Supplementary Materials:

Supplementary Methods

Adoptive transfer of T lymphocytes

For the isolation of T lymphocytes, splenocytes from allosensitized C57BL/6 Ly5.1 mice were stained with phycoerythrin (PE)-conjugated antibodies against CD19 (1D3), B220 (RA3-6B4), CD11b (M1/70), CD11c (HL3), NK-1.1 (PK136), Ter119/Erythroid cells (TER-119), CD117 (2B8) (1/200, all from BD Biosciences, Le Pont de Claix, France) and peridinin-chlorophyll protein (PerCP)-conjugated antibody against Thy1.2 (53-2.1) (BioLegend, London, United Kingdom).

After staining, the cells were negatively separated by LD magnetic columns with anti-PE Microbeads labeling (Miltenyi Biotec, Paris, France). The separated cell suspensions underwent cell sorting using an FACS Aria cell sorter (BD Biosciences). Five million purified T lymphocytes were transferred intravenously (IV) to the C57BL/6 RAG2 KO mice.

Evaluation of DSA avidity

DSA avidity was estimated by assessing the stability of preformed antigen–antibody complexes in the presence of increasing concentrations of the chaotropic agent urea. Low avidity antibodies are indeed more sensitive to the dissociating effects of chaotropic agents and their binding decreases at a lower urea concentration than high avidity antibodies. Briefly, 5.10^5 CBA splenocytes were incubated with the immune serum collected at the plateau of the humoral alloimmune response of C57BL/6 recipients of i) one CBA skin graft (primary response), ii) two consecutive CBA skin grafts (memory response), or iii) one CBA heart transplant (primary

response). The quantity of serum used in the 3 conditions was adjusted to saturate the allogeneic targets of the CBA splenocytes. The excess of DSA was washed and the DSA/alloantigen complexes were exposed to an increasing concentration of urea (0 to 4M) for 10' at RT. The amount of DSA that remained bound to the alloantigens at the surface of the CBA splenocytes was then evaluated using the flow cross match technique presented in **Supplementary Figure 1**.

Immune sera transfer experiments

The amount of DSA transferred and the frequency of the infusions was set to ensure that the titer of the circulating DSA remained stable and similar to the median value observed in wild type recipients grafted with allogeneic islets. Pooled sera from islet-grafted recipients could not be used to achieve these objectives because the DSA titer was too low.

Different models of allosensitization were compared (**Supplementary Figure 3A)**. Islet graft recipients generated a similar level of DSA compared to the recipients of a full thickness skin graft but this titer was several magnitudes lower than that observed after heart transplantation (**Supplementary Figure 3A**). Performing a second skin grafting procedure triggered a memory alloimmune response in the recipient, as demonstrated by the shorter graft survival (**Supplementary Figure 3B**) that increased the DSA titer up to a level similar to that observed after heart transplantation (**Supplementary Figure 3A**). However, pooled sera from mice iteratively grafted with allogeneic skin could not be used because memory response not only increased DSA titer but also DSA avidity (**Supplementary Figure 3C)**. In comparison with iterative skin grafting, DSA generated in response to a first heart transplantation were similar in titer, but their avidity was close to that observed after a first islet graft (primary alloimmune response in both cases, **Supplementary Figure 3C**). Furthermore, both the isotype composition and the cytolytic potential were similar for DSA generated after islet grafting and heart

transplantation (**Supplementary Figure 3D** and **3E**). Based on these findings, pooled sera from CBA heart recipients were used for the serum transfer experiments.

Flow cytometry

Fresh islets were dissociated by trypsin (0.05%)-EDTA (0.02%) solution (Sigma-Aldrich). Islet grafts collected from the renal subcapsular space were dispersed by a mixture of dispase (0.8mg/ml)/collagenase P (0.2mg/ml) and 0.1mg/ml DNAase (all from Roche, Meylan, France). Heart transplants harvested from recipients were digested by 500 unit/ml collagenase type II (Worthington, Lakewood, NJ) and 1 mg/ml collagenase/dispase (Roche).

