Changes in the flow properties of white blood cells after acute myocardial infarction

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SUMMARY Because they can obstruct blood vessels and release noxious substances, white blood cells may contribute to the development of tissue ischaemia. The flow properties of white cells were tested after myocardial infarction, by measuring the filtration rates of cell suspensions through 8 μm pore filters. Compared with mononuclear cells from age matched controls, mononuclear cells from patients with infarction showed impaired filterability within the first day after the onset of pain; this condition persisted for at least two days and by day 10 it was improved. On day 1, granulocyte filterability and the proportion showing morphological evidence of activation were nearly normal. By day 3 the flow resistance and activation had increased, but the changes seen depended on the age of the patient. The filterability and activation of granulocytes from patients aged <60 were significantly increased from day 1, whereas there were no changes in granulocytes from patients aged >60 years. Suspensions of unfractionated white cells showed changes intermediate between the mononuclear cells and granulocytes. A group of five patients who presented with chest pain but who were subsequently found not to have had an infarction showed no evidence of abnormal filterability or activation. The changes in filterability probably reflect white cell activation, which may have an adverse effect on the perfusion of the ischaemic myocardium.

It has been suggested that white blood cells, and neutrophilic granulocytes in particular, are major mediators of myocardial injury induced by ischaemia.1 When they are activated in the microcirculation these cells cause extensive tissue damage, for example by releasing oxygen free radicals and lysosomal enzymes.2 Neutrophils would be expected to invade the ischaemic myocardium as part of the inflammatory response, but they also become entrapped in capillaries in the early stages of myocardial infarction,3 and this response may be detrimental. In animal models of the ischaemic heart the role of white cells in mediating myocardial damage was suggested when infarct size was reduced if blood was depleted of white cells4 or if lipooxygenase metabolism of arachadonic acid (to form leukotrienes) was inhibited.5 A rheological mechanism may contribute to the trapping of white cells and to propagation of tissue injury. White cells are much more resistant to deformation than red cells and their entry into capillaries can take several seconds, giving rise to intermittent plugging even in the normal circulation.6 In animal studies when the perfusion pressure is lowered they become entrapped in the microcirculation of skeletal muscle causing prolonged disturbance of flow.7 Similar events were seen during experimental myocardial ischaemia in the dog. The capillaries became plugged by white cells, mainly granulocytes, and these were not dislodged by reperfusion.4 Furthermore, reperfusion increased the number trapped, possibly owing to activation of newly arriving cells by factors released by reaction of the earlier white cells with the endothelial wall.8 Activated cells had altered adhesive and mechanical properties,9 which increased their tendency to block narrow vessels. Therefore, in ischaemia there is a potential for the establishment of an ischaemic vicious cycle of cell trapping and activation, endothelial damage with release of chemotactic factors, and further activation and trapping of new cells.

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To investigate this possibility we measured the flow properties of white cells at intervals after myocardial infarction in patients. Previous studies have shown that the white cells of patients with severe ischaemia of the leg have abnormal flow properties attributable to activation by factors released by the ischaemic tissue. A similar process of cell activation and rheological alteration might be found after more acute myocardial ischaemia.

Patients and methods

Patients and blood sampling
Thirty patients were initially studied on the morning after admission to hospital with severe chest pain and a presumptive diagnosis of acute myocardial infarction (day 1, average time after start of pain = 18 hours). The diagnosis was confirmed in 25 of the patients by typical electrocardiographic changes and at least a twofold rise in the serum concentrations of cardiac enzymes; the other five had not had an infarction as judged by these criteria. Measurements were repeated two days later (in 21 patients with confirmed infarction and in five without) and approximately one week later (in 15 patients with confirmed infarction only). The age range of the patients was 34–88 years (mean (SD) 61 (11)). Fifteen age matched controls aged 36–92 (mean 56 (16)) were studied once. On every occasion, 20 ml venous blood was drawn and anticoagulated with edetic acid (1.5 mg/ml). Measurements were started within two hours of collection of the sample.

