Systemic Upregulation of Leukocyte Integrins in Response to Lower Body Ischemia-Reperfusion during Abdominal Aortic Aneurysm Repair

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Ischemia and reperfusion in myocardial infarction and stroke are associated with upregulation of leukocyte adhesion molecules, which contributes to tissue injury by facilitating leukocyte adhesion and infiltration in the affected tissues. Surgical repair of the abdominal aortic aneurysm involves clamping and declamping of the aorta, which necessarily results in ischemia and reperfusion of the lower half of the body. Given the large volume of the affected tissues and unimpeded venous return during reperfusion, we hypothesized that the procedure may result in upregulation of leukocyte integrins in the systemic circulation. To test this hypothesis, we studied neutrophil and monocyte surface densities of CD11b and CD18 in patients undergoing elective infrarenal abdominal aortic aneurysm repair. Serial blood samples were collected from the radial artery and femoral vein during the operation and leukocyte CD11b and CD18 surface densities were quantified by flow cytometry. Following reperfusion, CD11b expression in neutrophils and monocytes increased significantly in femoral venous and arterial blood. The mean time to peak expression of CD11b in neutrophils and monocytes during reperfusion was 34.4 and 31.4 minutes in venous and 38.5 and 36.4 minutes in arterial blood, respectively. Similar rises in CD18 expression on neutrophils and monocytes were observed in venous and arterial blood. The mean time to peak expression of CD18 in neutrophils and monocytes during reperfusion was 34.0 and 40.0 minutes in venous and 47.5 and 50.0 minutes in arterial blood, respectively.

Conclusion: Iatrogenic ischemia reperfusion during abdominal aorta aneurysm repair results in systemic upregulation of neutrophil and monocyte integrins, which peak approximately 30 minutes after declamping. The biological impact, if any, of this phenomenon is unknown and awaits future investigations.

Key words: leukocytes ■ CD11b/CD18 ■ adhesion molecules ■ inflammation ■ vascular endothelium

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Ischemia and reperfusion result in leukocyte activation, adhesion and infiltration, which can extend tissue injury in experimental stroke and myocardial infarction.[1,2] Activated neutrophils are considered the primary mediators of tissue destruction following ischemia and reperfusion.[3-8] This involves adhesion to the endothelial surface and tissue infiltration, followed by eventual release of toxic reactive oxygen metabolites and proteases by neutrophils.[4] The activation of neutrophils and monocytes is accompanied by an increase in cell-surface density of the adhesion molecules CD11b and CD18, which play an important role in leukocyte adhesion and infiltration.[5,6,9] The critical role of these adhesion molecules is clearly demonstrated by the observations that inhibition of leukocyte adhesion with antibodies directed against CD18 or the CD18/CD11 dimers can reduce the extent of injury.[1,2]

