Endovascular Approach to Abdominal Aortic Aneurysms Limits the Postoperative Systemic Immune Response


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Objectives: endovascular repair of abdominal aortic aneurysms (E-AAA) has in recent years developed as an alternative to the conventional open repair (C-AAA). Adverse outcomes following the open approach may relate to immune cell activation and the systemic inflammatory response syndrome (SIRS) and organ failure but the benefits in this respect of the endovascular approach are unclear. This study evaluated this question and focused on T-cell activation and function.

Design: prospective clinical study.

Materials: twenty patients undergoing abdominal aortic aneurysm repair (12 C-AAA and 8 E-AAA).

Methods: peripheral T-cell expression of surface markers CD69, CD62L and CD25 in vivo and Interleukin 2 (IL-2) and Interleukin-10 (IL-10) responses to the superantigen staphylococcal enterotoxin B (SEB) in vitro were measured preoperatively, 24 h and 1 week postoperatively.

Results: there was no significant increase \((p = 0.23)\) in the incidence of SIRS in the open compared with the endovascular group. Enhanced T cell activation occurred following C-AAA and this was associated with significantly greater IL-2 production in response to SEB, with no change in IL-10 production.

Conclusions: E-AAA attenuates proinflammatory T-cell changes compared with C-AAA repair. A reduction in T-cell activation and impaired responsiveness to superantigen suggests that the immunological sequelae of the endovascular approach to aneurysm repair is more favourable than after the open approach with potentially less risk of adverse outcomes. Proof of this thesis will require a larger prospective study.

Key Words: Vascular surgery; Endovascular; Aortic aneurysms; T-cells; Cytokines.

Introduction

Since Parodi’s initial report of intraluminal placement of a stent graft via remote access for abdominal aortic aneurysm (AAA) repair in 1991, endovascular AAA repair (E-AAA) has become an increasingly realistic alternative to conventional repair (C-AAA). Large clinical series and several research studies support both the feasibility of this approach and also benefits consequent on reduced bowel ischaemia and morbidity, a reduced hospital stay and decreased costs.2–4

The endovascular approach is, in contrast to the conventional approach, associated with minimal tissue trauma, a shorter occlusion time and no bowel handling. It is therefore a reasonable hypothesis that the systemic immune, metabolic and inflammatory response to endovascular aneurysm repair may be attenuated compared with open repair, which is associated with a marked immunoinflammatory cytokine response and evidence of endotoxaemia.5,6 This hypothesis, although supported by a compelling rationale and some scientific reports, is unproven at this time. Indeed, some studies suggest an enhanced immunoinflammatory response to E-AAA repair compared to open repair, with TNF-α and IL-6 implicated in its mechanism,11–14

The T-cell response to injury, and its characteristics, either pro-inflammatory or anti-inflammatory, is implicated in the systemic immune response to these stimuli. CD69 is the earliest detectable marker expressed by activated T-cells. CD25 is part of the IL-2 receptor complex; it reaches maximal expression 48 h following activation and slowly decreases in expression over the following 72 h. CD62L (L-Selectin) belongs to the selectin family of cell adhesion molecules and its presence identifies regulatory subpopulations of T-cells. CD4+CD62L+ T-cells mediate the majority of helper functions while CD8+CD62L+ T-cells include the suppressor cytotoxic subpopulation of T-cells.
Table 1. The definition of systemic inflammatory response syndrome according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference.\(^\text{18}\)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Definition</th>
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<tbody>
<tr>
<td>1. Temperature &gt;38 °C or &lt;36 °C</td>
<td></td>
</tr>
<tr>
<td>2. Heart rate &gt;90 beats per min</td>
<td></td>
</tr>
<tr>
<td>3. Respiratory rate &gt;20 breaths per min or PaCO(_2) &lt;32 mmHg</td>
<td></td>
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<tr>
<td>4. White blood cell count &gt;12,000/cu mm, &lt;4000/cu mm, or</td>
<td></td>
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<tr>
<td>&gt;10% immature (band) forms</td>
<td></td>
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</tbody>
</table>

Systemic inflammatory response syndrome (SIRS)=the systemic inflammatory response to a variety of severe clinical insults. The response is manifested by two or more of the following conditions:

The pro-inflammatory Type 1 helper (Th\(_1\)) response is characterised by the production of Interleukin 2 (IL-2) and activation of cell-mediated immune responses.\(^\text{15}\) The anti-inflammatory Th\(_2\) response is associated with the production of interleukin 10 (IL-10) and antibody formation.\(^\text{16}\) The development of either response appears to depend on the nature of the insult and the degree of injury. It has recently been suggested that early after major injury a Th\(_1\) profile predominates.\(^\text{17}\) The objective of this study was to elucidate the pattern of T-cell activation following E-AAA and C-AAA and to describe the differences in cytokine response to each of the surgical approaches.