Single cell suspensions were incubated with a blocking anti-mouse Fc receptor antibody (clone 2.4G2) for 20 minutes at 4°C and then with relevant fluorescent monoclonal antibodies: CD31 (clone MEC 13.3, 1/200, BD biosciences), CD19 (clone 1D3, 1/200, BD biosciences), H-2D^{k} (clone 36-7-5, 1/200, BD biosciences), kappa light chain (clone 187.1, 1/50, BD biosciences), CD45 (clone 30-F11, 1/400, BioLegend)) for 10 minutes at 4°C. Before acquisition, 0.1µg/ml 4',6-diamidino-2-phenylindole (DAPI) was added to the cell suspension to stain the dead cells. Sample acquisitions were made on an LSR II flow cytometer (BD Biosciences) and analyses were performed with FlowJo software version 10.0.8r1 (Tree Star Inc, Ashland, OR).

Pathological analyses

For analysis of H-2 expression by pancreatic islet cells, fresh islets isolated from C57BL/6 (H-2^{b}) or CBA (H-2^{k}) mice were embedded in Tissue-Tek OCT (Sakura Finetek, Villeneuve d'Ascq, France) and frozen in liquid nitrogen. Eight µm-thick cryosections were incubated for 20 minutes at 20°C with goat serum and then with 100µg/ml anti-H-2^{k} mAb (clone HB13) overnight at 4°C. Sections were washed and then incubated with 20µg/mL AF488-conjugated

goat anti-mouse IgG2a (Invitrogen) for 1h at 4°C. DAPI 1µg/ml was added to the sections to stain the cell nuclei. Images were taken at 20X magnification by Zeiss Axioimager Z1 (Zeiss, Marly le Roi, France) and acquired by Metamorph software version 7.8.13.0 (Molecular Devices LLC, Sunnyvale, CA). Image analysis was performed by ImageJ (NIH).

Pathological analyses of grafted islets were made as follows. After mouse euthanasia, followed by evacuation of blood by 40ml PBS infusion via abdominal aorta with incision of inferior vena cava, vessel painting was performed by infusing 300µg wheat-germ agglutinin conjugated with Alexa Fluor (AF) 488 (16µg/ml, Invitrogen) into abdominal aorta and then 4% paraformaldehyde (PFA) for fixation. Organs containing grafted islets (liver for model #1 or kidney for model #2) were harvested and 400µm-thick slices of fresh tissue were prepared with a vibratome. Tissue slices were postfixed in 4% PFA and permeated with 2% Triton-X 100 for 2 hours at 20°C. Afterwards, slices were incubated in 10% goat serum for blocking, followed by primary antibody incubation: polyclonal guinea pig anti-insulin antibody (1/50, Dako, Les Ulis, France) overnight at 20°C. Goat anti-guinea pig IgG secondary antibody conjugated with AF647 (1/200, Invitrogen) was then used to reveal insulin-positive cells for 6 hours at 20°C. Nuclei were stained with 1µg/ml DAPI for 1 hour at 20°C before applying the specimens to slides and then immersed in the optical clearing solution RapidClear 1.52 (SunJin Lab, HsinChu, Taiwan). Images were taken at 5X magnification by Zeiss Axioimager Z1 and 20X magnification by an LSM800 confocal microscope (Zeiss) with acquisition by ZEN software version 2.1 (Zeiss). Image analysis was performed by ImageJ.

For the histological assessment of the DSA-mediated lesions, heart transplants or left kidney (containing grafted islets) were harvested 30 days after the beginning of the DSA transfer, fixed in 4% buffered formalin for 24h and embedded in paraffin for hematoxylin and eosin stain and

