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# Adverse reactions during endovascular treatment of aortic aneurysms may be triggered by interleukin 6 release from the thrombotic content

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***Purpose:*** **It has been shown that endovascular aortic aneurysm repair might induce a significant inflammatory response, mainly involving tumor necrosis factor (TNF-α) release. This study determined in vitro whether these inflammatory responses could depend on white blood cell (WBC) activation caused by the aneurysmal mural thrombus.**
***Methods:*** **Mural thrombus specimens obtained from 10 different aortic aneurysms were weighed, homogenized, and assayed for interleukin 1β (IL-1β), interleukin 6 (IL-6), TNF-α, and soluble TNF receptor (sTNFRI).**
***Results:*** **Only high amounts of IL-6 (mean, 2973 pg/mL) were found. In contrast, after the addition of healthy donor WBCs to the thrombus mass supernatants, elevated levels of TNF-α (mean, 523 pg/mL) were seen. Theoretically, WBCs were stimulated by IL-6, resulting in TNF-α release. In additional experiments, it was proven that stimulated WBCs, induced by thrombus mass supernatants, synthesize TNF-α (mean, 796 pg/mL), and monoclonal antibodies against IL-6, prevented such TNF-α production (mean, 62 pg/mL).**
***Conclusion:*** **The biologic responses during endovascular repair may be explained by a release of IL-6 from the aneurysmal thrombus, causing WBC stimulation and production of TNF-α. More complex processes cannot be excluded, but the present findings suggest that restrictions of manipulations within the aneurysm may be advisable. (J Vasc Surg 1998;28:664-8.)**

Immunologic mechanisms play an important role in the pathogenesis of atherosclerosis and of atherosclerotic abdominal aneurysms. Inflammatory white blood cells (WBCs) invade the vessel wall and release cytokines. It has been shown that the abdominal aortic aneurysm (AAA) wall is able to produce interleukin 6 (IL-6)[1] and tumor necrosis factor (TNF-α).[2-4] The new endovascular approach to treat AAA[5] leaves the aneurysm intact with its intramural thrombus and induces a clot formation around the prosthetic device. Manipulations with introducers and catheters inside the aortic aneurysm might also affect the thrombus and subsequently induce microembolization or macroembolization, usually, however, without clinical consequences. Furthermore, release of toxic products and cytokines may occur. In previous studies, we have shown that some patients treated with an endoluminal procedure developed a clinical picture resembling a systemic inflammatory response syndrome (SIRS) during and after the insertion of the endovascular device,[6,7] correlated to an increased level of TNF-α, a mediator of SIRS.[8] One may speculate that cytokines present in the mural thrombus of an AAA are released during the endovascular procedure. The inflammatory process recruits new WBCs, and further augmentation of the WBC responses occurs, resulting in a cycle of receptor adhesion molecule alterations, cytokine release, inflammatory cell recruitment, and tissue damage.

This study determined in vitro whether the thrombotic content from aortic aneurysms contains cytokines and is able to activate WBCs.

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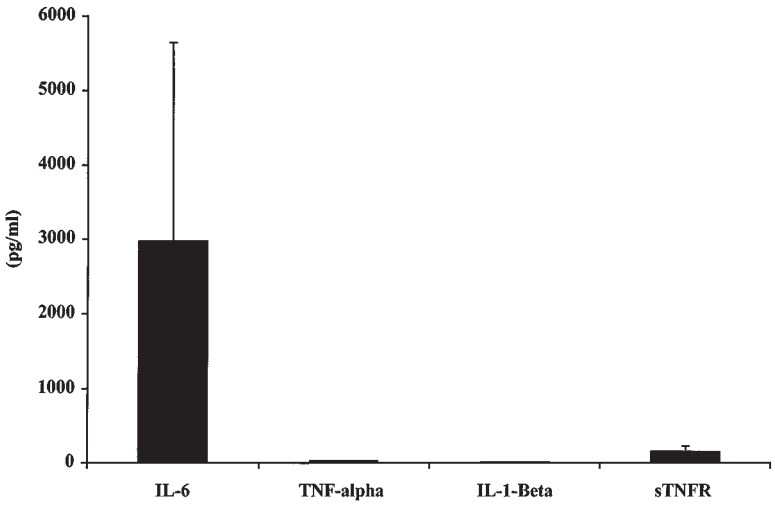


**Fig 1.** Ten infrarenal aortic thrombotic contents analyzed for cytokine levels. The cytokine concentrations are expressed as mean ± SEM.

