Immunopathology of CAV
... focus on Antibodies

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Banff meeting 2017; Concurrent Heart session “Reassessment of the pathology of CAV”
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Title: “Immunopathology of CAV” 15:50-16:10
Disclosures

None

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Content

- Evidence for B-cells and antibodies in CAV
- B-cell aggregates around epicardial coronary arteries
- Targets of antibodies produced in B-cell aggregates
- Local versus distant antibody production
  - progression of chronic rejection
  - potential implication for therapies
Cardiac Allograft Vasculopathy (CAV)

Vascular pathology after heart transplantation (HTx)

Limits the long term survival after HTx

Immune-mediated
- Cellular events
- Antibody mediated?

Ectopic Lymphoid Structures (ELS)
Immune processes in CAV
Role of donor cells and recipient cells...

B cells and antibodies

Inflammatory cytokines activate effector cells

Macrophage

Anti-inflammatory cytokines block cell activation

IL-6, IL-12, TNF, LT

T cell

B cell

Antigen presentation

T cell

B cell

Immunoglobulin secretion

Plasma cell

IL-10
B-cells and antibodies in CAV

- Relation between CAV and:
  - Donor Specific HLA Antibodies (DSA)
  - Non-HLA antibodies...
The composition of ectopic lymphoid structures suggests involvement of a local immune response in cardiac allograft vasculopathy

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Composition of Ectopic Lymphoid Structures (ELS)

Patient distribution

Time post transplantation

Extent Ectopic Lymphoid Structures

<6 months >6 months

Extrem Ectopic Lymphoid Structures
CAV lesion type and ELS are correlated

<table>
<thead>
<tr>
<th>Variables</th>
<th>Extent of ELS</th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELS-0</td>
<td>ELS-1 (0-300)</td>
<td>ELS-2 (&gt;300)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time post-transplant</td>
<td>n=13</td>
<td>n=20</td>
<td>n=5</td>
<td>p-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary cardiac diagnosis, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>n=4 (36)</td>
<td>n=5 (46)</td>
<td>n=2 (18)</td>
<td>0.266^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAV lesion type, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytic</td>
<td>3 (38)</td>
<td>1 (13)</td>
<td>4 (50)</td>
<td>0.002^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrotic</td>
<td>10 (33)</td>
<td>19 (63)</td>
<td>1 (3)</td>
<td>0.011^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-CAV type, No. (%)</td>
<td>n=13</td>
<td>n=20</td>
<td>n=5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-CAV1</td>
<td>3 (38)</td>
<td>1 (13)</td>
<td>4 (50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-CAV2</td>
<td>5 (36)</td>
<td>8 (57)</td>
<td>1 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-CAV3</td>
<td>11 (69)</td>
<td>1 (7)</td>
<td>0 (0)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Significant association between the type of neointimal lesion in the coronaries and ELS category (p=0.002).

- All patients with benign intimal thickening (BIT) were in the ELS-0 category and early after transplant (<6 months).
- 20/25 patients with ELS had fibrotic lesions. From which 19 were in the ELS1 category.
- 5/25 patients with ELS had lymphocytic lesions. Four of these 5 patients were in the ELS-2 category.

Histologic CAV type was significantly related to ELS category (p=0.011), where patients with H-CAV2 and H-CAV3 were mainly in the ELS1 category (57% and 69%, respectively).
ELS as Tertiary Lymphoid Organs (TLO)

- pNAD: High Endothelial Venules
- D2-40: Lymph vessels
- CD1a: Interdigitating dendritic cells
- CD21: Follicular dendritic cells
- LTβ: Lymphotoxin beta

Huibers et al. *JHLT.* 2015 May;34(5):734-45
Ectopic Lymphoid Structures contain many B cells

Memory B-cells
CD20 = green
CD27 = red

Huibers et al. JHLT. 2015 May;34(5):734-45
Cytokine profiles of Ectopic Lymphoid Structures (IL10, TGFβ)

Regulatory role of B cells:
- IL10
- IL35
- TGFβ (Dijke et al JHLT 2016)

Immune suppression in adventitia?
“compartimentalisation”
Active antibody production in ELS


Activation-induced cytidine deaminase

IgG

IgM

AID

IgG/CD138 merged
Isolation and Detection of IgG and IgM

ELISA IgG and IgM + Luminex measurements
- Patients selected according to IgG IHC
- Autopsy heart tissue
- Explanted heart tissue (Control)

Epicard with ELS → Myocard → Lysate Tissue → Bead shaker

Pre-chilled tube with micro beads
Quantification of antibody production in ELS

High levels of IgG and IgM in ELS

What are targets of these antibodies?

Donor HLA?

