UMC Utrecht

Immunopathology of CAV
… *focus on Antibodies*

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Banff meeting 2017; Concurrent Heart session "Reassessment of the pathology of CAV"
Wednesday 29 March 2017 15:00 – 18:30
Title: "Immunopathology of CAV" 15:50-16:10

Disclosures

None

The start of this work was supported by a travelling scholarship award from the ISHLT to A.J. Gareau in April 2014

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

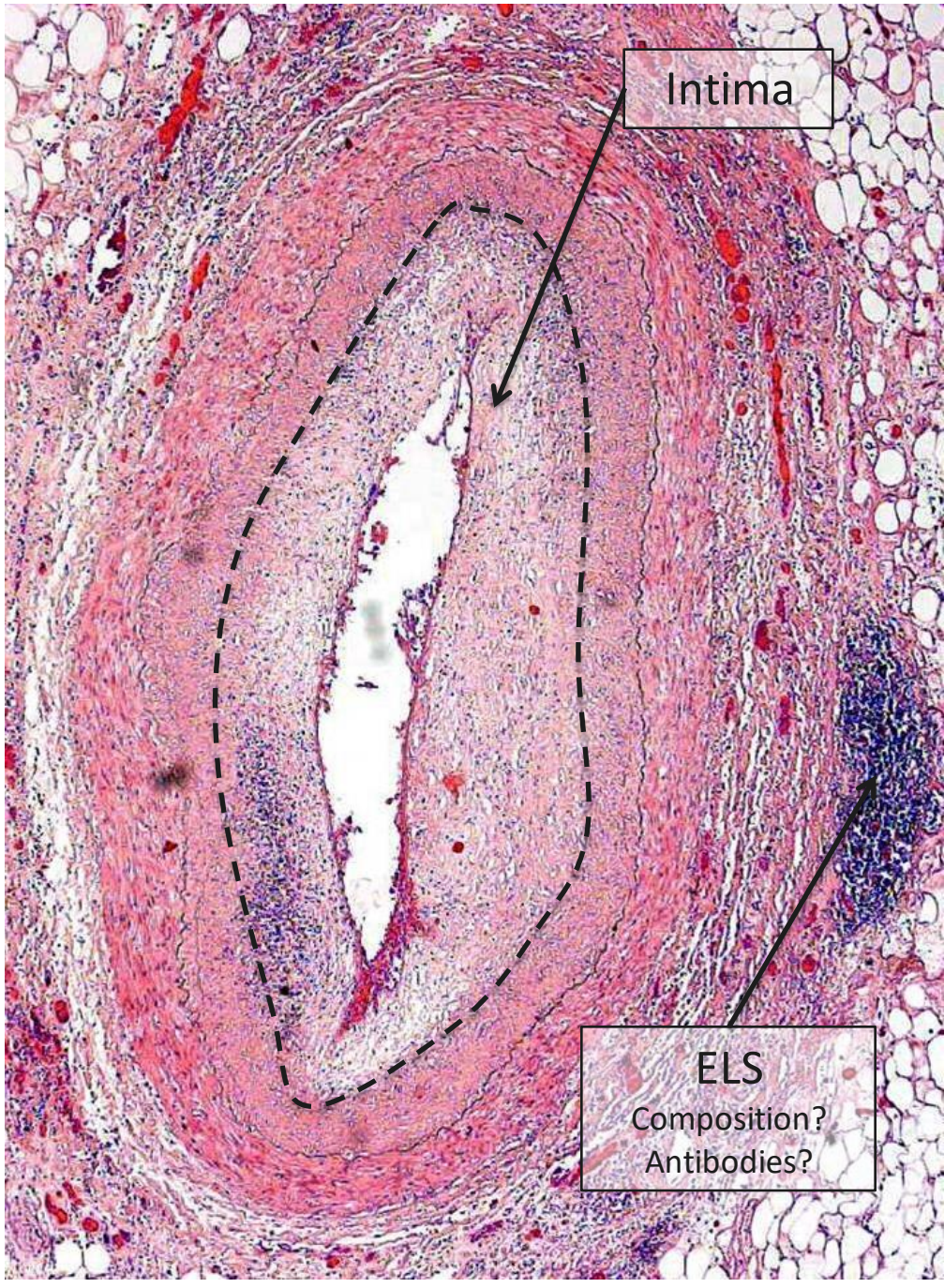
Cardiac Allograft Vasculopathy (CAV)

Vascular pathology after heart transplantation (HTx)

Limits the long term survival after HTx

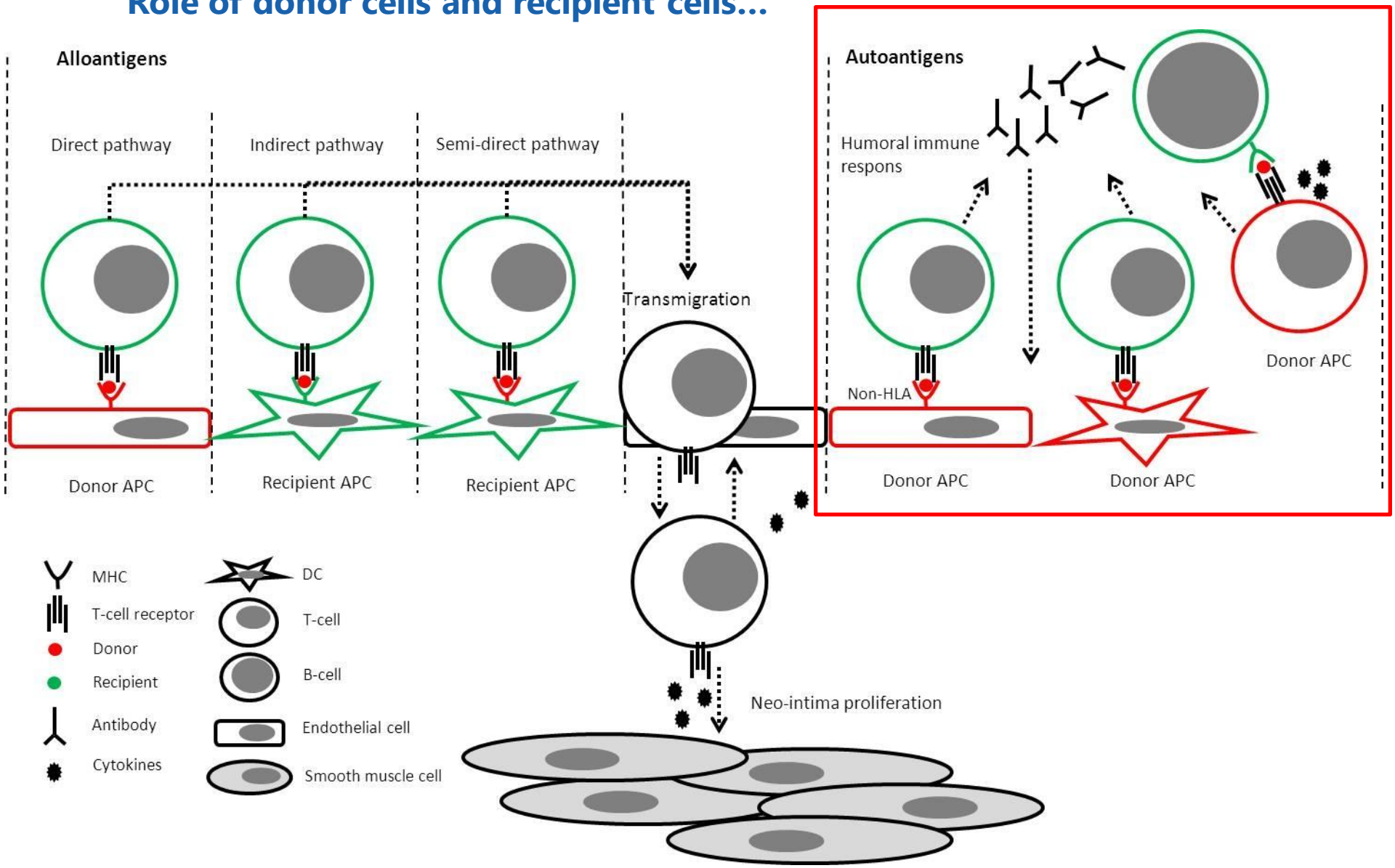
Immune-mediated
- Cellular events
- Antibody mediated?