## METHODS

**Specimens.** Thrombotic content was obtained from aortic aneurysms during 10 elective open aortic aneurysm repairs and was directly stored at –80°C. After defrosting, 1 g of each biopsy was homogenized for 3 minutes with a tissue homogenizator. Then 10 mL phosphate buffered saline (PBS, 1/15 mol/L, pH 7.2) was added to the homogenized thrombus, transferred to a 15 mL plastic tube, and centrifuged for 5 minutes at 1100 rpm. Afterward, each supernatant was washed 3 times in PBS, harvested, and stored at –80°C.

**Analyses of cytokine levels in thrombus mass supernatants.** The thrombus mass supernatants were assayed for IL-1β, IL-6, TNF-α, and soluble TNF-α receptor (sTNFRI) with the use of enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Minneapolis, Minn). These cytokines were chosen because they are proinflammatory and play a central role as mediators of the host response to infection and injury. Furthermore, in all experiments, each sample was assayed twice.

**TNF-α determination after addition of healthy donor WBCs to thrombus mass supernatants.** From healthy donors, 20 mL venous blood was withdrawn into heparinized 10 mL tubes. The monomorphonuclear and polymorphonuclear cells were obtained using a single step centrifugal procedure with a density gradient.[9] The cell fraction was diluted by 0.45% NaCl solution to restore normal osmolarity. The cells were then washed 3 times in culture medium (RPMI 1640; GIBCO, Life Technologies, Paisley, Scotland) and suspended in RPMI supplemented with 10% fetal calf serum and gentamicin (Sigma, St. Louis, Mo) to a final concentration of $1 \times 10^6$ cells/mL.

Then, 100 μL of the WBC suspension (cell concentration, $1 \times 10^6$/mL) was added to 100 μL thrombus mass supernatant and incubated for 6 hours at 37°C, 5% $CO_2$. Afterward, the samples were centrifuged for 5 minutes at 3000 rpm. The supernatant was harvested and stored at –80°C until the TNF-α assay (Quantikine, R&D Systems).

For positive control experiments, 180 μL cell suspension (cell concentration, $1 \times 10^6$/mL) was added to 20 μL lipopolysaccharide (LPS) *Escherichia coli* 055:B5 (Sigma) at a final concentration of 10 μg/mL.

For negative controls, 100 μL cells was added to 100 μL PBS.

**TNF-α measurements of thrombus mass supernatant preincubated with monoclonal antibodies against IL-6 (mAb-IL-6).** For this experiment, 90 μL thrombus mass supernatant was preincubated with 10 μL mAb-IL-6 (5 μL/mL in 0.05 mol/L carbonate buffer, pH 9.8) for 30 minutes at 4°C. Then 100 μL WBC suspension (cell concentration, $1 \times 10^6$/mL) was added, incubated, and centrifuged. As controls, 90 μL WBC suspension was added to 10 μL IL-6 (concentrations used were 1000, 2000, and 4000 pg/mL) (positive controls), and 100 μL WBC suspension was added to 100 μL PBS (negative control).