Donor Specific Antibodies (DSA) in ELS

<table>
<thead>
<tr>
<th>Time post-HTx (days)</th>
<th>HLA type</th>
<th>DSA (tissue)</th>
<th>non-DSA (tissue)</th>
<th>plasma time point before death</th>
<th>DSA (plasma)</th>
<th>non-DSA (plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>153 P</td>
<td>Class-I: A24(9), A31(19), B51(5), B55(22), CW9(3) Class-II: DR13(6), DQ1, DR52</td>
<td>neg</td>
<td>neg</td>
<td>3 days</td>
<td>A2, B40</td>
<td>B13, B27, B17</td>
</tr>
<tr>
<td>D</td>
<td>Class-I: A2, B7, B60(40), CW3, CW7 Class-II: DR4, DR5, DQ3, DQ4, DR53</td>
<td>neg, neg</td>
<td>DQ3, DQ4, DQ2</td>
<td>3 days</td>
<td>B7, DR3</td>
<td>B42, B54, B81, B82, A34, B55, B56, B57, Cw4, Cw6, Cw17, Cw18, DQ4, DQ2, DR12</td>
</tr>
<tr>
<td>372 P</td>
<td>Class-I: A2, A29(19), B44(12), B60(40), CW3 Class-II: DR13(6), DR7, DQ2, DR52, DR53</td>
<td>neg</td>
<td>neg</td>
<td>3 days</td>
<td>B7</td>
<td>B42, B54, B81, B82, A34, B55, B56, B57, Cw4, Cw6, Cw17, Cw18, DQ4, DQ2, DR12</td>
</tr>
<tr>
<td>D</td>
<td>Class-I: A2, A29(19), B44(12), B7, CW7 Class-II: DR4, DR5, DQ3, DR53</td>
<td>neg</td>
<td>neg</td>
<td>3 days</td>
<td>B7</td>
<td>B42, B54, B81, B82, A34, B55, B56, B57, Cw4, Cw6, Cw17, Cw18, DQ4, DQ2, DR12</td>
</tr>
<tr>
<td>2562 P</td>
<td>Class-I: A1, A2, B8, B38(16) Class-II: DR17(3), DR13(6), CW7, DQ1, DQ2</td>
<td>neg</td>
<td>neg</td>
<td>4 years</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>D</td>
<td>Class-I: A2, A23(9), B44(12), B60(40) Class-II: DR4, DR13(6), DQ3, DQ4, DR53, DR53</td>
<td>neg</td>
<td>neg</td>
<td>4 years</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>926 P</td>
<td>Class-I: A1, A2, B7 Class-II: DR15(2), DQ6(1)</td>
<td>neg</td>
<td>neg</td>
<td>1.5 years</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>D</td>
<td>Class-I: A2, A68(28), B51(5), B60(40) Class-II: DR4, DR11(5), DQ7(3), DR52, DR53</td>
<td>neg</td>
<td>neg</td>
<td>1.5 years</td>
<td>neg</td>
<td>neg</td>
</tr>
</tbody>
</table>

DSAs are present in 4 out of 21 tissue lysates of patients with ELS (1 or 2)

Tissue analysis corresponds to plasma analysis in 2 out of the 4 patients with DSAs (others time interval too big?)

All DSAs found in HTx patients with ELS are HLA-type II
Non-HLA antibodies (e.g. AT1R)

Angiotensin 2 Type-1 Receptor (AT1R)

Anti-AT1R antibodies are present in 11 out of 21 tissue lysates of patients with ELS (1 or 2)

Tissue analysis corresponds to plasma analysis prior to death

Anti-AT1R antibodies might already be present prior to transplant, already described in LTx

Budding et al. J Cyst Fibros. 2015
Antibodies in heart failure

Deposition of cardiac-specific antibodies


Progressive shift in B-cell subsets

Diagnosis: shift in antibody production

IgG

Control

Heart Failure

Antibody production

Relative production

IgA, IgM, IgG1, IgG2, IgG3, IgG4

Control

Heart Failure

Antibody production

Relative production

IgA, IgM, IgG1, IgG2, IgG3, IgG4
• Patients with ELS exhibit actively *antibody producing plasma cells*.

• These locally produced antibodies are in some cases directed against the donor **HLA-II type (DSA 19%)** and/or non-HLA antigens (e.g. AT1R >50%).

• **Local antibody-mediated rejection** may have major consequences for the graft.
Local vs distant antibody production

- Antibodies generated
  - Circulation (distant to graft)
  - Local (at side of rejection)

What is the most important site?

- Progression of chronic rejection
  - How do locally produced ab’s affect CAV?
  - How is ab production in ELS sustained?

- Therapeutic consequences
  - Do we need to target B cells, plasma cells, or T cells?
Local vs distant antibody production

- Affect on diagnostics
  - Measure in plasma
  - Measure in graft?

Kidney Intrgraft Donor-Specific Antibodies as Determinant of Antibody-Mediated Lesions and Poor Graft Outcome

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Introduction
Final remarks...

• What we measure in blood samples is not exactly what happens in the transplanted graft...
• The Endomyocardial biopsy visualizes only a local process and does not reflect the whole organ...
• Many rejection types interfere and affect graft failure:
  – Acute cellular rejection
  – Cardiac allograft vasculopathy
  – Antibody Mediated Rejection
• Present immunesuppression is focused on T cells and acute cellular rejection
• How can we adapt immunesuppression to a combined B and T cell regulation?
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