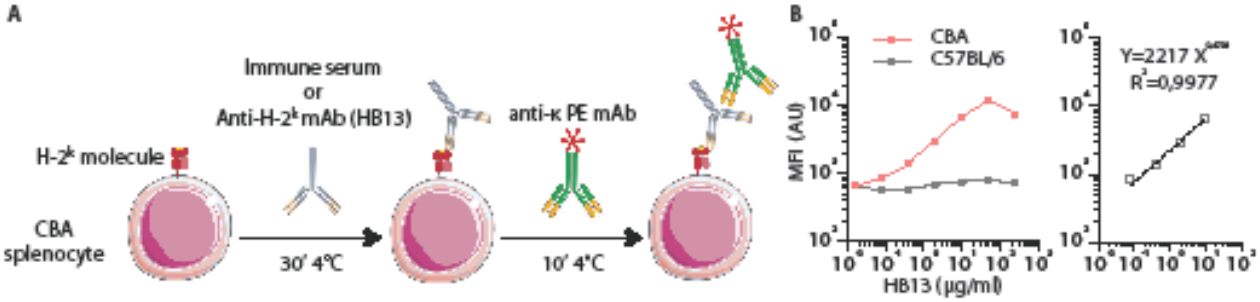
immunohistochemistry. Briefly, after antigen retrieval by heating in a citrate buffer, the following primary antibodies were used: anti-mouse CD31 (1/50; Dianova, Hamburg, Germany), anti-C4d (1/50; **DB Biotech,** Kosice, Slovakia), and anti-mouse CD68 (1/200; AbD Serotec, Kidlington, UK) to stain, respectively, the endothelial cells, C4d complement fraction, and macrophages. Sections were then revealed by Vectastain ABC HRP Kit (Vector, Peterborough, UK). The staining intensity was graded semi-quantitatively (0 to 5) by a trained pathologist (P.B.).

Supplementary Table 1: Characteristics of the study population

Variables	Whole cohort (n=49)	DSA (n=9)	No DSA (n=40)
Recipient characteristics			
Male, n (%)	25 (51.02)	3 (33.33)	22 (55.00)
Age (Years)	46.97 ± 10.03	44.89 ± 8.98	47.44 ± 10.30
Weight (Kg)	62.33 ± 8.83	55.22 ± 8.73	64.06 ± 8.04
BMI (Kg/m^2)	22.38 ± 2.78	19.70 ± 2.48	23.00 ± 2.48
Diabetes characteristics			
HbA1c (%)	8.13 ± 1.57	7.23 ± 0.66	8.34 ± 1.65
Insulin requirements (UI/Kg)	0.51 ± 0.192	0.63 ± 0.28	0.49 ± 0.16
Graft characteristics			
Nb of infusions	2.35 ± 0.69	2.33 ± 1.00	2.35 ± 0.62
IEQ/Kg	15,805 ± 9,167	21,346 ± 1,6462	14,458 ± 5940
Primary graft function			
ß score gain (points)	6.14 ± 1.44	6.44 ± 4.42	6.08 ± 1.46
Graft immunogenicity			
HLA mismatches (n)	16.85 ± 5.99	17.67 ± 7.23	16.66 ± 5.75
Immunosuppression			
Depleting induction, n (%)	20 (40.82)	6 (66.67)	14 (35.00)
Maintenance regimen, n (%)			
Sirolimus + CNI	18 (36.73)	2 (22.22)	16 (40.00)
MMF + CNI	26 (53.06)	6 (66.67)	20 (50.00)
Other	5 (10.20)	1 (11.11)	4 (10.00)

DSA : donor-specific antibodies ; BMI : body mass index ; HbA1c : Hemoglobin A1c ; IEQ : islet equivalent ; HLA : human leukocyte antigens ; CNI : calcineurin inhibitor ; MMF : mycophenolate mofetil