**Statistics.** The cytokine concentrations are expressed as mean ± SEM.

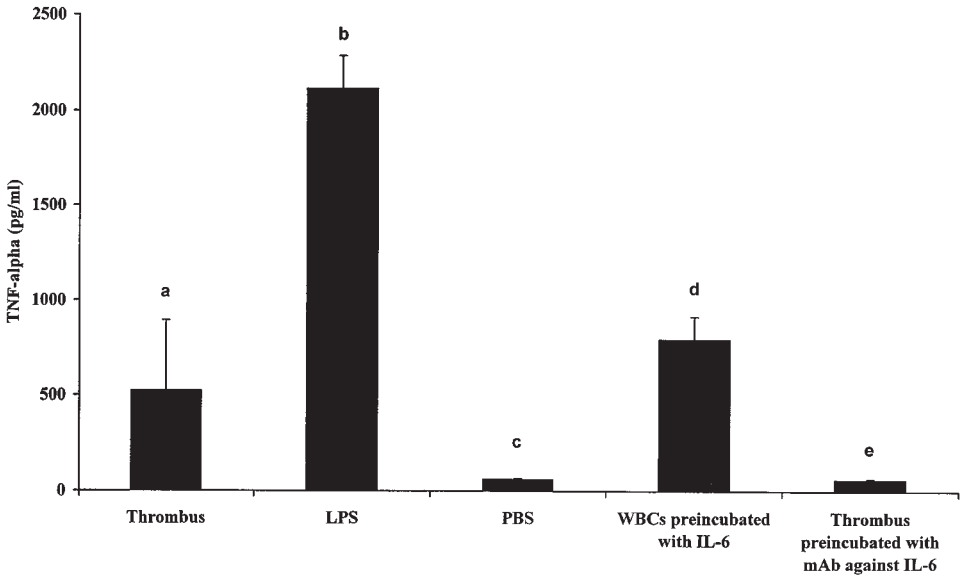


**Fig 2.** TNF-α measurements after addition of **a,** healthy donor WBCs to thrombus mass supernatants; **b,** LPS to WBCs (positive control); **c,** PBS to WBCs (negative control); **d,** preincubated WBCs with IL-6; **e,** preincubated WBCs with mAb against IL-6. All experiments were repeated 10 times. Each sample was analyzed twice. The results are expressed as mean ± SEM.

## RESULTS

**Cytokine levels in thrombus mass supernatant.** Only high amounts of IL-6 (mean, 2973 ± 2670 pg/mL) were found in thrombus mass supernatants (Fig 1). Screening for IL-1β, TNF-α, and sTNFRI showed no elevated or detectable levels. The detection limits were for IL-1β 8 pg/mL, IL-6 15 pg/mL, TNF-α 32 pg/mL, and sTNFRI 78 pg/mL (reference range for sTNFRI is 749–1966 pg/mL).

**TNF-α measurements after addition of healthy donor WBCs to thrombus mass supernatants.** By adding thrombus mass supernatants to healthy donor WBCs, elevated levels of TNF-α were recorded (mean, 523 ± 372 pg/mL) (Fig 2). Addition of LPS (positive control) caused about 4 times higher TNF-α values.

These results indicate that the thrombus mass supernatant stimulates WBCs to produce TNF-α. Because high levels of IL-6 were found in the thrombus mass supernatants, it is most likely that this cytokine has caused WBC activation. Therefore, the role of IL-6 was determined by 2 more experiments.

First, the addition of 1000 pg/mL IL-6 caused a significant release of TNF-α (mean, 796 ± 120 pg/mL), with no increase with the use of higher IL-6 concentrations. Second, preincubation of mAb-IL-6 with thrombus mass supernatant demonstrated no WBC stimulation, as seen by low TNF-α levels (62 ± 10 pg/mL), the results of which were equal to the negative controls (Fig 2).

## DISCUSSION

Sepsis, trauma, and more extensive surgical procedures cause pronounced inflammatory responses. Cytokines such as TNF-α and IL-6 are proinflammatory mediators of the host response, and IL-6 release is a general finding after surgery, whereas TNF-α release is seen only with complications. It is well recognized that TNF-α infusions are able to reproduce cardiovascular collapse signs,[10] whereas IL-6 may induce fever and cause acute phase protein synthesis[11].

In our early experience with endovascular treatment of an aortic aneurysm, a sudden blood pressure drop was recorded during insertion of the endovascular device in a considerable number of patients.[6,7] In these patients, TNF-α was detected immediately after implantation, to a maximum of 60 minutes later, and decreasing to zero values within 48 hours. In contrast, conventional surgery did not cause any TNF-α increase. IL-6 concentration increased in both groups, significantly more during open surgery, between 6 and 24 hours after clamping. Also, body temperature was elevated in some patients until the sixth postoperative day. There was no corresponding finding during open surgery. The conclusions from

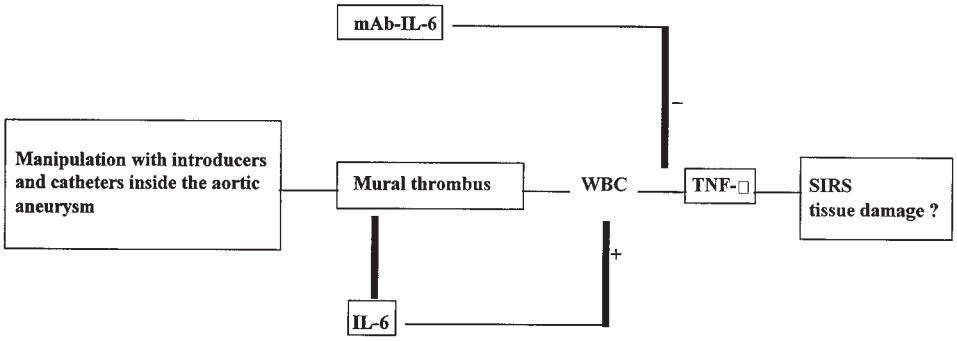


**Fig 3.** Possible cause of the biological responses caused by maneuvers with introducers and catheters into the mural thrombus of aortic aneurysm during endovascular repair. Monoclonal antibodies against IL-6 may prevent such response.