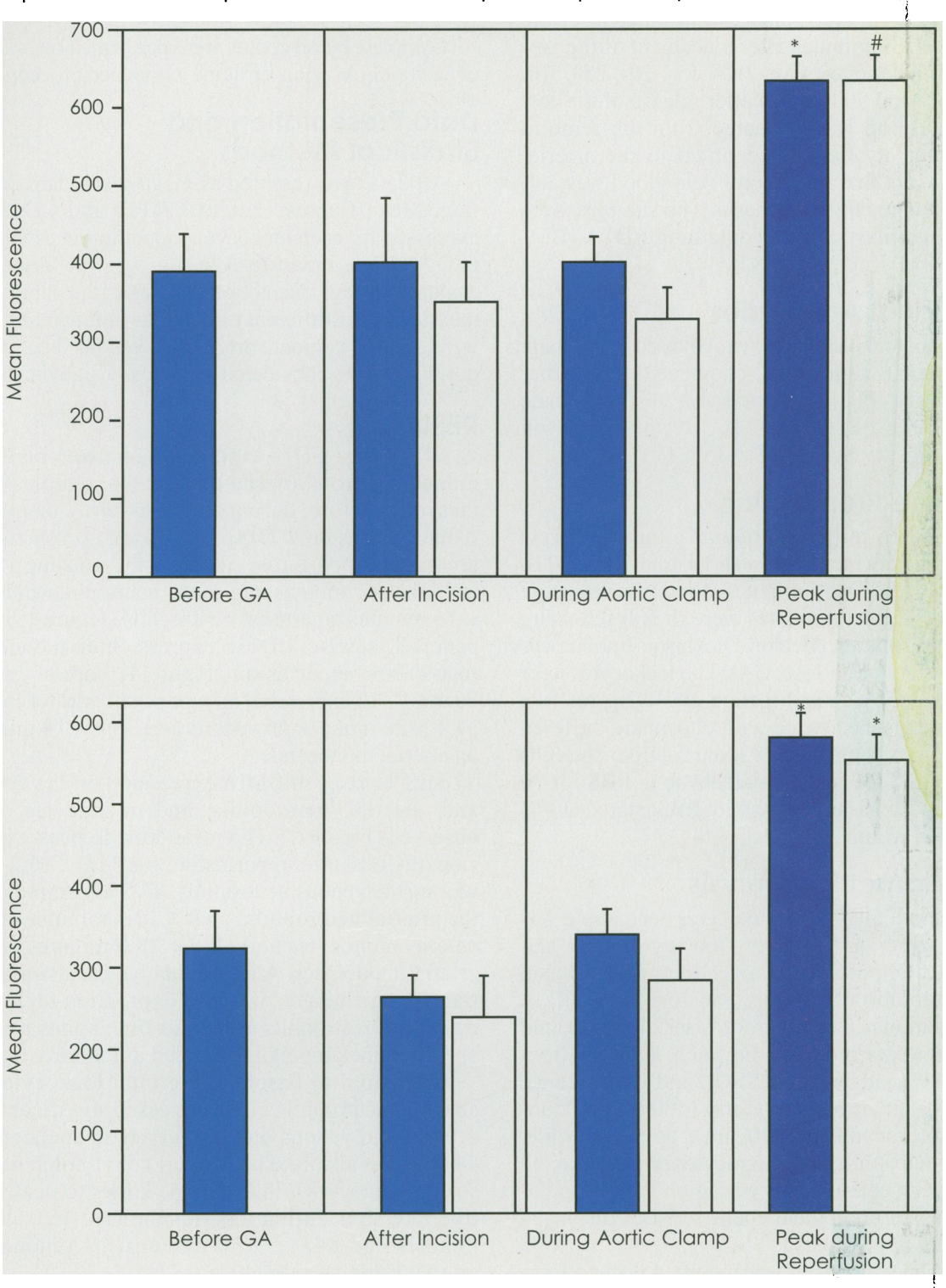
Surgical repair of abdominal aortic aneurysm involves extended clamping and declamping of the aorta, which necessarily results in ischemia and reperfusion of the lower half of the body. Given the large volume of the affected tissues and the unimpeded venous return from the ischemic sites following restoration of blood flow, we hypothesized that this surgical procedure would result in marked upregulation of leukocyte integrins in the systemic circulation. To test this hypothesis, we measured neutrophil and monocyte surface densities of CD11b and CD18 in serial blood samples obtained from radial artery (representing systemic blood) and femoral vein (representing blood draining the ischemic tissues) in a group of patients undergoing elective abdominal aortic aneurysm repair.

METHODS

Institutional human subjects committee approval and individual informed consent were obtained prior to the study. Eight Caucasian male patients aged 55–81 with past history of hypertension, tobacco smoking and arteriosclerotic cardiovascular disease undergoing elective infrarenal abdominal aortic

aneurysm repair were studied. Radial artery and pulmonary artery catheters were placed prior to induction of general endotracheal anesthesia. After the induction of general anesthesia, a right femoral vein catheter was placed. Each patient received 80–100 units per kilogram of heparin intravenously 10 min-

Figure 1. Mean CD11b expression on neutrophils (upper panel) and monocytes (lower panel) in the blood samples taken from the radial artery (dark bars) and femoral vein (open bars) before general anesthesia (GA), shortly after incision, during aortic clamping and at the peak observed after reperfusion compared to other time points from the same sample site; * p<0.01; # p<0.001



utes before application of the aortic clamp. Mean aortic cross-clamp time was 85 minutes (range 47–130 minutes), and mean duration of surgery was 241 minutes (range 150–315 minutes). There were no intraoperative complications.