Materials and Methods

Patient population

Twenty patients undergoing elective abdominal aortic aneurysm repair gave informed consent approved by the St James’s Hospital and Federated Dublin Voluntary Hospitals Joint Research Ethics Committee. All aneurysms were assessed preoperatively by aortography and contrast enhanced CT-scanning. Eight patients were suitable for E-AAA based on aortic morphology and 12 patients underwent conventional C-AAA. A Dacron graft (Brown, U.S.A.) was used in all C-AAA procedures (nine bifurcated and three straight grafts). An Excluder graft (Gore, Germany) was used in 5 E-AAA and a Talent graft (Meditronic, Germany) was used in 3 E-AAA procedures. All E-AAA grafts were bifurcated. All procedures were performed under general anaesthesia and C-AAA was performed with additional epidural anaesthesia. Anaesthesia and fluid therapy followed a standard protocol. The systemic inflammatory response syndrome was defined according to the ACCP/SCCM Consensus Conference Committee (Table 1).\(^\text{18}\)

Peripheral blood mononuclear cell isolation

Peripheral venous blood samples were obtained preoperatively (baseline sample), 24 h postoperatively, and 1 week following surgery (patients discharged from hospital within 1 week returned to hospital for phlebotomy at 1 week). These were collected in heparinised tubes, transported on ice and prepared for analysis within one hour of collection. Peripheral blood mononuclear cells (PBMC) were isolated by a standard density gradient technique and suspended in RPMI 1640 with Glutamine (Gibco Laboratories) supplemented with antibiotics (penicillin 100 u/ml and streptomycin) and 10% heat inactivated fetal calf serum (Boehringer-Mannheim, Mannheim, Germany) to a final concentration of 5 × 10\(^6\) cells/ml.

T-lymphocyte surface marker expression

Expression of cell surface markers on T-cells was determined using commercially available fluorescent monoclonal antibodies (PharMigen Europe, U.K.). Antibodies to three T-cell subpopulation markers were used: fluorescein isothiocyanate (FITC)-conjugated mouse anti-human monoclonal antibody to CD3 (T-cell antigen receptor), CD4 (T-helper cell), and CD8 (cytotoxic/surveillance T-cell). Expression of three T-cell activation markers was analysed using R-Phycocerythrin (R-PE) conjugated mouse anti-human monoclonal antibody to CD25, CD62L and CD69. Becton Dickinson Simultest™ Control, IgG\(_1\)/FITC/IgG\(_2a\)/IgG\(_2a\)/PE was used as a negative control (Becton Dickinson, Belgium). Aliquots (100 μl) of PBMC suspension were incubated in dark room conditions with saturating concentrations of monoclonal antibodies for 15 min at room temperature. The cells were washed twice with Facsflow (Becton Dickinson), fixed with 200 μl Cellfix (Becton Dickinson) and stored in darkness at 4°C. Flow cytometric analysis was performed within 24 h of fixing the samples.

Lymphocytes were identified by flow cytometry (FacScan, Becton Dickinson) using their morphological characteristics (forward vs side scatter resolution) and gated to exclude granulocytes and monocytes. Non-specific fluorescence was quadranted out using the control antibody and T-cell subpopulations were identified by expression of surface CD3, CD4 or CD8 along the x-axis of a dot plot. Expression of the activation markers CD25, CD62L and CD69 were read along the y-axis. Dual expression was expressed as a percentage of T-cells with the T-cell subpopulation marker.
Table 2. Patient demographics and clinical outcome. Where applicable values are median (range).

<table>
<thead>
<tr>
<th></th>
<th>E-AAA</th>
<th>C-AAA</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>M/F</td>
<td>3/5</td>
<td>7/5</td>
</tr>
<tr>
<td>Age</td>
<td>71 (71–72)</td>
<td>73 (68–79)</td>
</tr>
<tr>
<td>ASA</td>
<td>3 (1–4)</td>
<td>2 (1–4)</td>
</tr>
<tr>
<td>Duration of surgery (h)</td>
<td>3 (2–7)</td>
<td>3 (2–5)</td>
</tr>
<tr>
<td>Operative blood loss (ml)</td>
<td>300 (200–350)</td>
<td>1000 (650–1300)*</td>
</tr>
<tr>
<td>Blood transfused (units)</td>
<td>0 (0–2)</td>
<td>1 (0–4)</td>
</tr>
<tr>
<td>Duration ICU/HDU stay (days)</td>
<td>1 (0–1)</td>
<td>4 (2–7)*</td>
</tr>
<tr>
<td>Number of patients developing SIRS</td>
<td>1</td>
<td>4*</td>
</tr>
<tr>
<td>Postoperative hospital stay (days)</td>
<td>4 (3–7)</td>
<td>14 (12–15)*</td>
</tr>
</tbody>
</table>

ICU/HDU: intensive care unit/high dependency unit; SIRS: systemic inflammatory response syndrome. *p <0.05 vs E-AAA.