Sample preparation
Suspensions of white cells were prepared and their filterability was measured by a method described in detail elsewhere. Briefly, unfractionated white cells were separated from 10 ml whole blood by sedimentation under gravity. After sedimentation, a small fraction of these cells was fixed in 1% glutaraldehyde for subsequent morphological examination. At the same time, 10 ml blood was fractionated by centrifugation on two density gradients. The separated fractions, mononuclear cells (mixed lymphocytes and monocytes) and granulocytes, and the unfractionated cells were washed twice in phosphate buffered saline (Dulbecco A, Oxoid, London) plus 5% plasma and 1 g/l glucose, resuspended in this medium, and counted. The cell concentration was then adjusted to $5 \times 10^6$/ml and filtration measurements were carried out immediately. After filtration, the remaining granulocytes were fixed with glutaraldehyde.

White cell counts and differential counts were made on a Coulter Counter model S plus IV; whole blood differential counts were done on stained blood films. Fixed unfractionated white cells and granulocytes were examined by light microscopy and the percentage with pseudopodia or cytoplasmic irregularities was counted. These non-spherical cells were classified as "active".

Filterability measurements
Filterability was tested by measuring the flow rate of cell suspensions through filters (Nuclepore, Pleasanton, CA) with nominal pore diameters of 8 µm (actual mean diameter 7.2 µm; manufacturer's data). A St George's Filtrometer (Carri-Med, Dorking) was used, with a constant driving pressure equal to 3 cm of water. The device measures the flow rate of the suspension as a function of the volume filtered. The flow rates are expressed relative to the flow rate of the cell free suspending medium, which was separately tested for each filter.

Three flow variables were derived as previously described: the initial relative flow rate (extent of initial, rapid fall in flow rate), the slow particle resistance (subsequent rate of decrease in flow, percentage/ml), and the relative flow rate after 1 ml of the sample had been filtered. Theoretical considerations suggest that the initial relative flow rate characterises the flow resistance of the main population of rapidly flowing cells and the slow particle resistance characterises a small subpopulation of slowly flowing cells. The flow rate after 1 ml represents the contribution from both these types and also from any cells that permanently block the filter pores.

Statistical analysis
Group means were compared by a two tailed Student's $t$ test, and between group measurements made at different times by two tailed paired $t$ tests.

Results
White cell filterability was measured on days 1, 3, and 10 after acute myocardial infarction. The filterability of granulocytes and of unfractionated white cells was lower on day 3 than on day 1; it was improved again on day 10 compared with day 3. Monocyte filterability was equally reduced on days 1 and 3 and improved on day 10. These trends were seen in each flow variable. Figure 1 shows the results for the slow particle resistance, which also shows data for controls and for five patients who presented with chest pain but who showed no enzymatic or electrocardiographic evidence of infarction. These patients had normal white cell filterability on day 1 and no significant change on day 3.

Table 1 compares the white cell filterability measured for patients on day 3 with that of controls.
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![Graph showing flow properties over time]

Table 1 Filterability of white blood cells and percentage that are active on day 3 after infarction in 21 patients and in 15 controls (mean (SD))

<table>
<thead>
<tr>
<th>Filterability variables</th>
<th>Initial relative flow rate</th>
<th>Slow particle resistance (%/ml)</th>
<th>Relative flow rate after 1 ml</th>
<th>Active cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated cells:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI</td>
<td>0.80 (0.06)</td>
<td>362 (178)*</td>
<td>0.32 (0.17)**</td>
<td>28 (14)**</td>
</tr>
<tr>
<td>Controls</td>
<td>0.83 (0.04)</td>
<td>236 (62)</td>
<td>0.49 (0.07)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Granulocytes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI</td>
<td>0.82 (0.05)</td>
<td>202 (145)*</td>
<td>0.45 (0.23)*</td>
<td>30 (26)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.84 (0.03)</td>
<td>112 (46)</td>
<td>0.59 (0.12)</td>
<td>20 (20)</td>
</tr>
<tr>
<td>Mononuclear cells:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI</td>
<td>0.66 (0.12)**</td>
<td>692 (217)**</td>
<td>0.18 (0.11)**</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.82 (0.09)</td>
<td>523 (69)</td>
<td>0.32 (0.06)</td>
<td></td>
</tr>
</tbody>
</table>

Statistical comparisons are patients v controls: *p < 0.05, **p < 0.01. AMI, acute myocardial infarction.
considerable impairment developed by day 3 when each flow variable was statistically different from that in the controls and older patients. Their filterability then improved toward normal values by day 10.