Blood Collection

Blood from the radial artery catheter was collected before general anesthesia; 30 minutes after the skin incision; 30 minutes after placement of the aortic clamp; and at one-, five-, 10-, 15-, 20-, 25-, 30-, 40-, 50-, 60- and 90 minutes after release of the aortic clamp. Blood was collected from the femoral vein catheter at intervals identical to the arterial samples, except that no femoral vein blood was collected before general anesthesia. The samples were collected into plastic tubes containing EDTA (Becton-Dickinson, Rutherford, NJ).

Monoclonal Antibodies

The monoclonal antibodies (mAb) used in the study were antihuman mouse IgG, conjugated with either phycoerythrin (PE) or fluorescein isothiocyanate (FITC) (CD11b-PE, CD18-FITC, Becton-Dickinson Immunocytometry Systems, San Jose, CA).

Leukocyte Preparation

One-hundred-milliliter aliquots of arterial blood were immediately incubated with 10 ml of the CD11b and CD18 mAbs for 15 minutes at room temperature in the dark. The erythrocytes were then lysed using FACS lysing solution (Becton-Dickinson Immunocytometry Systems, San Jose, CA). The leukocytes were then separated by centrifugation at 500Xg for five minutes and washed twice with phosphate buffered saline (PBS) containing 0.1% sodium azide. The cells were fixed in 0.5% paraformaldehyde in PBS for 30 minutes at 4°C, washed twice with PBS, stored at 4°C and analyzed within 24 hours.[10]

Flow cytometric Analysis

A minimum of 10,000 leukocytes per sample was analyzed using a FACSort flow cytometer and LYSYS-II computer software (Becton-Dickinson Immunocytometry Systems, San Jose, CA). Each cell was illuminated by an argon laser (488 nm), and three parameters recorded for each: forward light scatter (FSC), side scatter (SSC), and fluorescence intensity for either phycoerythrin (580 nm) or fluorescein isothiocyanate (530 nm), as appropriate. Data for neutrophils and monocytes were extracted and analyzed separately by gating on these populations using morphological characteristics displayed on a dot plot of FSC versus SSC.

Fluorescence intensity was recorded using a four-decade, 1,024-channel logarithmic scale. Histograms of frequency versus fluorescence channel number were derived for each cell type, allowing calculation of mean fluorescence channel (MFC), which correlates directly with antigen expression. MFC was corrected for autofluorescence and nonantigen-specific antibody binding by using standard isotype control monoclonal antibodies.[10]

Complete Blood Counts

Complete blood counts were performed on aliquots of each sample using a routine automated procedure.

Data Presentation and Statistical Methods

All data are presented as mean ± standard deviation. Mean fluorescence of CD11b and CD18 are expressed for each leukocyte population in each sample. Multiple-measure ANOVA was performed to evaluate the significance of changes in the parameters measured over different time points and paired t-tests were done to compare arterial vs. venous data. P values <0.05 were considered statistically significant.

RESULTS

CD11b and CD18 expressions on neutrophils and monocytes from arterial and venous samples were measured before, during and after aortic clamping. After reperfusion, CD11b expression on venous and arterial neutrophils rose significantly, reaching a peak at 34.4 ± 27 minutes on venous neutrophils and 38.5 ± 16 minutes on arterial neutrophils (Figure 1, upper panel). Likewise, CD11b expression on monocytes rose during reperfusion (Figure 1, bottom panel). Peak CD11b expression on monocytes was reached at 31.4 ± 20 minutes on venous and 36.4 ± 14 minutes on arterial monocytes.