Measurement of lymphokine production

The cytokine response of patients’ adaptive immune function was measured using enzyme-linked immunosassays. Aliquots (500 μl) of patients’ T-cell suspensions were incubated for 48 h with an equal volume of either supplemented RPMI 1640 or Staphylococcus Enterotoxin B (SEB), a potent T-cell mitogen, in supplemented RPMI 1640 at a concentration of 200 ng/ml. We have found this concentration to be optimal for stimulation of T-cells (results not shown). Following the incubation the samples were centrifuged at 3000 rpm for 5 min. The resultant supernatant was stored at −70 °C until analysed.

ELISA experiments were performed in triplicate using standard kits to estimate the production of Interleukin 2 (Duotest, Becton Dickinson) and Interleukin 10 (Mabtech AB, Sweden). Briefly, ELISA plates (Nunc Maxisorb, Nunc Nangle International, Denmark) were coated with capture anti-cytokine antibodies, incubated with 1% BSA, and the unknown samples were added followed by a biotinylated detection antibody. Streptavidin-alkaline phosphatase conjugate and finally p-nitrophenyl phosphate were then added. An ELISA reader (Multiskan™ plus, Labsystems) at an absorbency wavelength of 405 nM was used to assess the alkaline phosphatase activity.

Results were presented as median (inter-quartile range) and non-parametric statistical testing was used. The data was analysed using SPSS statistical program. Mann–Whitney U-test was used to compare patients’ demographics and Chi squared test was used to compare incidence of SIRS between the two groups. Kruskal–Wallis test with Dunn’s post hoc testing was used for comparison of expression of activation markers and cytokine production at the three time points. p <0.05 was deemed statistically significant.

Patients’ demographics and clinical outcome

Patients’ demographics and clinical outcomes are described in Table 2. There was no mortality in either group. E-AAA resulted in significantly reduced hospital stay, reduced ICU/HDU stay and less operative blood loss. There was a decreased incidence of SIRS in patients treated with E-AAA compared with C-AAA, however this did not reach statistical significance (p = 0.29). The majority of episodes of SIRS occurred within the first 72 h of surgery. We observed no difference in expression of activation marker or cytokine production postoperatively between those patients who experienced episodes of SIRS and those who did not.

Expression of T-cell activation markers

A significant increase (Fig. 1) in the number of T cells expressing CD69 was observed 1 week after surgery in CD3+ (p = 0.02) and CD4+ cells (p = 0.035). In marked contrast, however, there was no significant increase in expression of CD69 at any time point following E-AAA (p<0.005 C-AAA vs E-AAA repair at day 7 postoperatively for CD3+ and CD4+ cells). No difference in expression of CD69 on CD8+ T-cells was observed in either group at any time point. In addition there was significantly increased expression of CD25 (Fig. 2) in the CD3+ subpopulation one week following C-AAA (p = 0.048) and this was significantly increased compared with E-AAA at one week postoperatively (p = 0.029). There was no significant change in CD62L expression in any T-cell subpopulation in either the
Fig. 1. Expression of CD69 on CD3⁺ (top graph), CD4⁺ (middle) and CD8⁺ (bottom) T-cells. Expression was significantly increased in CD3⁺ and CD4⁺ T-cells following C-AAA but not E-AAA 1 week postoperatively. *p < 0.05 vs preoperative expression of CD69. †p < 0.005 vs expression of CD69 on day 7 in E-AAA group.

Fig. 2. Expression of CD25 on CD3⁺ (top graph), CD4⁺ (middle) and CD8⁺ (bottom) cells. Expression was significantly increased 1 week after C-AAA when compared with both preoperative and 1 week post E-AAA production. *p < 0.001 vs preoperative expression of CD25. †p = 0.029 vs expression of CD25 on day 7 in E-AAA group.

Cytokine production by PBMC

After stimulation in vitro with SEB, PBMCs from patients undergoing C-AAA produced significantly greater IL-2 1 week postoperatively (210 (133–548) pg/ml) compared with preoperative levels (45 (25–64) pg/ml) and levels 24 h postoperatively (65 (21–280) pg/ml) (Fig. 3). This is in contrast to IL-2 production following E-AAA where we failed to observe any open or endovascular group at any other timepoint following surgery compared to preoperative levels.

Fig. 3. IL-2 production by PBMC stimulated with SEB. Production was significantly increased 1 week after C-AAA when compared with both preoperative and 1 week post E-AAA production. *p < 0.001 vs preoperative production of IL-2. †p = 0.048 vs production of IL-2 on day 7 post E-AAA.

