Circulating endothelial cells – from bench to bedside and back

Alexander Woywodt

Consultant Physician and
Nephrologist
Renal Unit
Lancashire Teaching Hospitals
NHS Foundation Trust
Preston, UK

Birmingham, April 15, 2008
Circulating endothelial cells

1. The technique: Principles, pitfalls, possible directions

2. An example of clinical use: Detection of CECs in vasculitis

3. CECs in transplantation and other diseases

4. Pathogenetic mechanisms
Let’s go back to 1999…

*The millennium bug (that never was)*

*Bill Clinton impeached by US senate*

Berlin

Hannover
Vasculitis: Pathology of small-vessel vasculitis

Interlobular artery in ANCA-associated small-vessel vasculitis

Johnson RJ, Feehally J.
Comprehensive Clinical Nephrology.
Mosby, London, 2000
Immunomagnetic isolation of CECs (F. Dignat-George, Marseille)

Target cell
Immunomagnetic isolation of CECs

Very easy indeed!
Immunomagnetic isolation: Encounter with the real world

- Very variable cell phenotype
- More than variable bead content; various thresholds have been used
- Non-specific binding of leukocytes via Fc receptor
- Inter-observer variations?

Very difficult indeed!
Immunomagnetic isolation: Encounter with the real world
**Staining**

Ulex europaeus  
(Gorse)  
Leguminosae

Ulex Lectins  
UEA-1  
UEA-2

binds $\alpha$-L-fucosyl residues of endothelial cells

Woywodt et al., Ann Haematol 2004
Summary: Technical issues

- Immunomagnetic isolation was devised for detection of rare events –
  
  CECs are rare. A double-stain technique may help

- 4°C and Fc-blocking agent to avoid binding of leukocytes

- First tube of blood must be discarded, store less than 4 hours

- No difference between arterial and venous samples.

- CVP lines? ICU patients?

- The issue of EPCs remains controversial

- There is a need for consensus
ANCA vasculitis: The disease

Vasculitis

[Images of medical scans and tissue samples]

[ANCA vasculitis: The disease]
Vasculitis: Difficult scenarios and the need for novel markers

- Patient with known vasculitis, ongoing immunosuppressive treatment, new problems: vasculitis or infection?
- ANCA can be positive in infectious disease
- Correlation between ANCA titres and disease activity not ideal
- Patient with multi-system disease of unknown origin
- Patient with suspected vasculitis and difficulties with biopsy (e.g. on ventilator, very obese, uncooperative)
- Soluble markers may be elevated in endothelial activation (as opposed to necrosis); thrombomodulin depends on renal function
Vasculitis: Pathology of small-vessel vasculitis

Interlobular artery in ANCA-associated small-vessel vasculitis

Johnson RJ, Feehally J.
Comprehensive Clinical Nephrology.
Mosby, London, 2000
Vasculitis: Results
Vasculitis: Results


circulating endothelial cell tissue factor immunocytochemistry; APAAP technique

circulating endothelial cell propidium iodide stain

86% tissue factor positive
84% necrotic
Vasculitis: Results

Vasculitis: Results

Spearman rank correlation with BVAS: 0.60 (95% CI 0.34-0.75, p<0.0001)
Vasculitis: What about relapse?

![Graph showing cell counts per ml for relapse, new-onset vasculitis, and healthy controls. P-values: p<0.001 for relapse vs. healthy controls, p=0.224 for new-onset vasculitis vs. healthy controls, P<0.001 for relapse vs. new-onset vasculitis.](image-url)

Vasculitis: What about granulomatous disease?

Vasculitis: Case vignette

- E.S. 43 years-old female
- sarcoidosis (clinical and radiological diagnosis, no biopsy)
- admitted elsewhere with rapidly progressive respiratory failure
- developed acute renal failure of unknown cause
- no NSAIDs, no aminoglycosides, no radiocontrast
- transfer to Hannover with the intention of LungTx
- high pressures / high PEEP. NO. Dialysis / volume removal
- high dose inotropic support
- CRP and PCT elevated. Piperacillin, Combactam, Levofox.
- CEC 92/ml (1.10.03)
- pANCA positive (3.10.03)
- renal biopsy: necrotising glomerulonephritis
Circulating endothelial cells are a potentially clinically useful marker in ANCA-associated small-vessel vasculitis. The number of circulating endothelial cells appeared to reflect disease activity during the course of therapy and correlated with BVAS scores. Patients with relapse and patients with new-onset vasculitis have similar cell numbers; patients with granulomatous disease have much less elevated cell numbers. The cell phenotype appears to be necrotic and pro-coagulant.

Vasculitis: Conclusion

- Circulating endothelial cells are a potentially clinically useful marker in ANCA-associated small-vessel vasculitis.
- The number of circulating endothelial cells appeared to reflect disease activity during the course of therapy and correlated with BVAS scores.
- Patients with relapse and patients with new-onset vasculitis have similar cell numbers; patients with granulomatous disease have much less elevated cell numbers.
- The cell phenotype appears to be necrotic and pro-coagulant.
Cardiovascular disease (CVD) is a major cause of mortality and morbidity in renal transplant recipients (Kendrick, *Am J Kidney Dis* 2001; 38 (Suppl 6): S36-S43)
Endothelial damage and renal transplantation

Vascular rejection

Calcineurine Inhibitors?