Ectopic Lymphoid Structures (ELS)

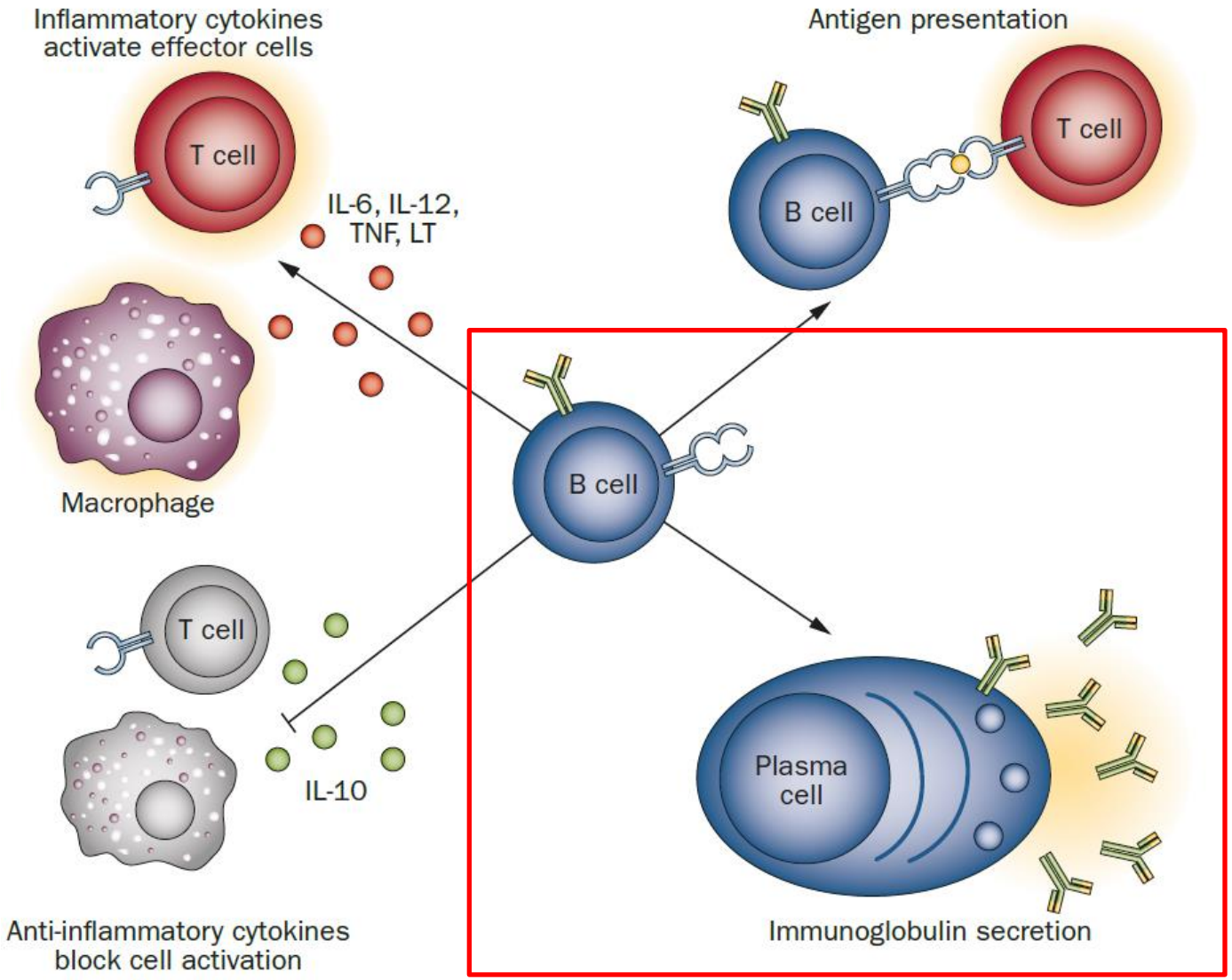


Immune processes in CAV

Role of donor cells and recipient cells…



B cells and antibodies



B-cells and antibodies in CAV

- Relation between CAV and:
  - Donor Specific HLA Antibodies (DSA)
  - Non-HLA antibodies…

American Journal of Transplantation 2016; 16: 111–120
Wiley Periodicals Inc.

doi: 10.1111/ajt.13

Late Failing Heart Allografts: Pathology of Cardiac Allograft Vasculopathy and Association With Antibody-Mediated Rejection

A. Loupy[1,2,*,†], C. Toquet[3,†], P. Rouvier[4,†], T. Beuscart[1], M. C. Bories[1], S. Varnous[5], R. Guillemain[6], S. Pattier[7], C. Suberbielle[8], P. Leprince[5], C. Lefaucheur[1], X. Jouven[1,2,9], P. Bruneval[1,2,10,†] and J. P. Duong Van Huyen[1,2,11,*,†]

Overall, antibody-mediated rejection was obser
in 19 (47.5%) failing heart allografts including
patients (40%) in whom unrecognized previ
episodes of subclinical antibody-mediated reject
occurred 4.5 ± 3.5 years before allograft loss.
planted allografts with evidence of antibody-media
rejection demonstrated higher endothelitis and mi

Donor-Specific Antibodies to Class II Antigens Are Associated With Accelerated Cardiac Allograft Vasculopathy: A Three-Dimensional Volumetric Intravascular Ultrasound Study

Yan Topilsky,[1] Manish J. Gandhi,[1] Tal Hasin,[1] Laurie L. Voit,[1] Eugenia Raichlin,[1] Barry A. Boilson,[1] John A. Schirger,[1] Brooks S. Edwards,[1] Alfredo L. Clavell,[1] Richard J. Rodeheffer,[1] Robert P. Frantz,[1] Sudhir S. Kushwaha,[1] Amir Lerman,[1] and Naveen L. Pereira[1,2]

Background. Although a link between donor-specific antibodies against human leukocyte antigens type II (DSA II+) and transplant glomerulopathy has been clearly established, its role in cardiac allograft vasculopathy (CAV) is unclear.
Methods. Donor-specific antibodies were evaluated using solid-phase single-antigen bead assay before transplantation in 51 heart transplant recipients. Coronary angiography and three-dimensional intravascular ultrasound were performed at baseline and approximately 1 year after the baseline examination.
Results. There were 4 (7.8 %), 11 (21.5%), and 2 (3.9%) patients who had DSA against donor class I (DSA I+), DSA

American Journal of Transplantation 2006; 6: 1781–1785
Blackwell Munksgaard

Minireview

doi: 10.1111/j.1600-6143.2006.01401.x

Role of Alloantibodies in the Pathogenesis of Graft Arteriosclerosis in Cardiac Transplantation

B. Soleimani[a], R. I. Lechler[c], P. I. Hornick[a,*,†] and A. J. T. George[b,†]

teriosclerosis. Vascular intimal thickening is the hallmark of the lesion seen microscopically in GA. This neo-intima, consisting of smooth muscle cells (SMC), fibroblasts, leuko-

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The Journal of Heart and Lung Transplantation
http://www.jhltonline.org

Effect of persistent versus transient donor-specific HLA antibodies on graft outcomes in pediatric cardiac transplantation

CrossMark

Claire A. Irving, MRCPCH, MD,[a] Vaughan Carter, PhD, FRCPath,[b] Andrew R. Gennery, MD, FRCPCH,[c] Gareth Parry, MB, FRCP,[d] Massimo Griselli, FRCS,[a] Asif Hasan, FRCS,[a] and C. Richard Kirk, MBBChir, FRCPCH[a]

B-cell aggregates around CAV arteries

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ORIGINAL PRE-CLINICAL SCIENCE

The composition of ectopic lymphoid structures suggests involvement of a local immune response in cardiac allograft vasculopathy