SuppFig1

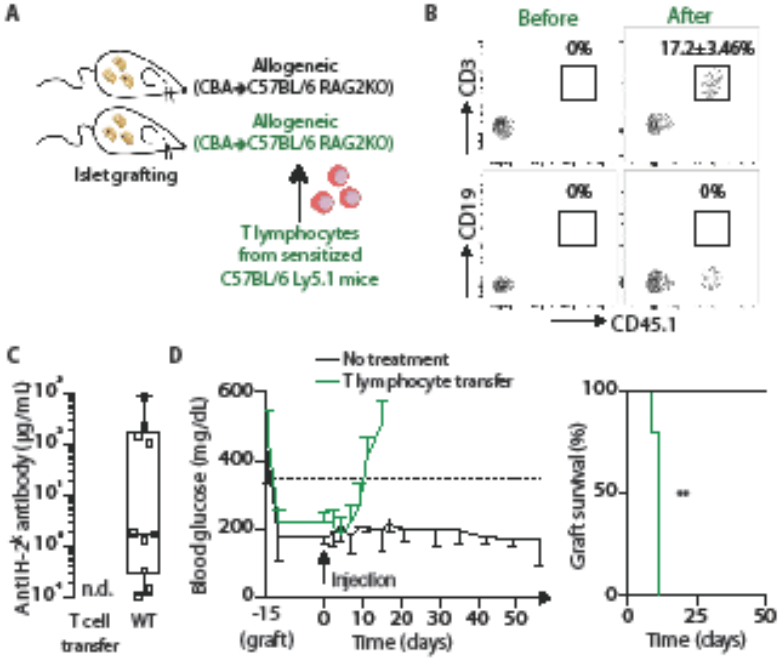

A
Immune serum
or
Anti-H-2k mAb (HB13)
anti-κ PE mAb
H-2k molecule
CBA
splenocyte
30' 4°C
10' 4°C
B
CBA
C57BL/6
MFI (AU)
HB13 (µg/ml)
Y=2217 X^0,0736
R²=0,9977

Supplementary Figure 1: Custom flow cross match technique to quantify DSA

A. CBA splenocytes were incubated with sera from sensitized recipients. Binding of DSA to CBA cells was revealed using a PE-conjugated secondary antibody.

B. A mouse mAb directed against H-2^k (clone HB13) was used to determine the mathematical relation between the mean fluorescence intensity (MFI) and the amount of DSA bound to CBA target cells.

SuppFig2


A
Allogeneic
(CBA→C57BL/6 RAG2KO)
Allogeneic
(CBA→C57BL/6 RAG2KO)
Islet grafting
T lymphocytes
from sensitized
C57BL/6 Ly5.1 mice
B
Before
After
0%
17.2±3.46%
CD3
CD19
0%
0%
CD45.1
C
Anti H-2k antibody (µg/mL)
n.d.
T cell transfer
WT
D
No treatment
T lymphocyte transfer
Blood glucose (mg/dL)
Injection
(graft)
Time (days)
Graft survival (%)
**
Time (days)

Supplementary Figure 2: T cell response is sufficient to reject allogeneic islets

