.17), CD8T cell - which was the main lymphocyte present (13.6±23.4 and 5.6±7.5, p=0.09), and CD20-B (5.4±16.7 vs 19.4±4.4, p=0.49), but more CD4T cells were present in the culprit (mean 6.2±7) than in the stable lesions (3.4±4.1) (p=0.04).

Comparing plaque versus adventitia in the same segments, in Group I more CD20B and total lymphocytes/mm² were present in the adventitia than in the plaque (34±58.1 vs

### Table I - Mean numbers of lymphocytes in intimal atherosclerotic plaques and adventitia of stable and unstable (culprit) coronary atherosclerotic segments from the same individuals

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Adventitia</th>
<th>Intimal plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culprit</td>
<td>Stable</td>
</tr>
<tr>
<td></td>
<td>lesion</td>
<td>lesion</td>
</tr>
<tr>
<td></td>
<td>(n=34)</td>
<td>(n=34)</td>
</tr>
<tr>
<td><strong>CD20-B</strong></td>
<td>34 ± 58.1</td>
<td>5.9 ± 13.2</td>
</tr>
<tr>
<td><strong>CD8-T</strong></td>
<td>20.5 ± 30.3</td>
<td>5 ± 5.7</td>
</tr>
<tr>
<td><strong>CD4-T</strong></td>
<td>16.2 ± 19.4</td>
<td>5.8 ± 7.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>69.5 ± 88.3</td>
<td>16.4 ± 21.2</td>
</tr>
</tbody>
</table>

* = Differences between culprit and stable lesions in the adventitia are significant; § - Differences between culprit and stable lesions in the intimal plaque are significant; * & = Differences between adventitia and intimal plaques in culprit lesions are significant. No other difference was significant.
Atherosclerotic segments from the same individuals. Atherosclerotic plaques and adventitia of stable and unstable (culprit) coronary atherosclerotic segments from the same individuals.

Adventitial and intimal inflammation of ruptured plaques

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Discussion

The role of inflammation in atherosclerosis could involve not only the pathogenesis of atherosclerotic plaques, but also their rupture. Concerning this last point, the intima has received much attention as a site of inflammation, while the adventitia has remained relatively unexplored.

In the present study, we compared ruptured plaques with stable ones. We chose lesions from the same patients, at a similar distance from the beginning of the coronary branch, and causing approximately the same degree of stenosis. Thus, the differences that would be found were probably related to the acute complication. We morphologically quantified some features that denote inflammation, namely the number of lymphocytes and microvessels, comparing the variations between ruptured and stable plaques both in the intimal lesions and in the corresponding adventitial zones. Interestingly, the differences were more prominent in the adventitia, where there was a significantly higher number of lymphocytes/mm² (due mostly to CD20-B cells) than in the plaques in the unstable group.

In an ongoing study, we compared another group of obstructive stable atheromatous coronary artery segments. These are from patients submitted to elective myocardial revascularization who died due to causes other than acute myocardial infarction. No significant histopathological differences was found with the present group II of stable plaques.

With ultrasound analysis, Depre et al found more neo-vessels in the plaques of patients with acute syndromes than in patients with stable coronary insufficiency. Kumamoto et al observed that the density of new intimal vessels correlated with the incidence of lumen stenosis and severity of inflammatory infiltrate. Zhang et al demonstrated a direct correlation between the increased amount of intimal microvessels and intimal thickness. Our findings agree with these data and may reflect increased angiogenic stimulation from lymphocytes. In this regard, activation can induce T cells to develop angiogenic mediators. In spite of predominating B cells, a great amount of T cells were present in the adventitia. The stimulus for B cells could be the presence of bacteria as we have demonstrated in a recent article. The most numerous inflammatory cell in the plaque is the macrophage, mainly in unstable plaques, as we have already reported.

However, apparently, macrophages are performing a phagocytic function because they are usually full of lipids (foam cells). In the adventitia, macrophages were also present but in small quantities.

To better understand the role of adventitial inflammation in atherosclerosis, it is worth calling attention to the fact that, as we showed in another study using the same patient group, as well as by intravascular ultrasound imaging, the ruptured plaques are bigger than stable lesions, and the segments containing them are dilated. Associating the present findings with the previous ones, we propose that rupture of coronary artery plaques usually occur in huge lipid plaques that form in an aneurysmatic dilated segment of the vessel as a consequence of pan-arteritis.
Although atherosclerosis is common in the abdominal aorta, aneurysms are quite infrequent. Many articles suggest that a great number of the elastic fibers must be destroyed for this type of lesion to occur. On the other hand, the main source of elastase, as well as of the other metalloproteinases that degrade extracellular matrix proteins, are the inflammatory cells. In accordance with our hypothesis that unstable lesions could be arteritis with aneurysmatic dilatation, aortic atherosclerotic aneurysms are also associated with a pronounced inflammation, composed mostly of plasma cells, and infectious agents such as Chlamydia pneumoniae have also been found.

Our hypothesis is consistent with data in the literature indicating that B cells are important for stimulating CD40L (CD154) in atherogenesis. In the present study, we observed a prominent B cell (CD20+ lymphocyte) infiltrate in the adventitia. Thus, we measured the external elastic membrane in silver-stained sections with common microscopy to check whether areas with severe inflammation have elastolysis and dilate. This membrane was thinner in the regions of the artery containing the greatest atherosclerotic plaques than in the remaining portions of the vessel; importantly, this difference was more prominent in the unstable segments (mean of 3.8 µm in the region of the ruptured plaque).
and 7.1 µm at the opposite side of the same segment; mean of 6.9 µm versus 8.5 µm in the control segments). The confocal laser microscopical analysis confirmed these findings.

Rupture of the EEM also occurred on the base of stable plaques, but to a lesser degree than on the unstable ones. We speculate that in the beginning of the lipid deposition process, vessel wall inflammation is already present. The healing of such inflammatory lesions, to circumscribe the lipid accumulation, would lead to development of stable atheromas, characterised not only by a fibrotic cap on the intimal surface, but also on the base and on the adjacent adventitia, explaining also the EEM fragmentation. This fibrotic healing tissue may cause vessel constriction (negative remodelling), also explaining severe obstruction caused by small fibrotic plaques (fig. 3).

It is noteworthy and traditionally reported that thrombosis is not frequent in the aorta, even with severe atherosclerosis, but is commonly found in aortic aneurysms. The usual explanation to for this is that in aneurysmatic areas the blood flow is slow. A role for fibrous cap degradation, linked to the inflammatory process, could be taught is these cases as well.

In conclusion, a contrast can be noted between areas containing ruptured and stable atherosclerotic plaques (fig. 3). Segments with stable plaques display either narrowing or absence of remodelling. In these lesions, the vessel shows medial thickening. Due to the whole vessel diameter narrowing, even small coronary artery intimal plaques could substantially obstruct the lumen. Unstable atherosclerosis would usually be related to a greater inflammatory infiltrate and increased number of microvessels at the adventitia. The involvement of this layer contributes to the characterisation of a process of pan-arteritis, which might be linked, by the action of elastase and other matrix-degrading enzymes, to dilation of the arterial segment (aneurysm). Thus, even large lesions could be associated with only moderate percent coronary obstruction, plaque rupture, and thrombosis.

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