Similar rises in CD18 expression on the venous and arterial neutrophils and monocytes were observed (Figure 2). The mean time to peak expression of CD18 after reperfusion was 34.0 ± 16.7 minutes on the venous neutrophils, 47.5 ± 33 minutes on the arterial neutrophils, 40.0 ± 11.5 minutes on the venous monocytes and 50.0 ± 28.0 minutes on the arterial monocytes. A representative bar graph illustrating the changes of CD18 expression on monocytes and neutrophils at various time points prior to and after unclamping is provided in Figure 3.

Compared to baseline, the total leukocyte and absolute neutrophil counts increased significantly in arterial and venous blood after aortic unclamping. Changes in absolute neutrophil count noted during surgery are shown in Figure 4. Times to peak neutrophil counts during reperfusion were 46.0 ± 30.0 minutes and 44.0 ± 29.0 minutes for venous and arterial samples, respectively.

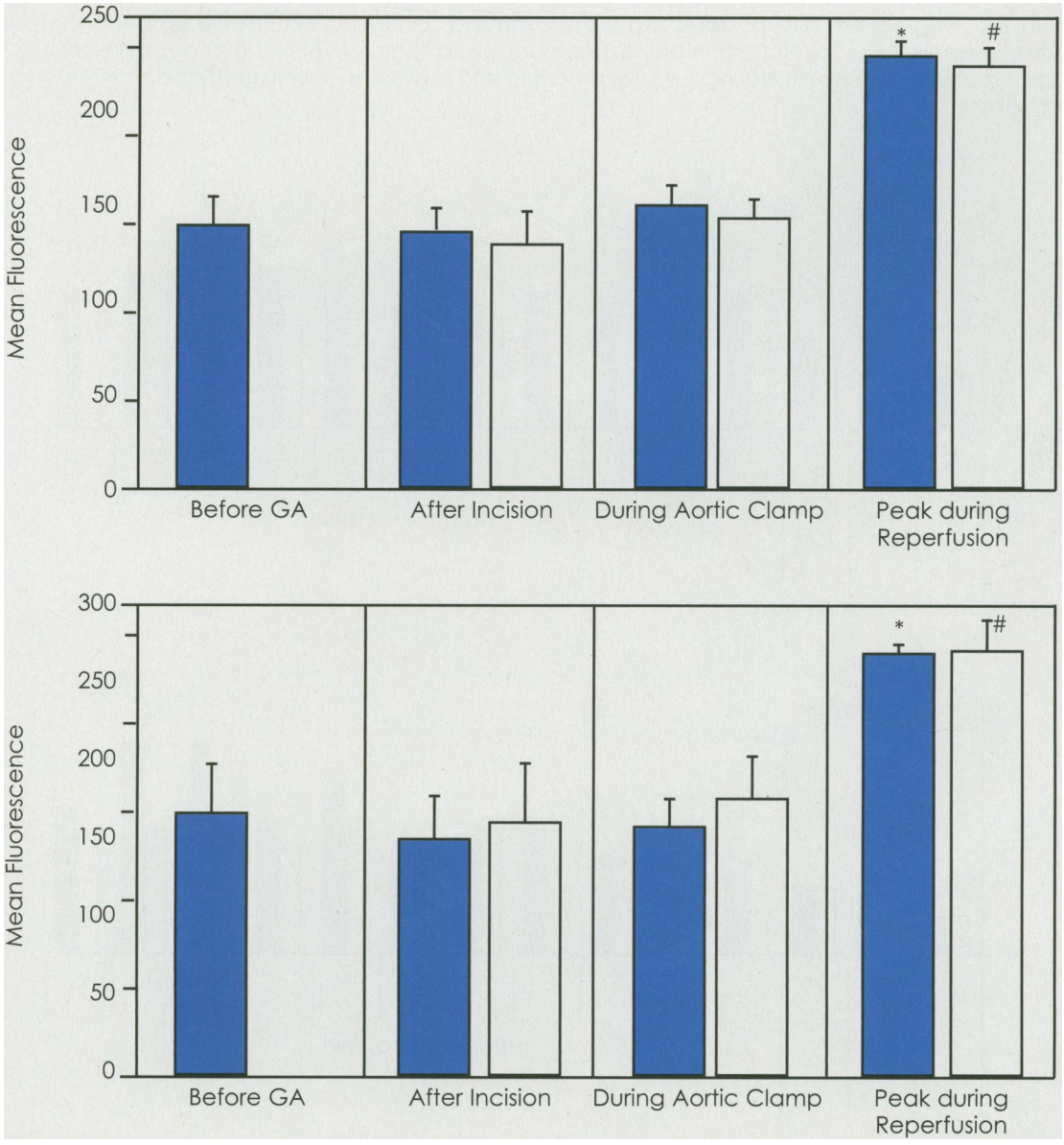
There were no significant correlations between