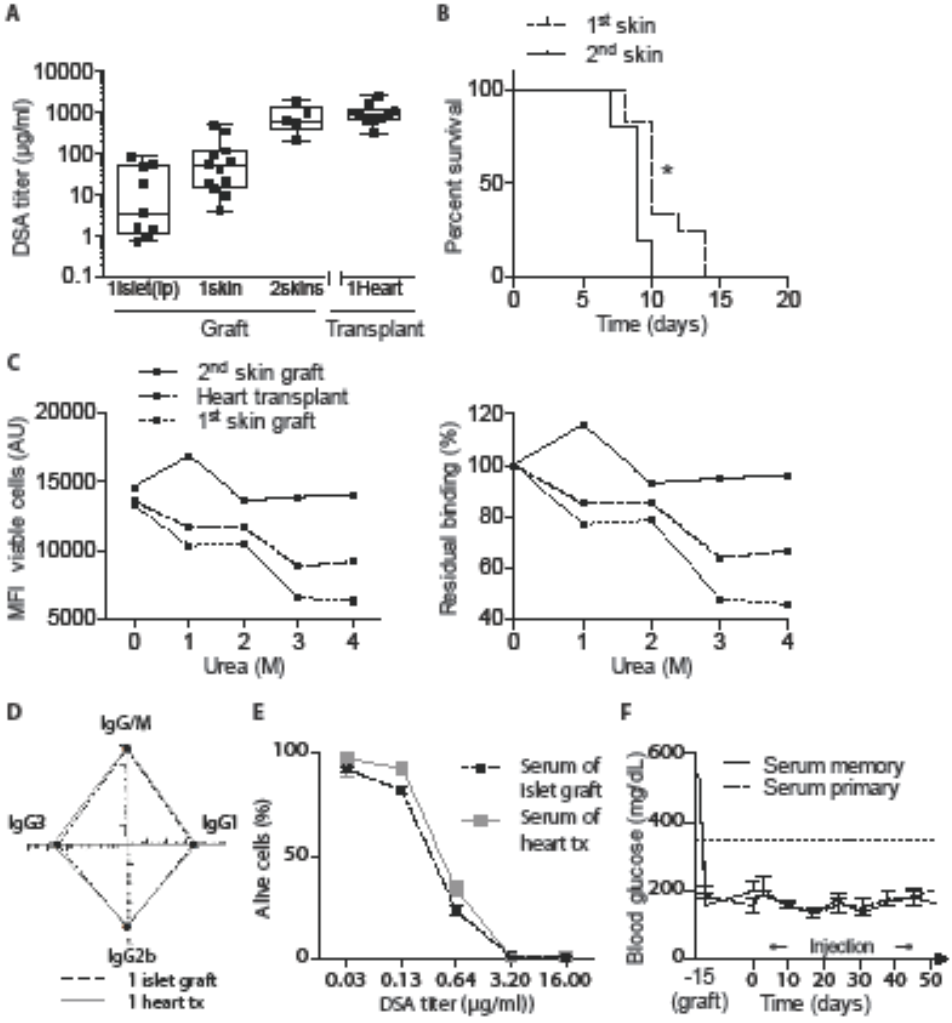
A. Five million alloreactive T lymphocytes were purified from the spleen of sensitized C57BL/6 Ly5.1 (CD45.1) mice and transferred IV into a C57BL/6 RAG2KO (CD45.2) recipient of a CBA islet graft.

B. The composition of the peripheral blood mononuclear cells (PBMC) was analyzed by flow cytometry before cell transfer and one week after islet graft rejection in transferred mice. Percentages (mean±SD) of circulating T (upper bi-plots) and B (lower bi-plots) lymphocytes are shown.

C. The titer of circulating DSA was measured using a flow cytometry cross match technique in transferred mice and wild type C57BL/6 recipients of CBA islets. n.d.: not detected.

D. ***Left:*** Evolution of glycemia (mean±SD) is shown for transferred (green; n=5) and control (black; n=4) groups. Islet graft loss was defined by fasting glycaemia > 350 mg/dL (dashed line). ***Right:*** graft survival curves for transferred (green; n=5) and control (black; n=4) groups are compared. **: p=0.0069; Log Rank test.

SuppFig3


A
DSA titer (µg/ml)
10000
1000
100
10
1
0.1
1Islet(lp)
1skin
2skins
1Heart
Graft
Transplant
B
1st skin
2nd skin
Percent survival
100
50
0
*
0
5
10
15
20
Time (days)
C
2nd skin graft
Heart transplant
1st skin graft
MFI viable cells (AU)
20000
15000
10000
5000
0
1
2
3
4
Urea (M)
Residual binding (%)
120
100
80
60
40
0
1
2
3
4
Urea (M)
D
IgG/M
IgG3
IgG1
IgG2b
1 islet graft
1 heart tx
E
Alive cells (%)
100
50
0
Serum of Islet graft
Serum of heart tx
0.03
0.13
0.64
3.20
16.00
DSA titer (µg/ml))
F
Blood glucose (mg/dL)
600
400
200
0
Serum memory
Serum primary
Injection
-15
(graft)
0
10
20
30
40
50
Time (days)

Supplementary Figure 3: Immune sera for transfer experiments

A. The flow cytometry cross match technique described in **Supplementary Figure 1** was used to quantify circulating DSA generated by wild type C57BL/6 recipients in response to various allosensitization procedures. Individual values measured at the peak of the humoral alloimmune response are shown.