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significant increase in production of IL-2 at any time point postoperatively, and the differences in IL-2 production following E-AAA and C-AAA became significant at one week postoperatively ($p=0.048$).

There was no difference observed in the production of IL-10 at any time point in either group.

**Discussion**

The conventional repair of abdominal aortic aneurysms is associated with a high incidence of postoperative systemic inflammation and a significant risk of SIRS and organ failure. The advent of endoluminal techniques for abdominal aortic aneurysm repair has changed the clinical outcome and morbidity from AAA repair without affecting mortality rates. The metabolic, cardiovascular and haematological response to E-AAA differs considerably to the transperitoneal approach although the effect on systemic immune function has not been fully elucidated.

In this study epidural anaesthesia was additionally used in C-AAA, this conceivably may be an influencing factor but, to the authors knowledge there is no additional effect on T-cell activity of spinal anaesthesia combined with general anaesthetic. The study shows that sustained activation of T-cells and a pro-inflammatory Th1 response is associated with the open repair and attenuated by the endovascular approach. This early T-cell response is similar to observations in patients after severe trauma and burn injury.

The T-cell response to E-AAA differs significantly from C-AAA, with evidence of a sustained expression of the activation markers CD69 and CD25 in the open group compared to the endovascular group. In a recent study of seven patients treated by E-AAA repair, Galle et al. reported a significant attenuation of T-cell activation responses in this group compared to an open group ($n=5$). One of the principle theoretical advantages of E-AAA is avoidance of aortic cross clamping and attenuation of the ischaemia-reperfusion injury that complicates C-AAA. Animal and transplant models have demonstrated that during ischaemia T-cells become activated, with upregulation of major histocompatibility complex (MHC) class II antigens and production of cytokines including IL-2 and INFγ. In this study significantly increased expression of CD69 1 week following C-AAA suggests persistent postoperative activation of T-cells, a phenomenon not observed in the E-AAA group. It is possible that the absence of cross clamping in the endovascular approach and the associated attenuated ischaemia-reperfusion injury underlies this difference.

This observation of T-cell activation does not, however, infer augmented function. Persistent activation can induce the anergic state implicated in post-traumatic sepsis and following burns. In this study, however, anergy was not induced and T-cell function was maintained after both E-AAA and C-AAA. Anergy following burn injury has been attributed to defective expression of the IL-2 receptor and diminished production of IL-2. In contrast, following either endovascular or conventional AAA repair the expression of interleukin-2 receptor did not diminish (and indeed reached significantly increased to significant levels 1 week following C-AAA) and this was associated with normal or increased production of IL-2 to further stimulation in vitro.

The cytokine response is evident in both open and endovascular repairs, but is enhanced in the open versus the endovascular group, consistent with a review of the literature to date. Cytokines produced by normal T-cells in response to the superantigen staphylococcal enterotoxin (SEB) include IL-2, but not IL-10. Th1 cells do not mount a normal response to SEB and Th1 cells respond in an augmented fashion. In this study a normal production of IL-2 and IL-10 in response to SEB was observed. In contrast, 1 week following C-AAA a significantly increased production of IL-2 in vitro without an increase in IL-10 production was noted. An intriguing possibility in man is that C-AAA may facilitate gram-positive translocation through gut hypoxia resulting in “priming” of T-cells to a proinflammatory response.

The behaviour of L-Selectin appears complex following AAA repair. Expression of CD62L is indicative of functionally suppressive T-cells (Th0) and is shed from activated cells. No expansion of the CD4+ / CD62L+ T-cell subpopulation following C-AAA occurred confirming that a shift to a predominantly Th2 phenotype had not occurred. Previous studies have reported a rapid loss of expression of CD62L on granulocytes during E-AAA with restoration of expression 24 h postoperatively. We were unable to demonstrate any differences in expression of CD62L following E-AAA. This is unlikely to be due to sample timing as expression of CD69 remained within baseline levels 24 h after surgery suggesting that T-cell activation had not yet occurred. We conclude that although granulocytes may display transient activation following E-AAA, T-cell activation occurs by a different mechanism.

In conclusion, this report demonstrates significant differences in T-cell response to endovascular and transperitoneal approaches to abdominal aortic aneurysm repair. Although T-cell responses are enhanced...
after both C-AAA and E-AAA repair, the open approach is associated with increased activation of T-cells and primes T-cells to an augmented proinflammatory response to superantigen compared with the endovascular approach. Given that we were unable to demonstrate a significant difference in T-cell response between those who experienced episodes of SIRS and those who did not, the importance of these findings requires further evaluation in a large cohort of patients. Nonetheless, the diminished T-cell responses in this small series with a trend towards reduced incidence of SIRS supports the thesis that the endovascular approach attenuates systemic immunoinflammation compared with the conventional approach, and offers further scientific rationale for endovascular approaches to aneurysm repair.

References


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