either the magnitude of rise or time to peak expression of the given receptors with the duration of aortic clamping.

DISCUSSION

CD11b and CD18 are members of the integrin family of adhesion molecules, which consist of transmembrane glycoproteins expressed primarily on neutrophils and monocytes. They form heterodimer complexes from the noncovalent union of the alpha and beta subunits before being transported to the cell surface. In this regard, the CD18 (beta2) integrin combines with three distinct alpha chains, namely CD11a, CD11b or CD11c.[1,2] The association of CD11b and CD18 forms the complement receptor, CR3 (or MAC-1) heterodimer, which participates in complement binding, phagocytosis and intercellular adhesion.[5,6] The sequence of this neutrophil-endothelial binding has recently been studied carefully. After an inflammatory stimulus, neutrophils roll along the postcapillary venules at velocities distinctly below that of flowing blood. After some rolling, the cells

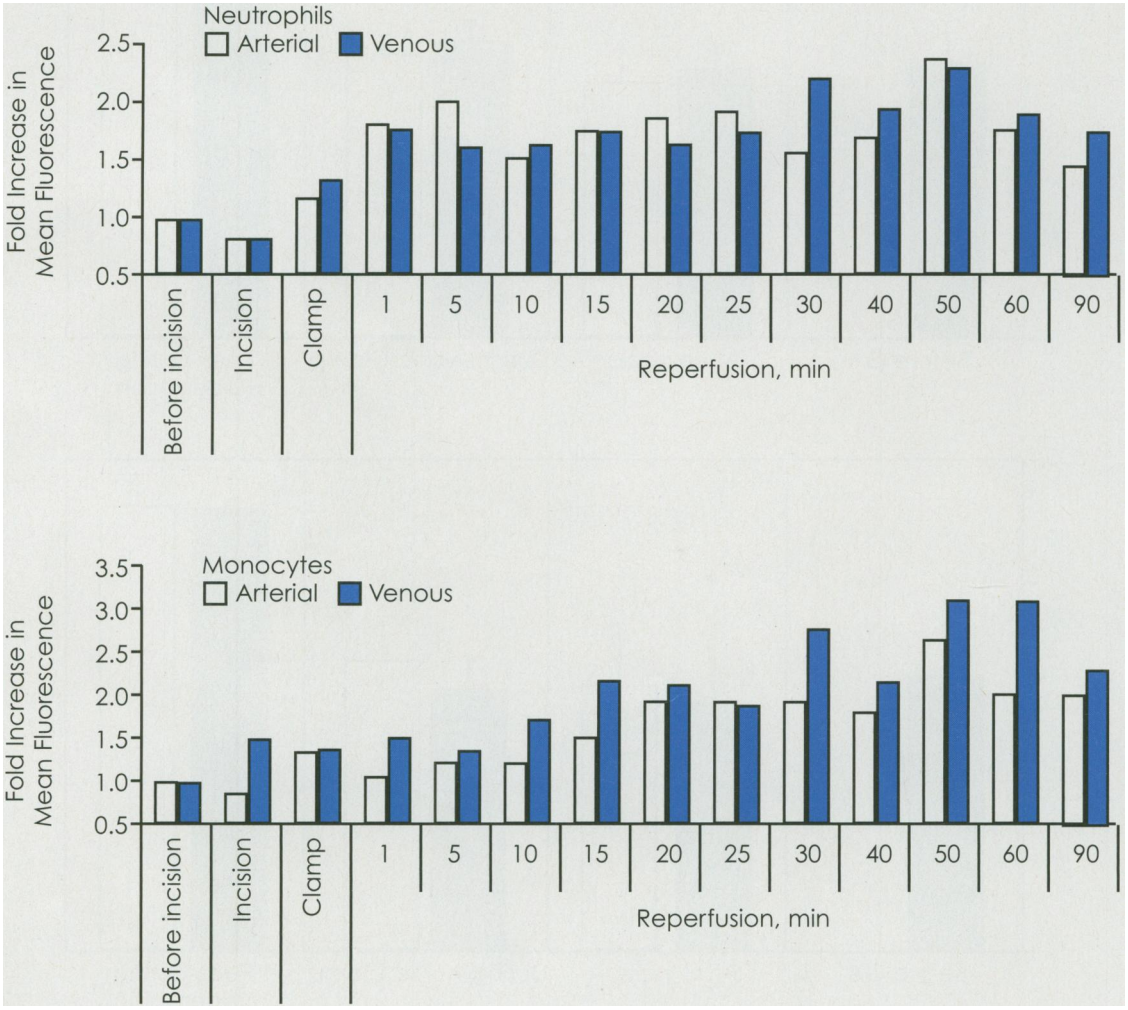
Figure 2. Mean CD18 expression on neutrophils (upper panel) and monocytes (lower panel) in the blood samples taken from the radial artery (dark bars) and femoral vein (open bars) before general anesthesia (GA), shortly after incision, during aortic clamping and at the peak observed after reperfusion compared to other time points from the same sample site; * $p<0.05$