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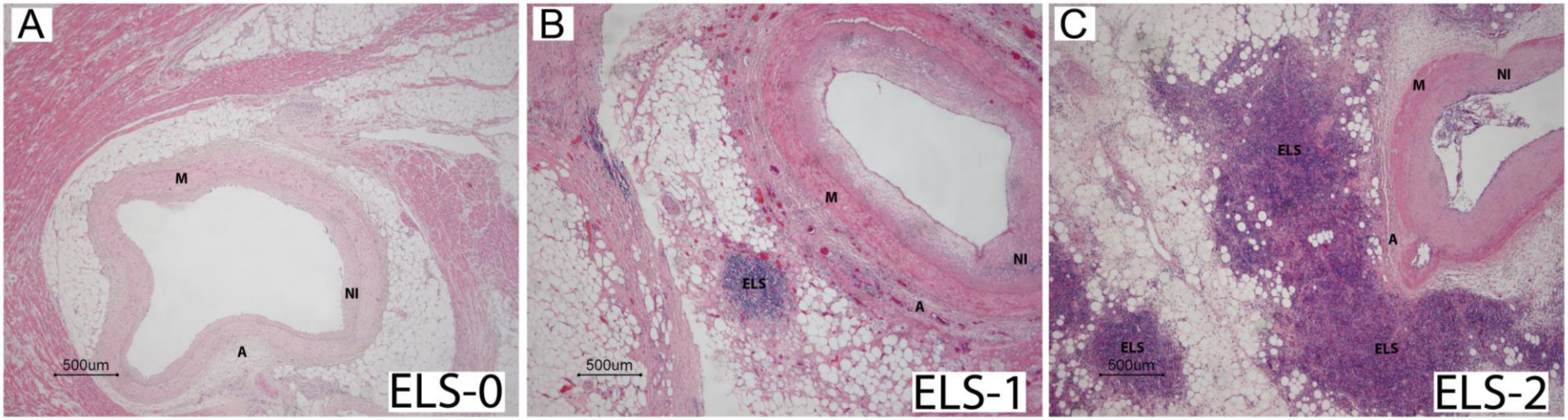
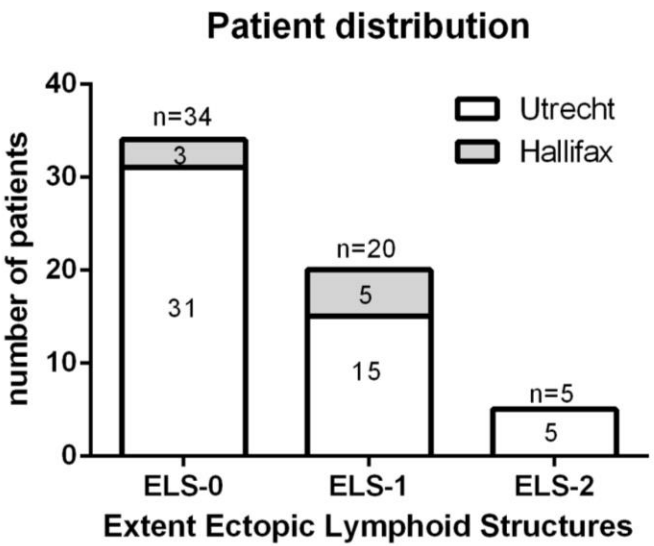
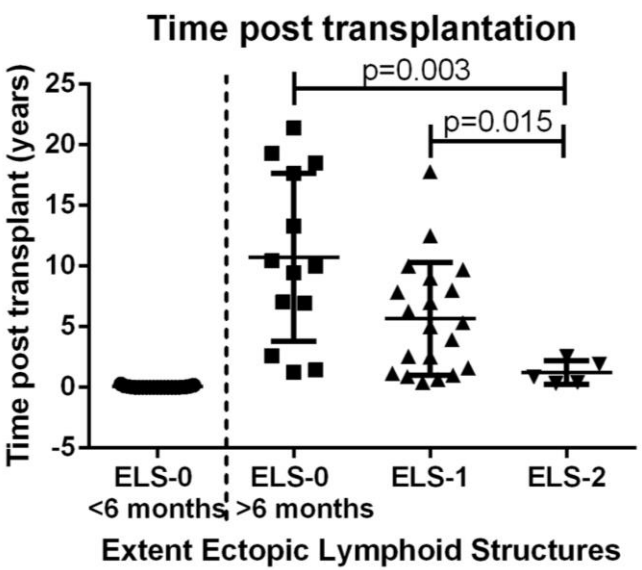
From the [a]Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands; the [b]Department of Pathology, Dalhousie University, Halifax, Nova Scotia, Canada; the [c]Department of Cardiology, University Medical Center Utrecht, Utretcht, The Netherlands; and the Departments of [d]Surgery; and [e]Microbiology & Immunology, Dalhousie University, Halifax, Nova Scotia, Canada.

Composition of Ectopic Lymphoid Structures (ELS)







CAV lesion type and ELS are correlated

Variables	Extent of ELS: ELS-0 (none)	ELS-1 (<300 μm²)	ELS-2 (>300 μm²)	
Time post-transplant	>6 months	All	All	*p*-value
Primary cardiac diagnosis, No. (%)	*n* = 13	*n* = 15	*n* = 4	0.266[b]
Dilated cardiomyopathy	4 (36)	5 (46)	2 (18)	
CAV lesion type, No. (%)	*n* = 13	*n* = 20	*n* = 5	0.002[b]
Lymphocytic	3 (38)	1 (13)	4 (50)	
Fibrotic	10 (33)	19 (63)	1 (3)	
H-CAV type, No. (%)	*n* = 13	*n* = 20	*n* = 5	0.011[b]
H-CAV1	3 (38)	1 (13)	4 (50)	
H-CAV2	5 (36)	8 (57)	1 (7)	
H-CAV3	5 (31)	11 (69)	0 (0)	
Female/male	1 (100)	0 (0)	0 (0)	
CMV status patient/donor, No. (%)	*n* = 12	*n* = 15	*n* = 3	0.314[b]
−/−	2 (33)	4 (66)	0 (0)	
−/+	2 (25)	3 (38)	3 (38)	
+/−	4 (57)	3 (43)	0 (0)	
+/+	4 (44)	5 (56)	0 (0)	
Immune suppression, No. (%)	*n* = 13	*n* = 15	*n* = 4	0.118[b]
Cyclosporin	12 (43)	14 (50)	2 (7)	
Tacrolimus	1 (25)	1 (25)	2 (50)	
Rejection episodes, No. (%)	*n* = 13	*n* = 15	*n* = 4	0.449[b]
Yes	10 (48)	8 (38)	3 (14)	
Quilty in EMB, No (%)	*n* = 13	*n* = 15	*n* = 5	0.053[b]
Quilty A or B or both	13 (43)	14 (47)	3 (10)	
Time post-HTx (days)	*n* = 13	*n* = 20	*n* = 5	0.002[c]
Median (IQR)	3,659 (1,746–6,595)	1,886 (468–3,194)	315 (135–812)	
Relative NI lesion size	*n* = 13	*n* = 20	*n* = 5	0.133[d]
Mean (SD)	0.42 (0.15)	0.51 (0.13)	0.46 (0.07)	
CAV lesion type, No. (%)	*n* = 13	*n* = 20	*n* = 5	0.002[b]
Lymphocytic	3 (38)	1 (13)	4 (50)	
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H-CAV type, No. (%)	*n* = 13	*n* = 20	*n* = 5	0.011[b]
H-CAV1	3 (38)	1 (13)	4 (50)	
H-CAV2	5 (36)	8 (57)	1 (7)	
H-CAV3	5 (31)	11 (69)	0 (0)	
Cause of death, No. (%)	*n* = 13	*n* = 20	*n* = 5	0.337[b]
CAV	3 (27)	7 (64)	1 (9)	
Acute rejection	2 (22)	4 (44)	3 (33)	
Other	8 (44)	9 (50)	1 (6)	