B. C57BL/6 (syngeneic negative control; n=3) or CBA skin was grafted to wild type C57BL/6 recipients. Graft survival curves for first (dashed line; n=12) and second skin grafts (solid line; n=5) are compared. *: p=0.0177; Log Rank test.

C. The avidity of DSA generated by wild type C57BL/6 recipients during an alloimmune response against a first skin graft (dotted line), a first heart transplant (dashed line), or a second skin graft (solid line) were compared by assessing the stability of DSA binding to CBA splenocytes in the presence of increasing concentrations of urea. In presence of a chaotropic agent, low avidity antibodies generated during primary response dissociated from their targets in contrast with high avidity antibodies generated during memory response.

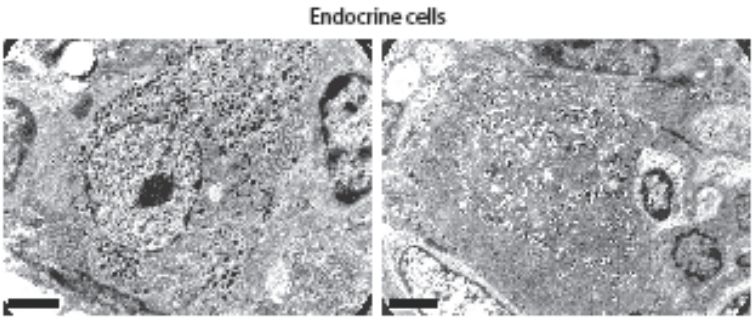
D. Heavy chain isotype repertoire of DSA generated by wild type C57BL/6 recipients in response to a CBA heart transplant (solid line) or a CBA islet graft (dashed line) were compared. DSA of different isotypes were quantified by flow cytometry as in **Supplementary Figure 1**, values are expressed in MFI (mean±SD).

E. The cytotoxic potentials of immune serum of wild type C57BL/6 recipients sensitized with either a CBA heart transplant (solid line) or a CBA islet graft (dashed line) were compared *in vitro* using complement-dependent cytotoxic assay. (mean±SD)

F. Blood glucose level was measured twice weekly in C57BL/6 RAG2KO recipients of a CBA islet graft. Evolution of glycemia (mean±SD) is shown for mice transferred with immune serum

collected during a primary (low avidity DSA, dashed line; n=3) or memory (high avidity DSA, solid line; n=3) alloimmune response.

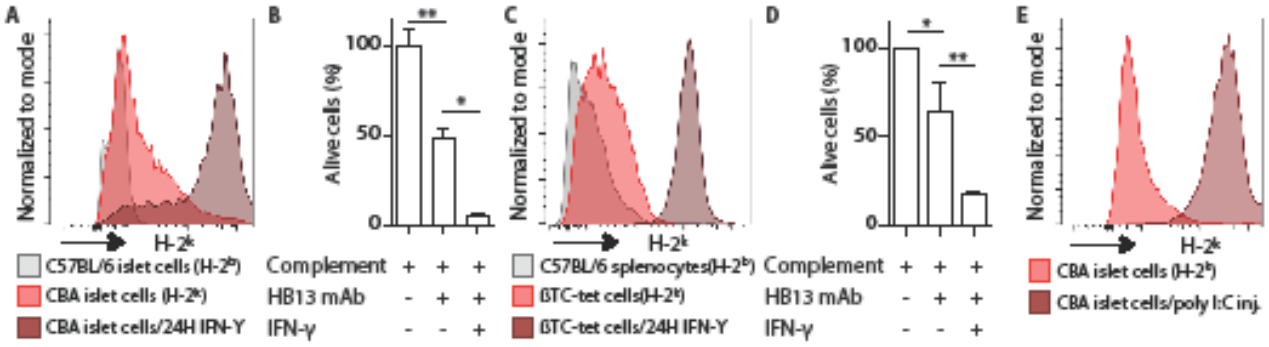
SuppFig4



Supplementary Figure 4: Impact of DSA on graft endocrine cells

Transmission electron microscopy was used to assess the ultrastructural integrity of endocrine cells of CBA islet grafts, 30 days after beginning the HB13 transfer. (scale bar=2μm).