stop, change shape and extravasate into extravascular tissue. The initial capture of the traveling neutrophils is mediated through the expression of another family of adhesion molecules known as selectins. Firm adhesion and transmigration of the leukocytes, however, does not occur unless the integrin molecules are engaged and bind to their endothelial ligands.[1] Thus, a primary function of the CD11b/CD18 adhesion complex (CR3) is the facilitation of neutrophil-endothelial binding through its interaction with endothelial ligands, such as CD54 (ICAM-1, intercellular adhesion molecule 1). This interaction is a critical step in the neutrophil response to inflammatory stimuli that can ultimately result in tissue damage.

CD11b and CD18 have been widely implicated in the pathogenesis of local and distant (especially pulmonary) neutrophil-mediated tissue injury arising from ischemia and reperfusion.[4,7,11-18] Pretreatment of animals with monoclonal antibodies against CD11b/CD18 has been shown to decrease the infiltration of neutrophils into reperfused ischemic tissues and to limit tissue injury.[13-16,18,19] In addition, in animal models, pretreatment with CD18 antibody lowers pulmonary sequestration of neutrophils and mitigates the associated rise in lung permeability following lower-torso ischemia and reperfusion.[13] Also, in an animal model, blockade of CD18 during reperfusion has been shown to decrease edema and subsequent necrosis of the previously ischemic tissue.[18] Though experiments have demonstrated a role for leukocyte CD11b and CD18 in ischemia/reperfusion injury via the use of monoclonal antibodies against those receptors,

Figure 3. Representative bar graphs depicting CD18 expression on blood neutrophils (upper panel) and monocytes (lower panel) in radial artery blood (dark bars) and femoral venous blood (open bars) obtained at multiple time points during surgical procedure in a patient undergoing abdominal aorta aneurysm repair.