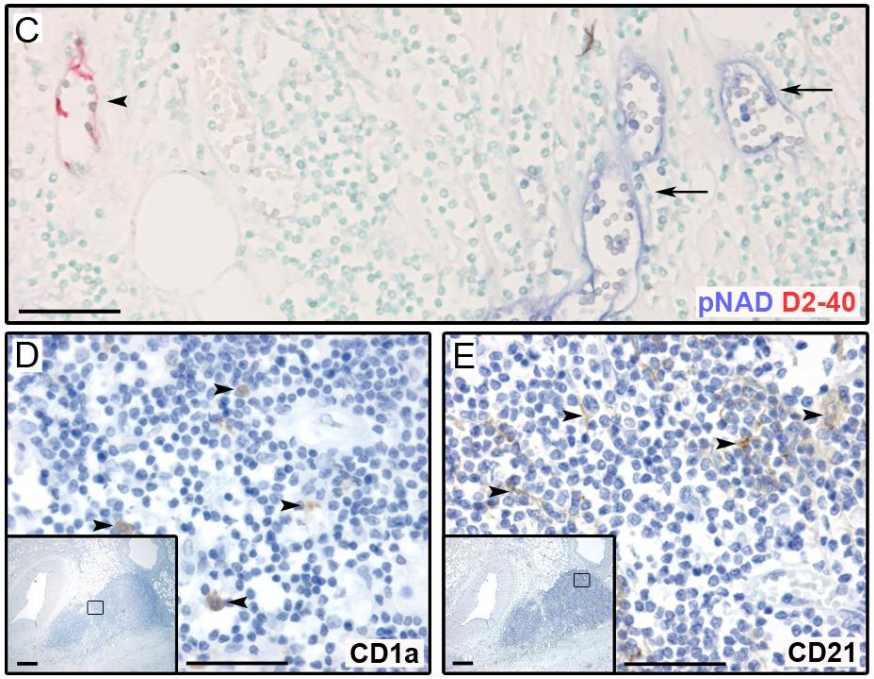
CAV, cardiac allograft vasculopathy; CMV, cytomegalovirus; ELS, ectopic lymphoid structures; EMB, endomyocardial biopsy; H-CAV, histologic cardiac allograft vasculopathy; HTx, heart transplantation; IQR, Interquartile range; LVAD, left ventricular assist device; NI, neointima; SD, standard deviation.
[a]Group size for the variables varies due to incomplete medical records.
[b]Fisher's exact test.
[c]Kruskal-Wallis test.
[d]Analysis of variance.

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

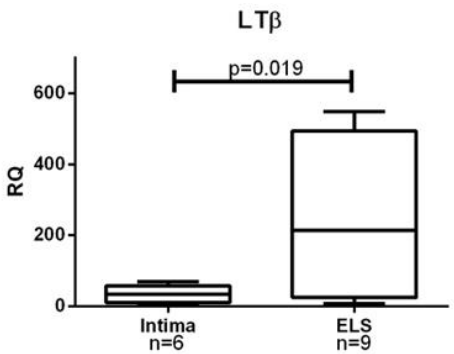
- All patients with benign intimal thickening (BIT) were in the ELS-0 category and early after transplant (<6 months).
- 20/25 patients with ELS had fibrotic lesions. From which 19 were in the ELS1 category.
- 5/25 patients with ELS had lymphocytic lesions. Four of these 5 patients were in the ELS-2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

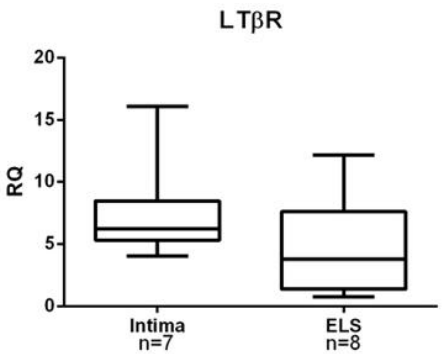
Histologic CAV type was significantly related to ELS category (p=0.011), where patients with H-CAV2 and H-CAV3 were mainly in the ELS1 category (57% and 69%, respectively).

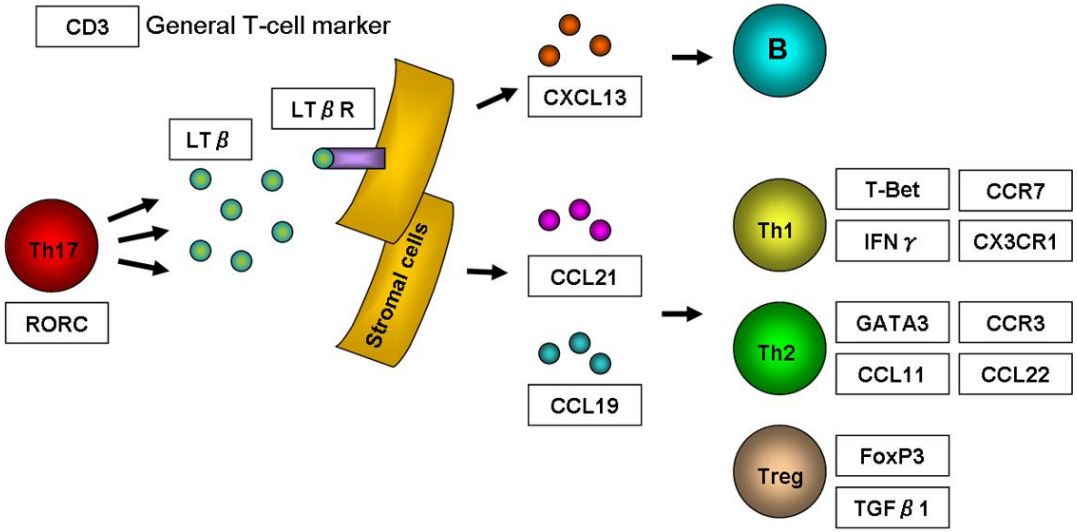
ELS as Tertiary Lymphoid Organs (TLO)



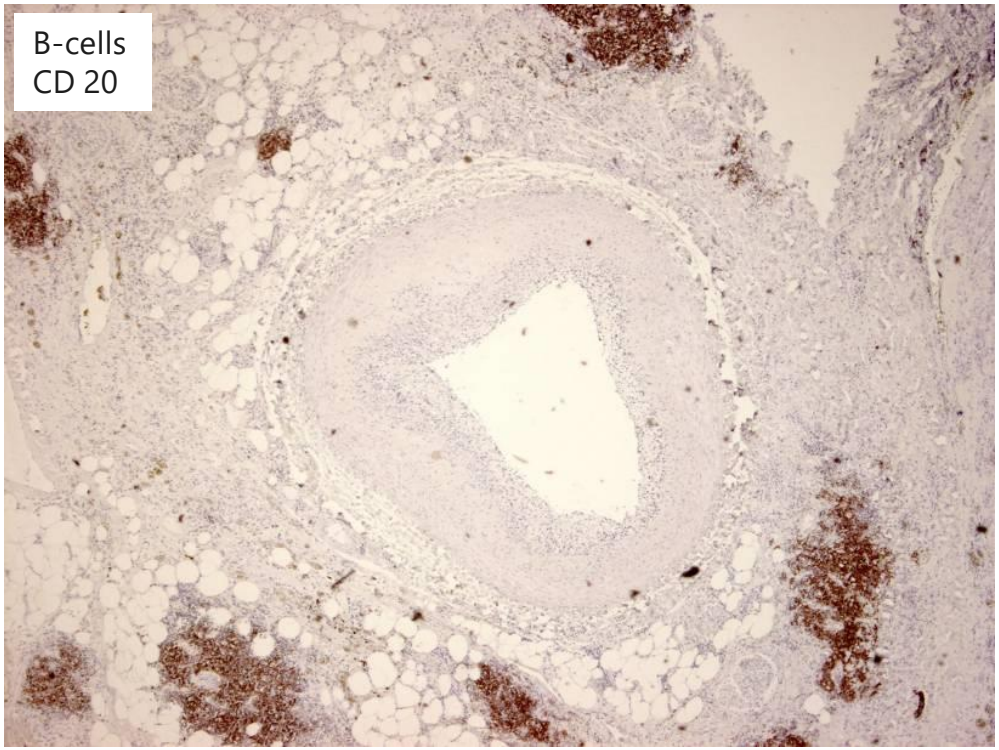
pNAD	High Endothelial Venules
D2-40	Lymph vessels
CD1a	Interdigitating dendritic cells
CD21	Follicilar dendritic cells
LTβ	Lymphotoxin beta