SuppFig5


A
Normalized to mode
H-2k
C57BL/6 islet cells (H-2b)
CBA islet cells (H-2k)
CBA islet cells/24H IFN-Y
B
Alive cells (%)
100
50
0
**
*
Complement + + +
HB13 mAb - + +
IFN-γ - - +
C
Normalized to mode
H-2k
C57BL/6 splenocytes(H-2b)
βTC-tet cells(H-2k)
βTC-tet cells/24H IFN-Y
D
Alive cells (%)
100
50
0
*
**
Complement + + +
HB13 mAb - + +
IFN-γ - - +
E
Normalized to mode
H-2k
CBA islet cells (H-2k)
CBA islet cells/poly I:C inj.

Supplementary Figure 5: Boosting the expression of allogeneic targets on islet cells increased *in vitro* DSA-mediated cytotoxicity

A. The H-2^k expression level was measured by flow cytometry on cell suspension from freshly isolated C57BL/6 (H-2^b, grey, negative control) or CBA (H-2^k, red) pancreatic islets. The same experiment was conducted on cell suspension from CBA (brown) pancreatic islets after 24 hours culture in 0.5 ng/ml IFN- γ (brown). Representative histograms are shown.

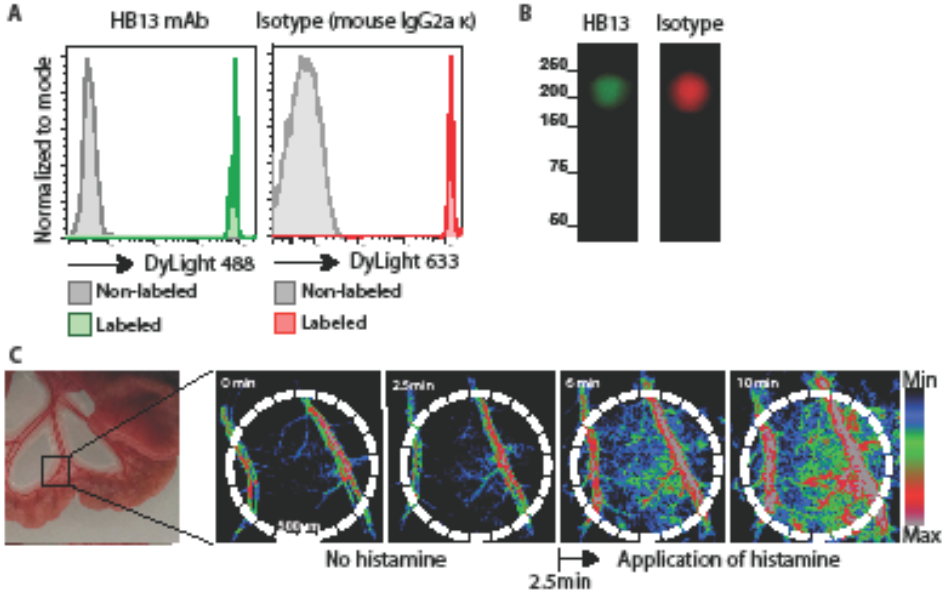
B. Complement-dependent cytotoxicity potential of HB13 was evaluated in vitro on dissociated CBA pancreatic islet cells pre-incubated or not with IFN- γ. Mean±SD. *: $p<0.05$, **: $p<0.01$; one-way ANOVA.

C. The level of H-2^k expression was measured by flow cytometry on a H-2^k restricted β cell line (βTC-tet) pre-incubated (brown) or not (red) with IFN- γ. C57BL/6 (H-2^b, grey) splenocytes were used as a negative control. Representative histograms are shown.

D. Complement-dependent cytotoxicity potential of HB13 was evaluated in vitro on βTC-tet cells pre-incubated or not with IFN- γ. Mean±SD. *: $p<0.05$, **: $p<0.01$; one-way ANOVA.

