Site of Intimal Rupture or Erosion of Thrombosed Coronary Atherosclerotic Plaques Is Characterized by an Inflammatory Process Irrespective of the Dominant Plaque Morphology

Allard C. van der Wal, MD; Anton E. Becker, MD; Chris M. van der Loos, PhD; Pranab K. Das, PhD

Background
The study was designed to verify the concept of plaques "at risk" and whether inflammation could play a role in plaque rupture and thrombosis.

Methods and Results
In 20 patients who had died of acute myocardial infarction, the thrombosed coronary artery was identified and the site of plaque rupture was traced in serial sections. The cellular characteristics of the fibrous cap at the immediate site of rupture were analyzed and compared with the adjacent cap tissue by use of monoclonal antibodies reactive with macrophages, T lymphocytes, and smooth muscle cells. A deep intimal rupture, extending into the lipid core, was encountered in 12 plaques, whereas 8 had superficial erosions only. Ten atherosclerotic plaques had a distinctly attenuated fibrous cap covering a large atheroma, 7 showed a thick fibrocellular cap overlying a lipid pool, and 3 showed a fibrocellular lesion without a clear lipid core. Macrophages, and to a lesser extent T lymphocytes, were the dominant cells at the immediate site of either rupture or superficial erosion in each instance. These sites, moreover, were always characterized by abundant expression of HLA-DR antigens on both inflammatory cells and adjacent smooth muscle cells, suggesting an active inflammatory reaction. In terms of overall cellular composition of the ruptured plaques, the dominant cell types were macrophages and T cells in 11, smooth muscle cells in 3, and mixtures of both in 6.

Conclusions
The underlying atherosclerotic plaque morphology in complicated coronary artery lesions causing acute myocardial infarction is heterogeneous with respect to both plaque architecture and cellular composition. However, the immediate site of plaque rupture or erosion is always marked by an inflammatory process. This suggests that inflammation plays a role in destabilizing the fibrous cap tissue and, thus, in enhancing the risk of coronary thrombosis. (Circulation. 1994;89:36-44.)

Key Words • atherosclerosis • myocardial infarction • plaques

Thrombosis of coronary arteries causing acute myocardial infarction is a consequence of a complicated atherosclerotic lesion.1,2 Most coronary thrombi are associated with tears that enter a large pool of extracellular lipids.3,4 Indeed, the risk of plaque rupture appears to be related to the composition of the atherosclerotic plaque. However, the precise mechanisms causing plaque rupture are not fully understood.5 The fibrous cap of ruptured plaques is often infiltrated by foam cells,1,4,6,7 which are largely of macrophage origin.8 The same population of blood-derived monocytes/macrophages that transforms in the lesions into foam cells interacts with T lymphocytes and smooth muscle cells via growth factors and cytokines.9 The occurrence of an inflammatory response in atherosclerotic lesions, which is mediated at least to some extent by cellular immune mechanisms, is now well appreciated.10 Thus far, however, the significance of these inflammatory processes for the development and pathological outcome of lesions is unsettled. In analogy with other chronic inflammatory diseases with tissue breakdown as a major feature, the inflammatory activity in atherosclerotic lesions could also be involved in localized destabilization processes of cap tissues. On the basis of such considerations, proteolytic enzyme release by macrophages has been suggested as a mechanism involved in cap weakening.4,11 The cellular composition of advanced atherosclerotic plaques is known to be heterogeneous,12,13 but detailed information about the cellular constituents at the immediate site of plaque rupture is not yet available.

See p 503

We investigated the cellular components of recently ruptured atherosclerotic plaques with cell-specific monoclonal antibodies in an attempt to evaluate further the potential role of an inflammatory process in the pathogenesis of intimal tearing. For this purpose, we studied thrombosed coronary arteries obtained from patients who had developed an acute myocardial infarction and died (mean time interval between onset of symptoms and death, <1 day). Phenotypic characterization of the cells at the immediate site of plaque rupture was performed and compared with that of overall cellular makeup of the entire plaque.
Methods

Tissue Sampling

The hearts of 20 patients who died of acute myocardial infarction and in which a thrombosed coronary artery was found at autopsy were used for this study. Infarcted myocardium was identified with a nitroblue tetrazolium enzyme staining technique on heart slices cut perpendicular to the left ventricular long axis. Roentgenograms of the heart were taken to identify calcifications in the coronary arteries. Only thrombosed coronary arteries that correlated with recent regional and transmural infarction were selected for this study. There were 20 sites (left anterior descending coronary artery, n=9; left circumflex artery, n=8; right coronary artery, n=3), one site in each case.