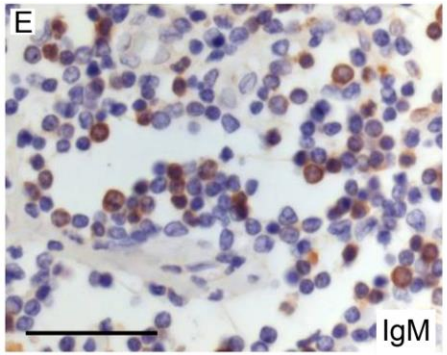


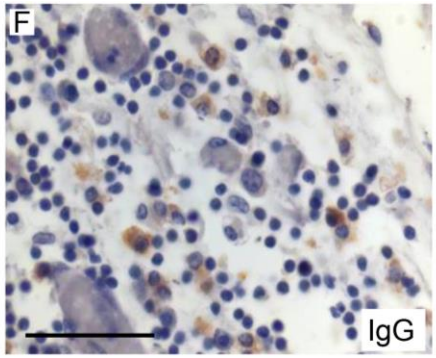


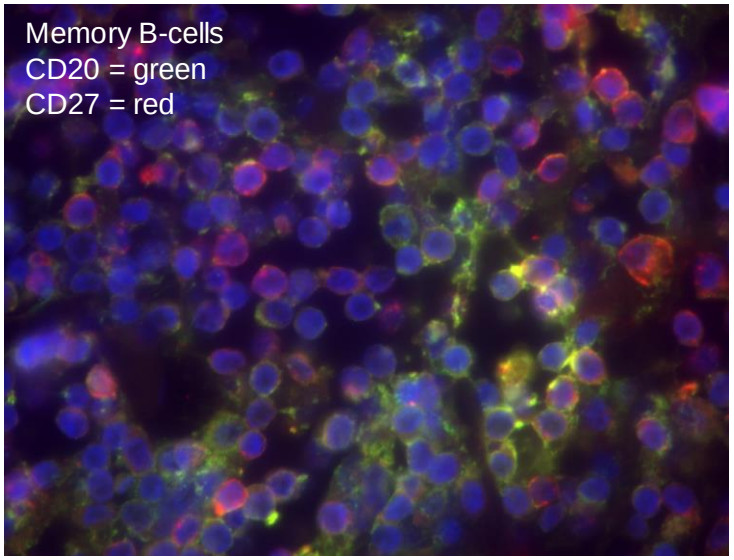


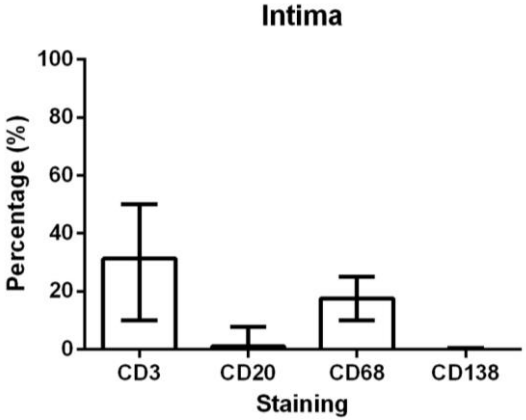
Ectopic Lymphoid Structures contain many B cells

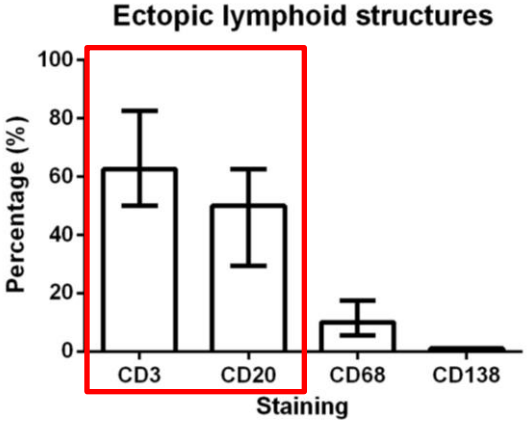




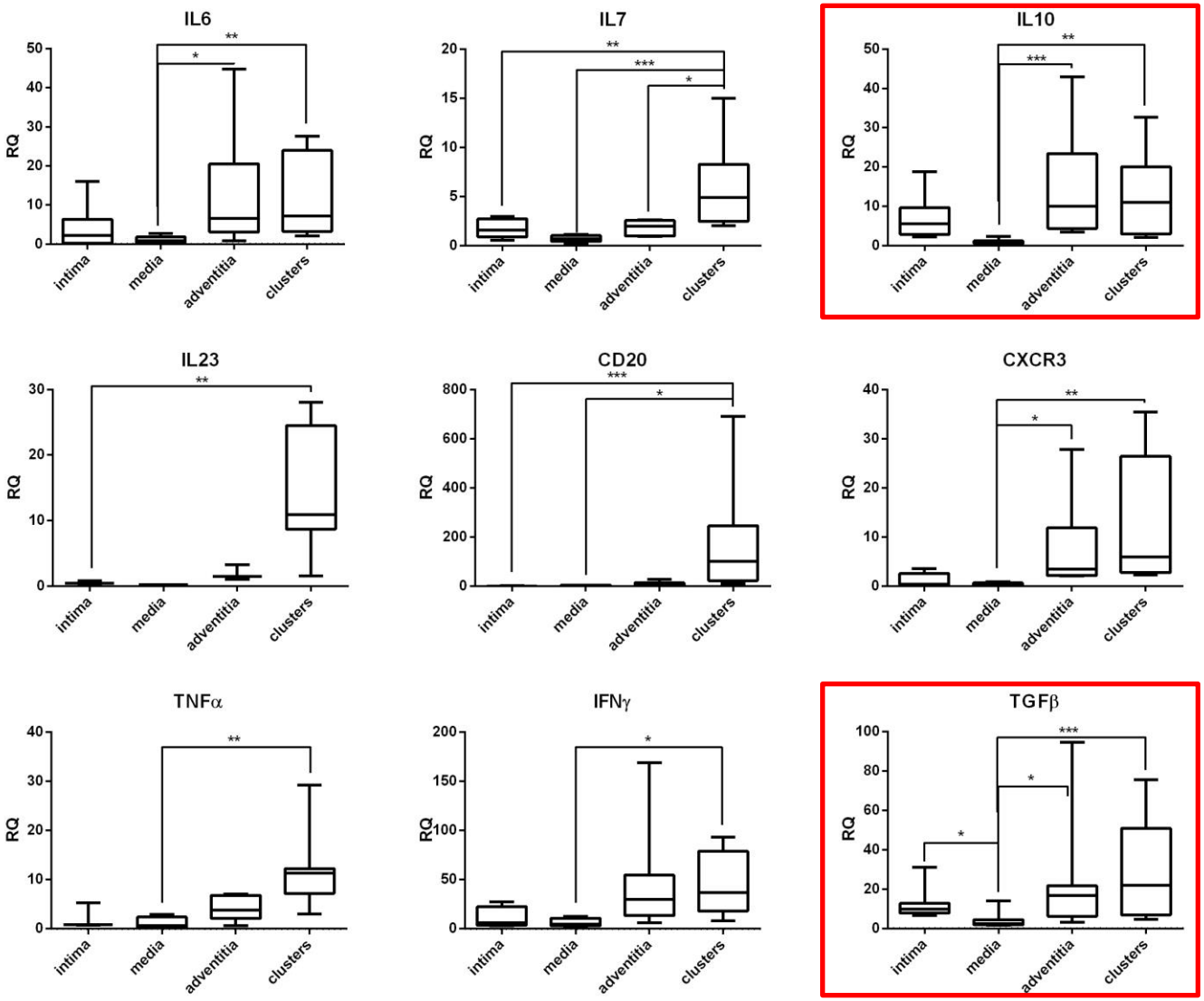








Cytokine profiles of Ectopic Lymphoid Structures (IL10, TGFb)