Woywodt et al., Transplantation 2003

Woywodt et al., Hypertension 2003
Endothelial damage and HSCT
**Endothelial damage and HSCT: Results**

![Image of a graph showing cells/ml against different groups: TBI n=14, Bu/Cy n=14, reduced intensity n=11, healthy controls n=22. The graph includes statistical comparisons: p<0.05, n.s. p<0.01.]

Other diseases: Thrombotic microangiopathy and pre-eclampsia

Am J Obstet Gynecol 2007;198
### Other diseases

<table>
<thead>
<tr>
<th>Condition (reference)</th>
<th>Method†</th>
<th>Number of CECs/ml²</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary Angioplasty (19)</td>
<td>IB</td>
<td>6–10</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Sickle cell anaemia (23)</td>
<td>IB</td>
<td>13.2 – 22.8³</td>
<td>2.6</td>
</tr>
<tr>
<td>Rickettsial infection (24)</td>
<td>IB</td>
<td>5–1,600</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Thrombotic thrombocytopenia (25)</td>
<td>IB</td>
<td>6–220</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Acute coronary syndromes (26)</td>
<td>IB</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>Behcet’s disease (27)</td>
<td>IB</td>
<td>0–25</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Chronic venous insufficiency (28)</td>
<td>SC</td>
<td>1.001</td>
<td>514</td>
</tr>
<tr>
<td>Aortoarteritis (29)</td>
<td>SC</td>
<td>58</td>
<td>16⁴</td>
</tr>
<tr>
<td>Septic shock (32)</td>
<td>DC</td>
<td>16.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Breast cancer and lymphoma (33)</td>
<td>FC</td>
<td>6,800–39,100</td>
<td>1,200–7,900⁵</td>
</tr>
<tr>
<td>Systemic lupus erythematosus (34)</td>
<td>DC</td>
<td>32</td>
<td>5</td>
</tr>
<tr>
<td>Renal transplantation (36)</td>
<td>IB</td>
<td>24 – 72⁴</td>
<td>6</td>
</tr>
<tr>
<td>Thalassaemia (37)</td>
<td>DC</td>
<td>45</td>
<td>4⁷</td>
</tr>
<tr>
<td>Inflammatory vasculitis (38)</td>
<td>IB</td>
<td>136</td>
<td>5</td>
</tr>
<tr>
<td>Kawasaki disease (39)</td>
<td>IB</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Pulmonary hypertension (40)</td>
<td>DC+IB</td>
<td>30</td>
<td>3.5</td>
</tr>
<tr>
<td>Peripheral vascular disease (42)</td>
<td>IB</td>
<td>1.1–3.5⁸</td>
<td>0.9</td>
</tr>
<tr>
<td>Bone marrow transplantation (44)</td>
<td>IB</td>
<td>16 – 44⁹</td>
<td>8</td>
</tr>
<tr>
<td>Systemic sclerosis (45)</td>
<td>FC</td>
<td>243–375</td>
<td>77</td>
</tr>
<tr>
<td>Systemic lupus erythematosus (46)</td>
<td>FC</td>
<td>89</td>
<td>10</td>
</tr>
</tbody>
</table>

Blann AD et al.  
Thromb Haemost. 2005  
Feb;93(2):228-35
Phenotype studies

FITC-UEA-1

C3-Tissue Factor

Laser-capture microdissection
Pathogenetic mechanisms I

[Diagram showing various biological processes related to disease, including mechanisms like necrosis, detachment, and apoptosis.]
Pathogenetic mechanisms II

Circulating endothelial cell (necrotic)


NF-kappa b
Toll-like receptor 2

Healthy endothelium

IL-1β
IL-6
TNFα

? Permeability?
Pathogenetic mechanisms III

Figure 5. Envelopment of apoptotic or necrotic ECs by HMVEC-1 cells and HUVECs. (A) Exposure of HMVEC-1 cells to necrotic HMVEC-1 cells for 3 hours resulted in engulfment of labeled cell fragments. (B) After 6 hours of co-incubation phagocytosed particles appear in the lysosomes around the nucleus (arrows). (C) CM-Dil labeled HMVEC-1 cells were incubated with CMFDA-labeled apoptotic HMVEC-1 cells. After 1 hour apoptotic cells were engulfed by HUVECs (white arrowheads). The yellow arrow indicates an apoptotic cell that binds to the surface of a HUYEC. (D) CM-Dil stained HUVECs were exposed for 2.5 hours to apoptotic HMVEC-1 cells. The micrograph shows a confocal image of a cell engulfing an apoptotic (green) cell. A cross-section of the whole cell is shown in the micrograph at the bottom. Bars represent 50 μm (A-C) or 20 μm (D).
Repair

Bone marrow

Ischemia

VEGF
EPO
PDGF-CC
Ang-1/2

EPC

BM
Detachment
Homing
Maturation

HDAC
HoxA9

SDF1
CXCR4
CD 18
ICAM-1
Repair II

Where to go from here

- More on EPCs
- Studies into cell phenotype
- Panel of markers (microparticles, novel soluble markers)?
- Mechanisms of detachment?
- Interactions with other cell subsets (healthy endothelium, thrombocytes...)

Studies into cell phenotype
Torsten Kirsch, PhD, Uta Erdbruegger, MD
Kristin Wyss, Michaela Beese, technicians
Frank Streiber, MS
Heide Regelsberger, lab technician
Marion Haubitz, MD
Hermann Haller, MD
Astrid Borgnes, Ph.D., Dynal, Oslo, Norway
Prof. Francoise Dignat-George, Marseille, France
Dr. Andrew Blann FRCPath, Birmingham