The time interval between onset of cardiac symptoms and death was well documented in 17 patients and varied from 0 to 2 days (mean interval, <1 day). In 3 patients, the interval was less certain but did not exceed 3 days. The time interval between death and autopsy ranged from 3 to 12 hours. The ages of the patients ranged from 32 to 86 years, with a mean age of 62.8 years.

Tissue Processing

All 20 coronary arteries showed some degree of calcification. Thus, they were carefully dissected from the epicardial fat, fixed in formalin, and slowly decalcified in EDTA. Thrombosed segments of the coronary arteries were sliced into 3-mm segments and embedded in paraffin. The paraffin blocks were serially sectioned at 6-μm thickness, and every 50th section was stained with an elastic–van Gieson stain to detect the site of plaque rupture. The remaining sections from the site of the intimal tear were then mounted for immunostaining.

Immunocytochemistry

A streptavidin-biotin complex/horseradish peroxidase technique was used as previously described.13 Endogenous peroxidase activity was blocked with methanol+0.3% H2O2. Horseradish peroxidase activity was visualized with diaminobenzidine as chromogen. The monoclonal antibodies used are listed in Table 1. In case of anti-HLA-DR staining, the immunoreactivity of the CR3/43 antibody was enhanced with 10 mmol/L citrate, pH 6.0, for 15 minutes at 100°C under microwave application.14,15 Sections of vessel wall where the specific antibody had been omitted or replaced by an irrelevant antibody of the same isotype served as negative controls.

Results

Histopathology

The coronary atherosclerotic plaques underlying recent luminal thrombosis showed a large lipid core in 17 of 20 cases. In 10 of these, the plaque contained virtually no fibrous cap, whereas 7 presented a substantial fibrous cap overlying the lipid core. In the remaining 3 cases, the plaque was composed of fibrocellular tissue without a clear lipid core.

In 12 plaques, the fibrous cap had completely ruptured, with the fissure extending into the lipid core.

Table 1. Monoclonal Antibodies Used

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity Relevant to the Present Study</th>
<th>Dilution</th>
</tr>
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<tbody>
<tr>
<td>1A4</td>
<td>Smooth muscle actin</td>
<td>1:100</td>
</tr>
<tr>
<td>HAM56</td>
<td>Macrophages; some endothelial cells</td>
<td>1:50</td>
</tr>
<tr>
<td>CR3/43</td>
<td>MHC class II molecules on macrophages, endothelium, activated T cells, and subpopulation of smooth muscle cells</td>
<td>1:50</td>
</tr>
<tr>
<td>LCA</td>
<td>CD45 present on all lymphocytes</td>
<td>1:50</td>
</tr>
<tr>
<td>UCHL-1</td>
<td>CD45RO present on memory T cells and subpopulation of macrophages</td>
<td>1:100</td>
</tr>
</tbody>
</table>

MHC indicates major histocompatibility complex. Antibodies were derived from Dakopatts, Glostrup, Denmark.

Table 2. Histopathological and Immunocytochemical Details of 20 Complicated and Thrombosed Atherosclerotic Lesions in Coronary Arteries of Patients Who Died of Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Type of Plaque Rupture</th>
<th>Type of Lesion Underlying Thrombosis</th>
<th>Dominant Cell Type at Site of Rupture</th>
<th>Dominant Cell Type of Adjacent Plaque Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Macrophage/T Cell</td>
<td>SMC/Collagen</td>
</tr>
<tr>
<td>Plaque rupture with hemorrhage into lipid core, n=12</td>
<td>Lipid-rich without substantial cap</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Solid cap overlying atheroma</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Fibrous</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Lipid-rich without substantial cap</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Superficial erosion and/or intimal flap, n=8</td>
<td>Solid cap overlying atheroma</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Fibrous</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>