this study were that endovascular aortic aneurysm repair induced a significant inflammatory response, mainly involving TNF-α and differing from the findings during open aneurysm repair[7].

These findings may theoretically be explained in various ways. One possibility is that the materials building up the stent-supported graft may cause inadvert reactions. In preliminary experiments on surface adhesion molecule expression, however, the components of the device did not cause any increased expression of CD11b/CD18 on WBCs. Another possibility is that manipulations inside the aortic aneurysm may cause release of various toxic products. It is known that bacteria, mainly *Staphylococcus epidermidis*, contaminate a proportion of aortic aneurysm thrombi, reported at 5% to 25%.[12-16] However, taking this low incidence rate into account, it seems less reasonable to assume that bacteremia explains our findings.

Manipulation with large introducers inside the aneurysm might cause release of cytokines, and endothelial damage may also take place during insertion of the instruments. The concept that the mural thrombus is an inert, "non-living" material could be challenged. The present study showed that high amounts of IL-6 are present in the thrombotic contents of aortic aneurysms. The thrombus supernatant also stimulated WBCs to produce TNF-α. These results suggest that IL-6 is most likely the main source to induce TNF-α production by WBCs. Evidence that IL-6 plays a central role in the biologic response was found in the control experiments. Direct WBC activation by IL-6 caused TNF-α release and inhibition of IL-6 by monoclonal antibodies demonstrated to reduce the TNF-α amount to a level equal to negative controls (Fig 3). Although TNF-α has been found in human atheromas,[17,18] we did not find any increased levels in the thrombus mass supernatants. A direct release of TNF-α into the circulation is, therefore, less likely.

Attempts have been made to correlate isolated TNF-α levels with patient prognosis. Although the findings have not been consistent, circulating levels of TNF-α have been associated with poor clinical outcome in patients with sepsis or burns.[19-22] However, plasma levels of TNF-α have not been useful in predicting outcome when assessed in conjunction with simple clinical and laboratory variables.[23] The clinical effects of high transient TNF-α release in patients undergoing an endovascular AAA repair are unknown. Theoretically, it might be possible to limit the TNF-α release using monoclonal antibodies to adhesion molecules on WBCs. By blocking these adhesion molecules, it is possible to improve survival rates and minimize tissue injury associated with experimental hemorrhagic shock[24] and sepsis.[25] Moreover, a clinical impression exists that in patients who have aneurysm without thrombi or with only small thrombi, or when a "non-touch" technique has been possible, the adverse reactions (both blood pressure decreases and cellular responses) have been limited or absent. Therefore, the morphology of the aneurysm per se and the experience of the surgeon may be of importance. However, other explanations for our findings cannot be excluded. Such possibilities include direct compression of the graft to portions of the thrombus and more specific endothelial damage during insertion of the device and also at the level of the nonthrombotic part of aorta, where the proximal part of the stent graft is pushed out into the vessel wall.

The findings of TNF-α release systemically in our clinical studies and a primary IL-6 release from

the thrombus mass in vitro in the present study may not seem similar, but this is probably because only transient IL-6 releases took place during the placement of the stent grafts, and no blood samples have been taken during this short period. This transient IL-6 release may have activated WBCs, with a subsequent upregulation of adhesion molecules and a further activation of the cytokine cascade.

In conclusion, the mural thrombus of an aortic aneurysm contains high amounts of IL-6. Manipulation with introducers and catheters inside the mural thrombus might release IL-6 and stimulate WBCs to produce TNF-α, which is able to mediate a systemic inflammatory response. To avoid or decrease any adverse reactions during endovascular repair of an AAA, restricted manipulations within the vascular system seem advisable. Whether monoclonal antibodies can prevent inflammatory responses has to be investigated.

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