The correlation between age and the filterability of mononuclear cells was less. Patients aged <60 years showed a consistently slower flow, but the difference was only barely significant (>=0.033 for the different flow variables). In the controls there was an opposite tendency towards a lower flow and greater activation with increasing age but this was not statistically significant.

Haematological indices were also abnormal in the patients (table 2). On day 1 the white cell count and the percentage of granulocytes were higher and the percentage of lymphocytes was lower than in the controls. Subsequently these variables returned towards normal.

Discussion

We found abnormalities in white cell behaviour after myocardial infarction. The granulocyte response was delayed and dependent on the patient’s age. Significant impairment of flow developed by day 3 and was then inversely correlated with patient age. Generally, patients over 60 years of age showed no changes in filterability during the whole measurement period. On the other hand, patients aged <=60 years did show significantly impaired granulocyte flow on day 3. The filterability of granulocytes correlated well with the number of “active” cells seen microscopically. Thus the changes in flow properties of granulocytes probably reflected activation, with this activation response being greater in younger patients.

The flow properties of the mononuclear cells were abnormal at the first test. Monocytes are far more resistant to flow than lymphocytes, judged both from the filterability measurements13 and from studies of micropipette aspiration.14 In vitro, most mononuclear cells seem to be morphologically active, and
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are then much more resistant to micropipette deformation than the "passive" spherical monocytes. Thus changes in the filterability of the mononuclear suspension may reflect rapid activation of the monocytes after infarction. However, the ratio of monocytes to lymphocytes in the mononuclear suspension was also increased after infarction because of the shift in white cell differential count (table 2). This ratio correlated with impaired mononuclear filterability in individual patients on day 1 but not on day 3 or day 10. Also, the ratio returned to normal more quickly than the mononuclear suspension filterability. Thus the changes in the filterability of the mononuclear suspension probably result from a combination of monocyte activation and a shift in the monocyte/lymphocyte ratio.

Unfractionated mixtures of white cells showed a response intermediate between the separated granulocytes and mononuclear cells, as might be expected. Again, filterability correlated with the number of cells judged to be morphologically active.

The dependence of the white cell response on age, which was pronounced in granulocytes but less evident in mononuclear cells, was unexpected and in the opposite direction to that found in controls (in whom dependence was weak). It was not simply the result of previous infarction. When patients who had suffered previous infarction were compared with those in a similar age range who had not had previous infarction, there were no significant differences in any of the variables of filterability (data not shown). It is possible that the cells from older patients were less able to respond to the stimulus of ischaemia and tissue damage. Previous studies have suggested that the immune response is impaired in older people.15

Activation, margination, and diapedesis of neutrophils occur within 60 minutes of coronary artery occlusion in dogs.16 Margination will raise vascular flow resistance and facilitate diapedesis. Capillary plugging by granulocytes has been seen on a similar time scale of hours.10 Plugging is thought to arise at first because the reduced perfusion pressure is not capable of deforming the white cells sufficiently for them to flow through narrow vessels. Thus rapid changes in the flow behaviour and activity of the white cells may contribute to the early stages of ischaemia and tissue damage, and could be important determinants of the final infarct size.1 In the present study, rheological changes in white cells were seen in peripheral blood after infarction. This response probably represents a "diluted" version of what occurs in the myocardium. Also, those cells which were maximally stimulated may have become trapped and were therefore not detectable in peripheral blood. The granulocyte changes were not evident until day 3 and this could correspond to the acute phase of the inflammatory response. White cells may be involved earlier in the ischaemic process, but this was not detectable by our methods. None the less, monocyte filterability was altered from an early stage.

Because white cells are able to inhibit flow in the microcirculation, the rheological alterations seen here may worsen perfusion of the infarcted myocardium. At the same time they probably reflect functional changes in the cells, which may take part in the initiation and propagation of tissue damage. Thus treatment which inhibits white cell activation in the early stages of infarction may be beneficial, even though the normal white cell response may be essential at later stages of healing.

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