*SMC indicates smooth muscle cell.
Fig 1. Histological sections. A, Detail of an atherosclerotic plaque with a large lipid core (+), an attenuated fibrous cap (F), and a central rupture (arrows), with luminal thrombus (T) and hemorrhage into the lipid core. Elastic tissue stain. B, The same area stained with HAM56 reveals accumulation of macrophages in the fibrous cap at the site of rupture. C, The same site stained with 1A4 reveals virtual absence of smooth muscle cells in the fibrous cap. The media shows positive staining. Original magnification, ×8.
Fig 2. Histological sections. A, Detail of atherosclerotic plaque with a large lipid core (*) and a thick fibrous cap (F) with sudden transition to an attenuated site with rupture (arrow). The site is marked by a thrombus (T). Elastic tissue stain. B, Same area stained with anti-CD68 reveals accumulation of macrophages at the site of rupture, whereas the adjoining thick fibrous cap is devoid of macrophages. C, Same area stained with 1A4 reveals absence of smooth muscle cells in the lacerated area and in the thick fibrous cap. Positivity is obtained in the media and overlying musculoelastic layer. Original magnification, A, ×16; B and C, ×12.
Fig 3. This page and facing page. Histological sections. A. Detail of an atherosclerotic plaque stained with HAM56, showing intimal erosion containing macrophages (arrows) and adherent thrombus (T). The area covers a thick fibrous cap (F), itself overlying a lipid core (+). B. Same area stained for HLA-DR reveals marked positivity in the superficial parts of the eroded intima, corresponding with the sites of accumulated macrophages. The macrophages bordering on the atheroma are strongly positive also. C. Same area stained with 1A4 reveals absence of smooth muscle cells in the superficial (eroded and macrophage-containing) intima, contrasting with positive staining of the media and the occasional presence of smooth muscle cells in the deeper layers of the intima (arrows). D. Detail of the superficial part of the intima stained with LCA shows lymphocytes (black dots). Foam cell macrophages are visible because of the hematoxylin counterstain. Original magnification, A through C, ×15; D, ×90.
by van der Wal et al  Inflammation and Ruptured Atherosclerotic Plaques

these cases, the ruptured cap was associated with a recent intraplaque hemorrhage. In some cases, part of the lipid core, together with macrophages and lymphocytes, had been extruded and was found within the thrombus. In 8 plaques, no such rupture of the fibrous cap could be found, despite mounting of all sections. Intraplaque hemorrhage was absent also. In these instances, the fibrous cap at the site of the thrombus showed an eroded surface characterized by loss of the endothelial lining and injury of the superficial intimal layers, with incorporation of platelet/fibrin thrombi. At these sites, the superficial layers of the cap were highly cellular.

None of the thrombi showed organization. Four thrombi contained detached fragments of a cellular cap with many foam cells. The findings are summarized in Table 2.

Immunocytochemical Findings

The results of immunophenotyping of the cellular constituents of the plaques will be described in relation to the type of intimal tear at the site of the thrombus. The findings are summarized in Table 2.

Plaque Rupture Extending Into the Lipid Core (n=12)

In 8 of 12 plaques, the fibrous cap at the site of rupture was extremely attenuated and composed almost entirely of macrophages (HAM-56+) mixed with lymphocytes (CD45+) (Fig 1). Macrophages outnumbered lymphocytes by far. Smooth muscle cells (1A4+) were either completely absent or present in occasional small clusters (Fig 1).

In 4 cases, the atherosclerotic plaque showed a thick and well-developed fibrous cap that was either acellular and composed entirely of extracellular matrix or mixed with a cellular component composed predominantly of smooth muscle cells. In each of these, however, the site of rupture showed a discrete area dominated by tightly packed foamy macrophages intermingled with T cells (Fig 2). At these sites, there were no smooth muscle cells.

In none of the cases had a rupture occurred in an area dominated by smooth muscle cells. The site of plaque rupture was always characterized by strong expression of HLA-DR antigens, which contrasted markedly with the low or absent expression of HLA-DR elsewhere in the fibrous cap. HLA-DR expression was most abundant on macrophages (foam cell and non–foam cell) and lymphocytes, but HLA-DR+ smooth muscle cells also occurred, although always limited to cells immediately adjacent to sites with inflammatory infiltrates and rupture.

Polymorphonuclear leukocytes, which were occasionally found in the fibrous cap at the site of rupture, did not stain with the antibodies used.

Plaques With Intimal Erosion (n=8)

These lesions were confined to the superficial parts of the fibrous cap. The site of the lesions was contiguous with the overlying thrombus and in each instance contained accumulations of macrophages intermingled with T cells (Fig 3). Smooth muscle cells were absent. The overall plaque morphology, however, varied markedly. Two plaques showed an extensive atheroma and a thin, attenuated fibrous cap with erosion. In the remaining six cases, the surface erosion, dominated by macrophages and T cells, overlay a band of connective tissue characterized by a mixture of smooth muscle cells and variable numbers of macrophages and T cells. In three of these six, a fibrous cap covered an atheroma (Fig 3), whereas the remaining three plaques were basically fibrocellular and composed predominantly of smooth muscle cells, without a clear lipid core (Fig 4).

Strong HLA-DR expression was found on macrophages and lymphocytes at the site of erosion and on cells, both inflammatory and smooth muscle, in the adjacent connective tissue layers (Figs 3 and 4).