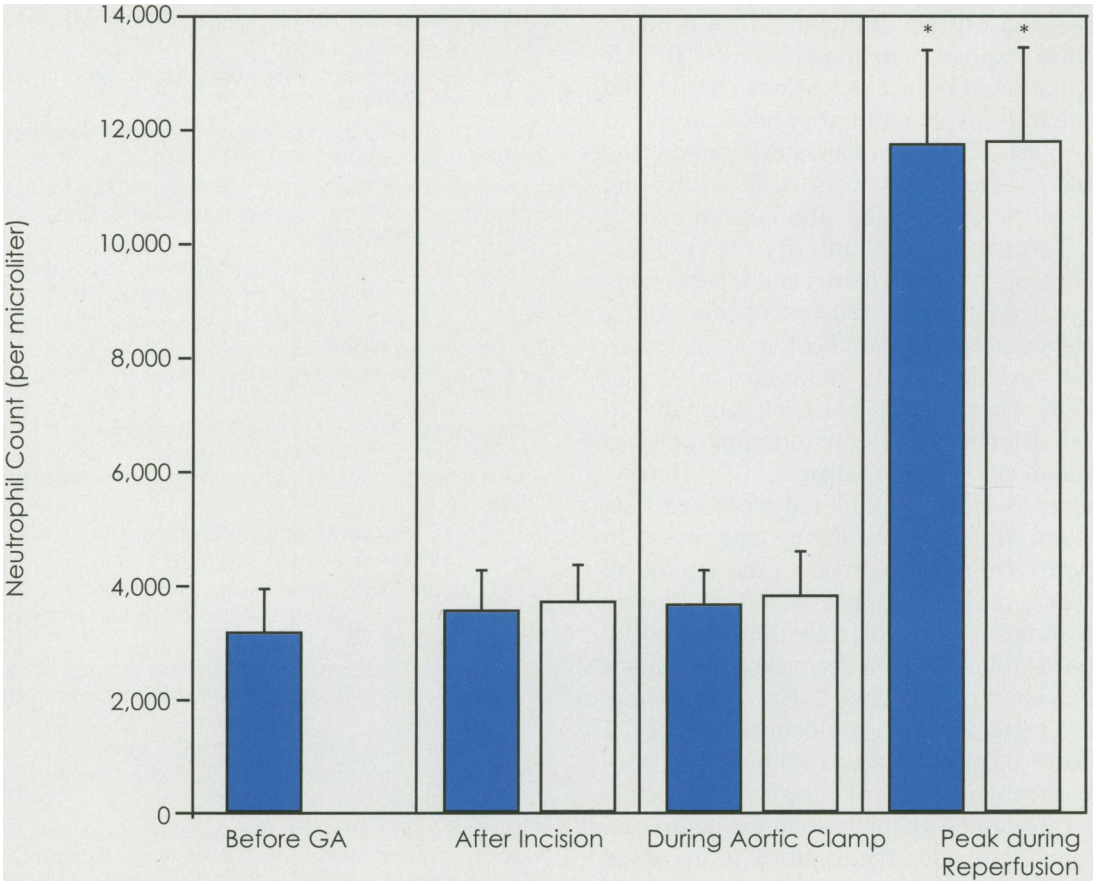
there is a paucity of data regarding the actual cell surface expression of CD11b and CD18 during ischemia/reperfusion, especially in humans. Recently, individuals with unstable angina were found to have increased surface expression of CD11b/CD18 on the circulating monocytes following coronary angioplasty.[20] Similarly, patients undergoing an acute ischemic stroke were noted to have increased expression of CD18 on circulating neutrophils.[21]

We found that surface expressions of CD11b and CD18 on neutrophils and monocytes increase during reperfusion following aortic clamping, confirming our original hypothesis. The magnitude of rise and the time to peak receptor expressions did not correlate with the duration of aortic clamping, which ranged between 47–130 minutes in the present study. In addition, there were no differences in the receptor expression between neutrophils and monocytes in the venous blood draining the ischemic lower extremity and those found in the systemic arterial blood.

In a previous study, Hill et al.[22] reported significant upregulation of neutrophil CD11b expression in patients undergoing supraceliac, aorta clamping for aorta aneurysm repair in single samples obtained 90 minutes after reperfusion. Upregulation of neutrophil CD11b expression following supraceliac aorta clamping reported by those investigators is consistent with results found in our patients undergoing infrarenal aorta clamping. In addition, the present study extended the findings of Hill et al.[22] by measuring both CD11b and CD18 expression on neutrophils and monocytes at multiple time points.