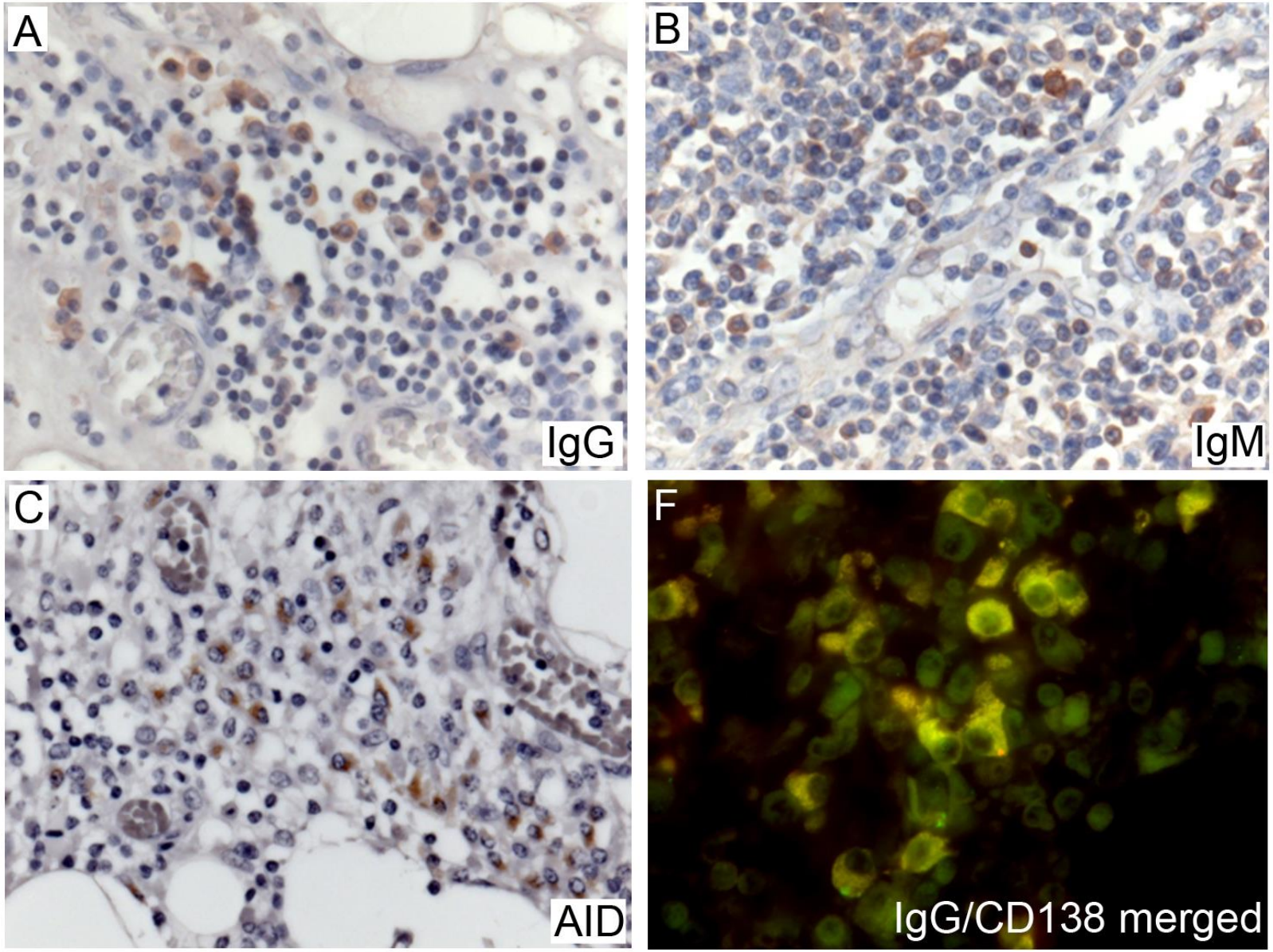
Regulatory role of B cells:

- IL10
- IL35
- TGFbeta

(Dijke et al JHLT 2016)

Immune suppression in adventitia?
"compartimentalisation"