Discussion

The pathogenetic mechanisms that cause intimal ruptures in atherosclerotic plaques responsible for thrombosis are not fully understood. In addition to biomechanical factors, such as shear stress and vasospasm, destabilizing changes in the tissues of the fibrous cap may be important. The presence of large numbers of foamy macrophages in lesions underlying thrombosis was reported a long time ago and was recently con-
Fig 4. This page and facing page. Histological sections. A, Cross section through a coronary artery that contains an extensive fibrocellular plaque (FP), with intimal erosion and thrombus (T). The boxed area is shown in higher magnification in B through E. B, HAM56 stain shows accumulation of macrophages in the fibrocellular lesion. C, The boxed area stained with 1A4 shows smooth muscle cells diffusely throughout the fibrocellular lesion and within the media. D, Same area stained with LCA reveals lymphocytes confined to the area that also contained macrophages. E, Same area stained for HLA-DR reveals strong positivity at the site of accumulated macrophages and lymphocytes and in the fibrocellular tissue containing smooth muscle cells. Original magnification, A, x15; B through E, x25.
firmed by Richardson et al.,4 who also speculated on the possibility of enzymatic degradation of the fibrous cap by macrophages, causing weakening of the fibrous cap before rupture.

The present study confirms that the vast majority of ruptured plaques (17 of 20) contained a substantial core of extracellular lipids and cellular debris, as previously reported.1,2,4,18 The fibrous cap overlying the lipid core, however, appeared to be highly variable in both thickness and cellular constituents. Atherosclerotic plaques with thin or virtually nonexistent fibrous caps showed a surface zone dominated by macrophages (mostly foam cells) intermingled with T lymphocytes. Superficial erosion or complete rupture into the lipid core was found to be the immediate cause of thrombosis in this type of lesion. Conversely, atherosclerotic plaques with a substantial fibrous cap showed marked heterogeneity with respect to their cellular makeup. The fibrous caps in these lesions consisted mainly of extracellular matrix (mostly collagen), with variable amounts of smooth muscle cells. However, at the site of erosion, corresponding with the overlying thrombus, the superficial parts of the cap were dominated by macrophages and, to a lesser extent, T lymphocytes. Similarly, at sites of deep plaque rupture, the fibrous cap showed a localized accumulation of macrophages and T cells, with loss of smooth muscle cells. It thus appears that the site of plaque rupture is invariably associated with the occurrence of large numbers of macrophages and T cells and lack of smooth muscle cells. In 8 plaques, the immediate site of rupture was distinct from the adjacent fibrous cap because of the inflammatory process. The 3 plaques that were composed almost solely of fibrocellular tissue also presented inflammatory cells in relation to the site of erosion. Our own studies of advanced but otherwise uncomplicated atherosclerotic lesions also revealed that some lesions are composed mainly of smooth muscle cells and collagen but occasionally with local areas dominated by inflammatory cells (unpublished information). On the basis of our present observations, therefore, one may argue that these particular types of lesions should also be considered as “plaques at risk,” in addition to plaques with large atheromatous pools and virtually absent fibrous cap.

Since all plaques were obtained from patients who died shortly after the complicating event (mean time interval, <1 day) and no organization of the thrombi could be detected, one has to assume that the lympho-
cytes and lipid-laden macrophages were present in the intima at the time of thrombus formation. Both T cells and macrophages expressed class II major histocompatibility complex antigens abundantly, and the site of the intimal tear always could be distinguished from the surrounding plaque tissue by an increase in the number of HLA-DR 
+ cells. Macrophages always outnumbered the lymphocytes in these complicated plaques. The expression of HLA-DR molecules on T cells indicates activation of these cells. The activated T cells may trigger neighboring macrophages to synthesize and secrete tissue-degrading enzymes, such as metalloproteinases. Recently, the expression of stromelysin-1 gene in foamy macrophages was reported. However, apart from these enzymes, toxic substances such as free oxygen radicals and oxidized lipid products could result in damage of the connective tissues of the fibrous cap. Neutrophils, which were found occasionally in the tissues of the lesion near the thrombus, are also capable of destroying cap tissue, since they are capable of producing a large number of lytic enzymes. These cells are rarely encountered in intact plaques. It is most suggestive, therefore, that they enter the plaque tissue shortly after the rupture. Indeed, neutrophil activation has also been observed at sites of laceration shortly after percutaneous transluminal coronary angioplasty.

Another important finding is the almost complete absence of smooth muscle cells at sites of rupture or erosion, since, after all, these are the cells considered to produce the connective tissue matrix in atherosclerotic plaques. An overall decrease in smooth muscle cells has also been reported in ulcerating atherosclerotic plaques of the abdominal aorta compared with intact lesions. One may hypothesize that a decrease in the number of smooth muscle cells induces local changes in the production of extracellular matrix components, which may contribute to local weakening of the cap.

This study has shown that plaque rupture causing thrombosis, whether as a superficial erosion or as a deep fissure, is associated with a localized inflammatory process that does not necessarily reflect the overall cellular composition of the plaque.

References


Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology.

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