The mechanism responsible for upregulations of neutrophil and monocyte CD11b and CD18 expressions during reperfusion is not entirely clear, and several factors may be involved. For instance, some investigators have suggested that neutrophil activation may be due to the release from the ischemic tissues of inflammatory mediators, such as activated complement products, reactive oxygen species, cytokines, (IL-1, IL-6, IL-8 and TNF-α) thromboxane-B2 and leukotriene-B4.[3,5,6,24-31] In fact, increased plasma levels

Figure 4. The absolute neutrophil count (ANC) of blood samples taken from the radial artery (dark bars) and femoral vein (open bars) before general anesthesia (GA), shortly after incision, during aortic clamping and at the peak observed after reperfusion compared to other time points from the same sample site; * p<0.001



of IL-6, IL-8 and thromboxane-B2 (but not TNF-α or C3a) have been observed following lower-body reperfusion in humans undergoing aortic aneurysm surgery.[29,32] These events could potentially stimulate surface expression of CD11b and CD18 observed in the present study. This supposition is supported by an earlier study published by Swartbol et al.,[23] who demonstrated that plasma obtained after aortic declamping from patients undergoing aorta aneurysm repair can activate CD11b and CD18 expressions on leukocytes obtained from normal volunteers. Together, these observations suggest that activation of the circulating populations of these cells may occur via the systemic action of an inflammatory mediator(s) as opposed to or in addition to local activation and/or release of the sequestered leukocytes from the ischemic tissues.

The observed activation of circulating monocytes is noteworthy. Even though monocytes have a central role in most inflammatory responses, their activation and regulation during ischemia and reperfusion, especially in humans, have not been clearly elucidated. As noted above, Qi et al. have observed evidence of increased surface expression of CD11b/CD18 on circulating monocytes following coronary angioplasty.[20] In vitro studies have shown that monocytes subjected to hypoxia produce IL-1 upon reoxygenation. Moreover, monocytes preconditioned to anoxia exhibit augmented IL-8 production following exposure to hyperoxia.[33,34] In our study, upregulation of monocyte surface CD11b and CD18 was quantitatively and temporally similar to that of neutrophils. Thus, it appears that production of inflammatory mediators from cells within the affected tissues can lead to activation of circulating monocytes. This can, in turn, amplify the systemic inflammatory response to ischemia and reperfusion.

Earlier studies have reported leukopenia during reperfusion of the ischemic lower body in animal models and in humans undergoing abdominal aneurysm repair.[13,32,35] This phenomenon has been attributed to sequestration of leukocytes in the reperfused tissues and in the pulmonary microvasculature.[13,32,35,36] However, our patients as a group exhibited increased total leukocyte and neutrophil counts during reperfusion. In addition, there was no difference in the leukocyte counts between venous and arterial blood. In two patients, an initial leukocytosis shortly after aortic unclamping was followed by a decrease in the leukocyte and neutrophil counts later during reperfusion. Even in these patients, leukocyte counts did not fall below that observed prior to aortic clamping. Our findings are consistent with those of Gadaleta and associates, who demonstrated an increase in the neutrophil count five- and 30 minutes after removal of the aortic clamp during abdominal aortic aneurysm repair.[27] Therefore, it appears that any local or pulmonary sequestration of leukocytes was offset by a concurrent leukocytosis caused by aortic unclamping.

It is of interest that no significant correlation was observed between the duration of ischemia (aortic clamping) and the magnitude and rapidity of the rise in the integrin expression during reperfusion. The precise reason for this lack of correlation is not clear. However, it is possible that the shortest duration of ischemia (47 minutes) used in the present series was sufficient to elicit a maximal response.

In summary, lower-body reperfusion occasioned by unclamping of the aorta following repair of abdominal aortic aneurysms results in marked leukocytosis in both local venous effluent and systemic arterial blood. This is associated with upregulation of neutrophil and monocyte integrins that peak approximately 30 minutes after reperfusion. The biological impact of this phenomenon, if any, is currently unknown and requires further investigation.

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