Active antibody production in ELS

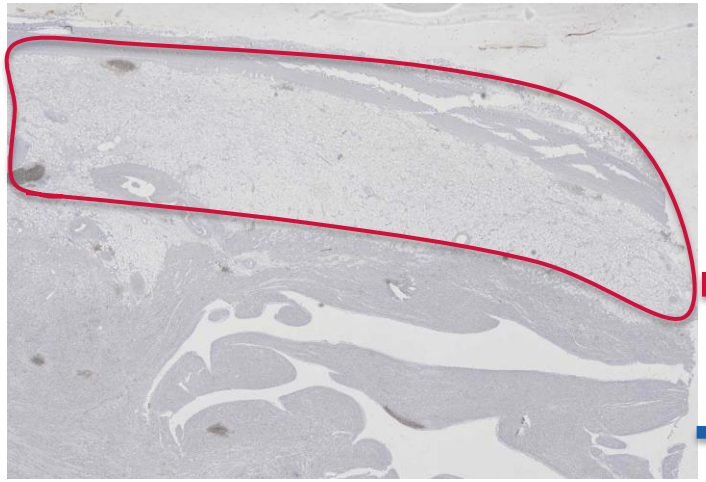


Activation-induced cytidine deaminase

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

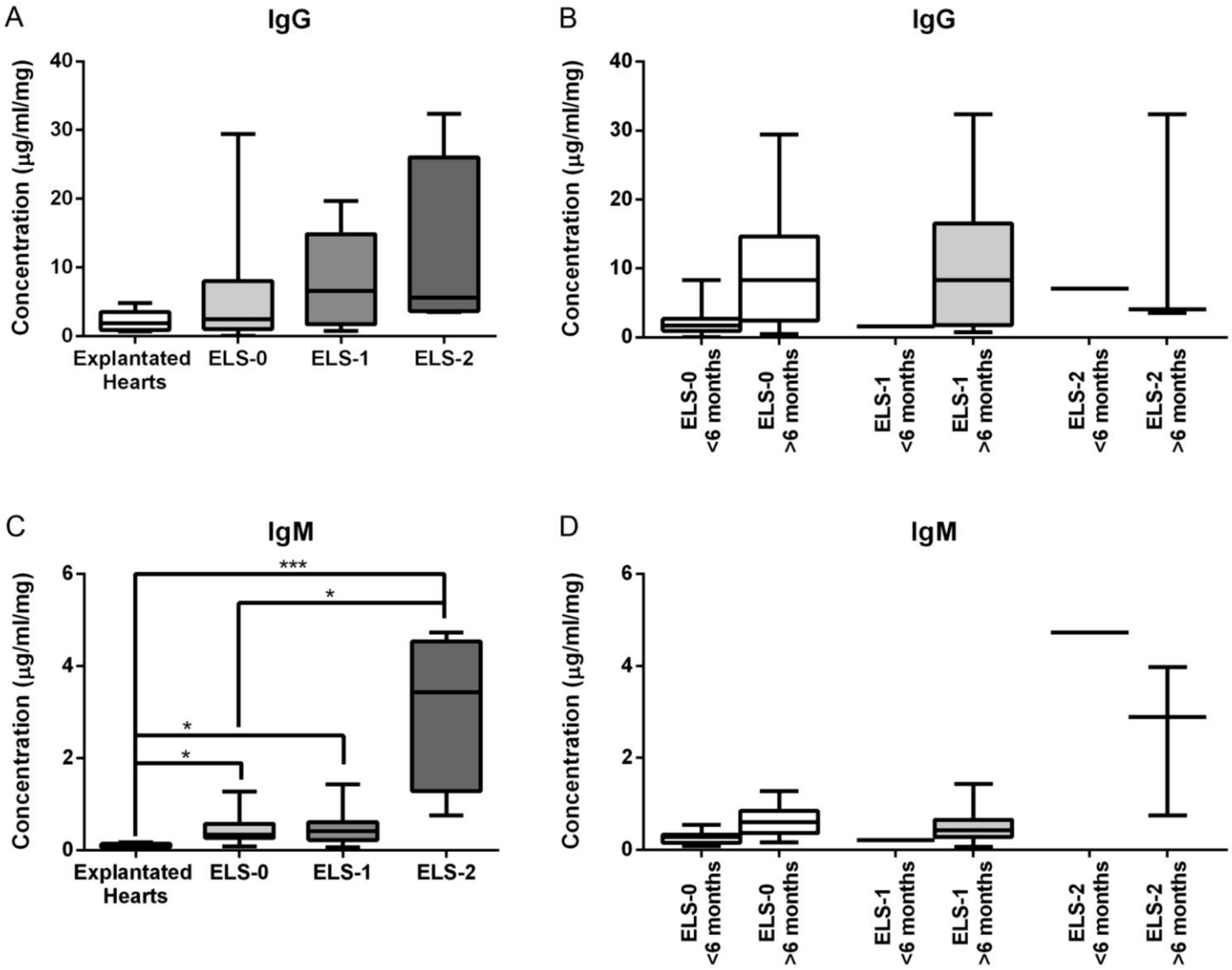
Myocard

Pre-chilled tube with micro beads

Lysate Tissue

Bead shaker

Quantification of antibody production in ELS



CAV + ELS

N=21

↓

High levels of IgG and IgM in ELS

↓

What are targets of these antibodies?

Donor HLA?

Donor Specific Antibodies (DSA) in ELS

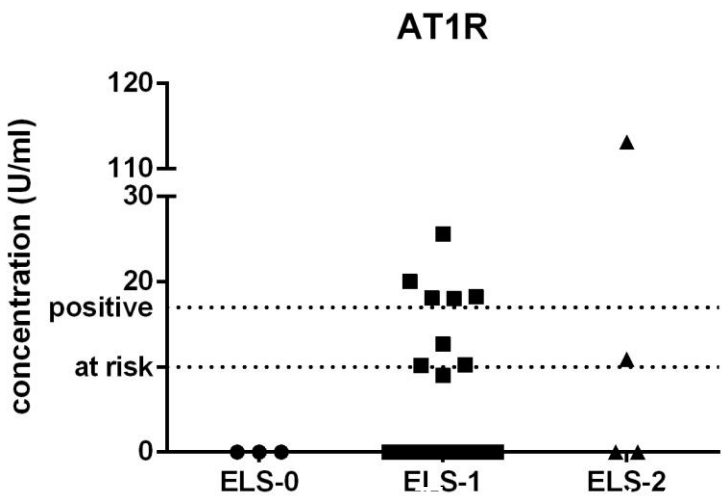
Time post-HTx (days)		HLA type	DSA (tissue)	non-DSA (tissue)	plasma time point before death	DSA (plasma)	non-DSA (plasma)
153	P	Class-I: A24(9), A31(19), B51(5), B55(22), CW9(3) Class-II: DR13(6), DQ1, DR52			3 days		
	D	Class-I: A2, B7, B60(40), CW3, CW7 Class-II: DR4, DR8, DQ3, DQ4, DR53	neg DQ3, DQ4	neg DQ2		A2, B40 DQ3, DQ4, DR53, DR4, DR8	B13, B27, B17 DQ2, DR9
372	P	Class-I: A2, A29(19), B44(12), B60(40), CW3 Class-II: DR13(6), DR7, DQ2, DR52, DR53			3 days		
	D	Class-I: A3, A29(19), B44(12), B7, CW7 Class-II: DR4, DR9, DQ3, DR53	neg DQ7(3)	neg neg		B7 DQ3, DR4	B42, B54, B81, B82, A34, B55, B56, B57, Cw4, Cw6, Cw17, Cw18 DQ4, DQ2, DR12,
2562	P	Class-I: A1, A2, B8, B38(16) Class-II: DR17(3), DR13(6), CW7, DQ1, DQ2			4 years		
	D	Class-I: A2, A23(9), B44(12), B60(40) Class-II: DR4, DR13(6), DQ1, DQ3, DR52, DR53	neg DQ3, DR53	neg neg		neg neg	neg neg
926	P	Class-I: A1, A2, B7 Class-II: DR15(2), DQ6(1)			1.5 years		
	D	Class-I: A2, A68(28), B51(5), B60(40) Class-II: DR4, DR11(5), DQ7(3), DR52, DR53	neg DQ7(3)	neg DQ2, DQ4		neg neg	neg neg

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)

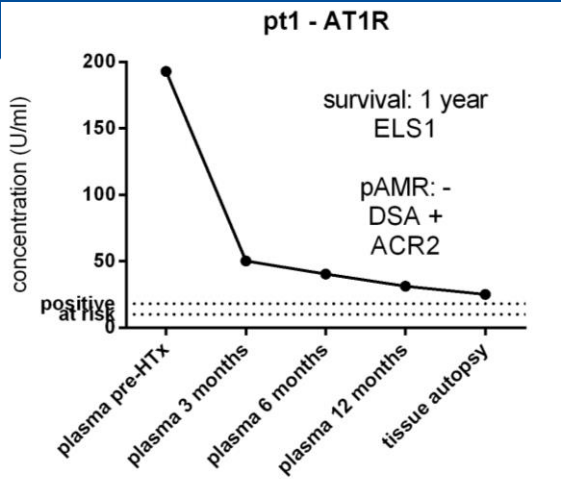


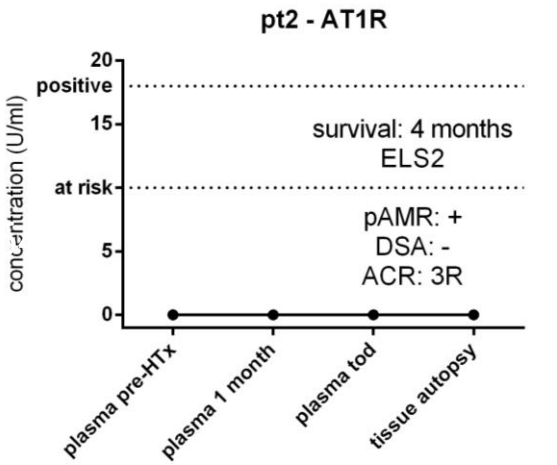
Angiotensin 2 Type-1 Receptor (AT1R)

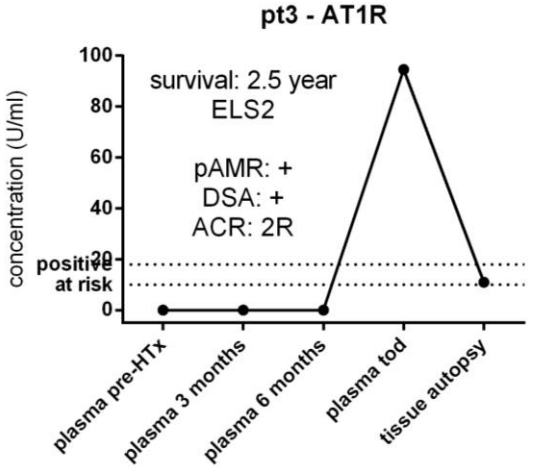
Anti-AT1R antibodies are present in 11 out of 21 tissue lysates of patients with ELS (1 or 2)