E. The level of expression of H-2k was analyzed on dissociated CBA pancreatic islet cells purified from donors injected (brown) or not (red) with polyI:C. Representative histograms are shown.

SuppFig6

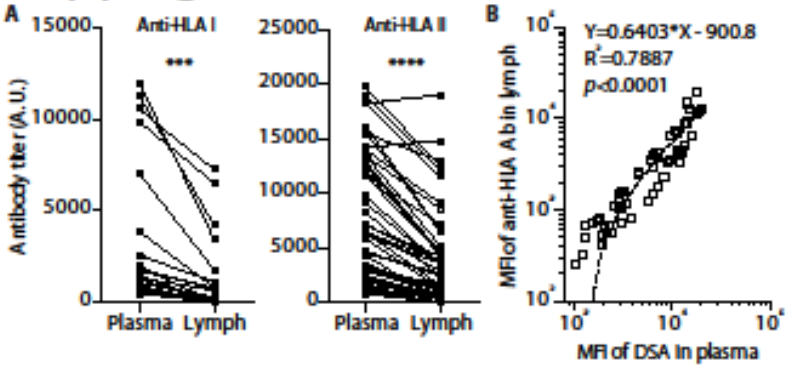

A
HB13 mAb
Isotype (mouse IgG2a κ)
Normalized to mode
DyLight 488
DyLight 633
Non-labeled
Labeled
Non-labeled
Labeled
B
HB13
Isotype
260
200
150
75
60
C
0 min
2.5min
5 min
10 min
Min
Max
No histamine
2.5min
Application of histamine

Supplementary Figure 6: Vascular sequestration of IgG in vivo

A. & B. HB13 was labeled with DyLight 488; isotype control mAb was labeled with DyLight 633. Efficiency of fluorescent conjugation for the two mAbs was evaluated by flow cytometry using an anti-mouse compensation particle set (BD Biosciences) (**A**) and SDS-PAGE followed by direct analysis of gel fluorescence (**B**).

C. Fluorescently labeled IgG was injected IV to C57BL/6 mice and time-lapse intravital microscopy was used to analyze the diffusion of antibodies outside the vasculature by monitoring DyLight 488 fluorescence in mesentery. Time 0 was set as 1 minute after the fluorescent mAb injection. Two and half minutes after the beginning of recording, a solution of histamine (100nM) was applied on the observed tissue to locally increased vascular permeability. ***Left:*** Macroscopic view of the observed area (black dashed square). ***Right:*** representative images of intravital microscopy showing vascular sequestration of HB13 before histamine application and diffusion of mAb outside the vessels afterward. White dashed circle indicates the position of histamine application.

SuppFig7


A
Anti-HLA I
***
Antibody titer (A.U.)
15000
10000
5000
0
Plasma Lymph
Anti-HLA II
****
25000
20000
15000
10000
5000
0
Plasma Lymph
B
Y=0.6403*X - 900.8
R²=0.7887
p<0.0001
MFI of anti-HLA Ab in lymph
MFI of DSA in plasma

Supplementary Figure 7: Vascular sequestration of anti-HLA antibodies in transplanted patients

A. Comparison of anti-HLA antibody content of paired plasma and lymph samples. MFI measured in solid phase assay (***left:*** anti-HLA I, ***right:*** anti-HLA II) was used as a surrogate for antibody titer for each anti-HLA specificity. ***: p=0.0003, ****: p<0.0001; paired t-test.

B. Linear regression was used to determine the relation between plasma and lymph titer for each anti-HLA specificity.

Supplementary Movie 1: Vascular sequestration of IgG in vivo

Fluorescently labeled IgG was injected IV to C57BL/6 mice and time-lapse intravital microscopy was used to analyze the diffusion of antibody outside the vasculature by monitoring DyLight 488 fluorescence in mesentery. Time 0 was set as 1 minute after the fluorescent mAb injection. Two and half minutes after the beginning of recording, a solution of histamine (100nM) was applied on the observed tissue to locally increased vascular permeability.