Tissue analysis corresponds to plasma analysis prior to death

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







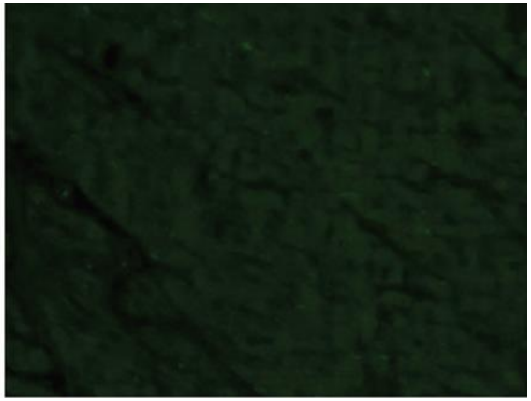
Budding et al. *J Cyst Fibros. 2015*

Antibodies in heart failure

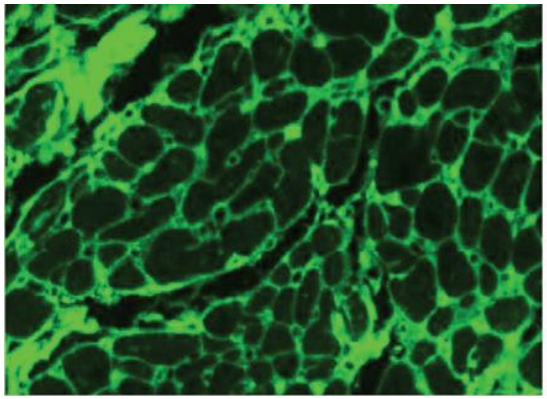
Deposition of cardiac-specific antibodies

K.A. Youker *et al*, European Heart Journal (2014)

IgG

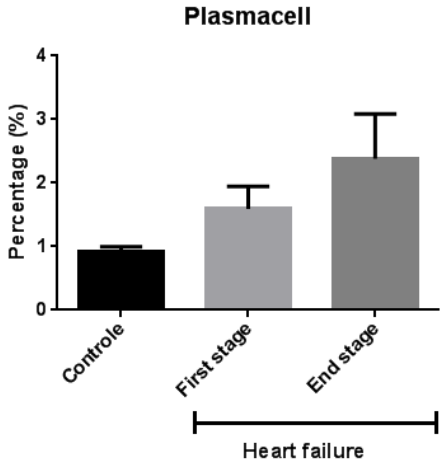


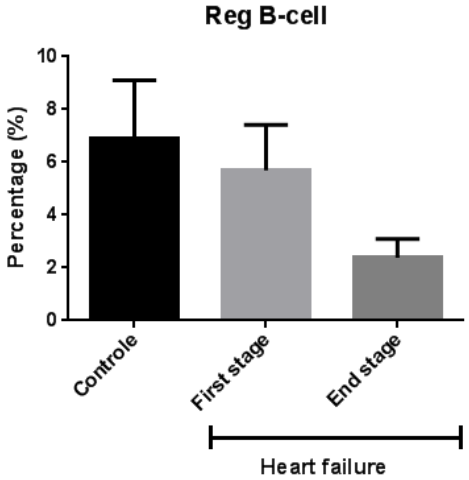
Control



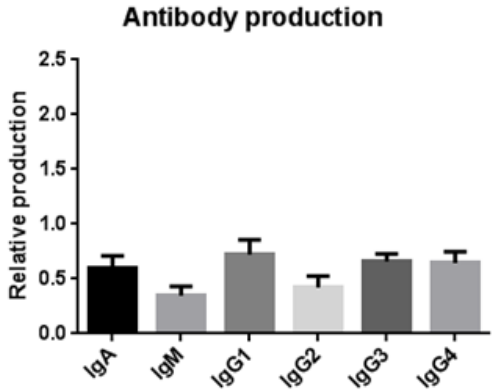
Heart Failure

Progressive shift in B-cell subsets

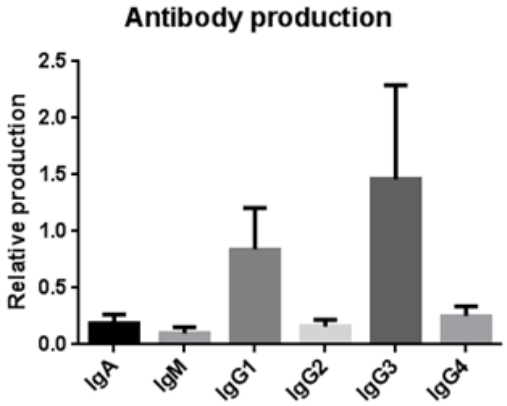




Diagnosis: shift in antibody production

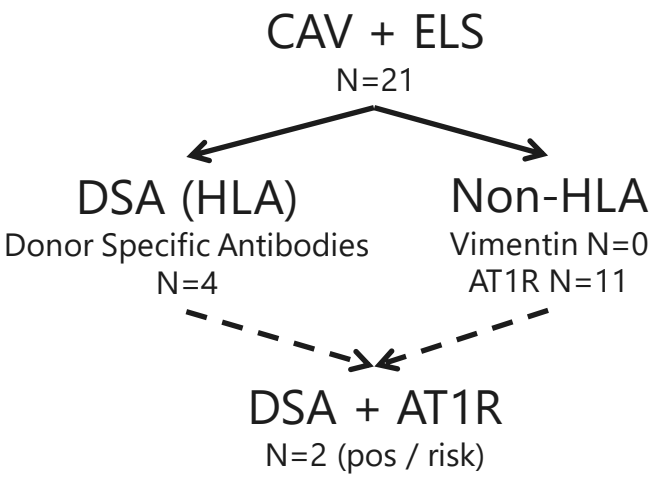


Control



Heart Failure

Conclusion



- 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.

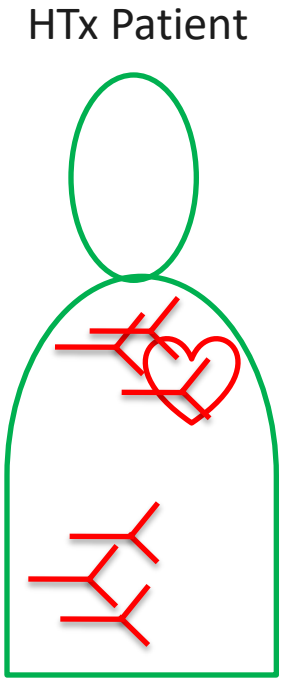
Local vs distant antibody production

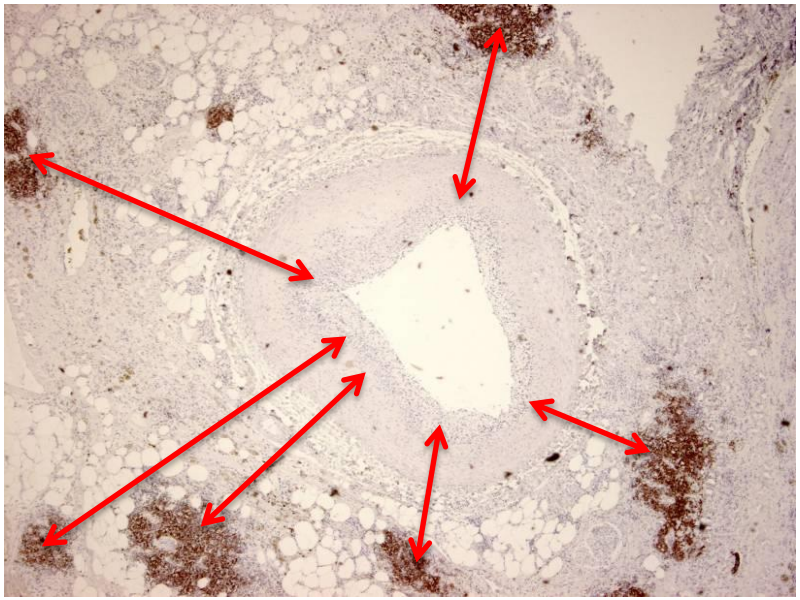
- Antibodies generated
  - Circulation (distant to graft)
  - Local (at side of rejection)

  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?

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

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lesions; CAMR, chronic antibody-mediated rejection; cg, glomerulopathy; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; g, glomerulitis; gDSA, intragraft DSA; IFTA, interstitial fibrosis and tubular atrophy; isDSA, immunodominant serum DSA; KTR, kidney transplant recipients; ptc, peritubular capillaritis; SAFB, single antigen flow bead; sDSA, serum DSA; ∑sDSA-MFI, sum of the mean fluorescence intensity of the different circulating DSA

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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 proces 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 immunesuppresion is focussed on T cells and acute cellular rejection
- How can we addapt immunesuppression to a combined B and T cell regulation?

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