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MHC Class I Masking to Prevent AMR in a Porcine Kidney Transplantation Model in Alloimmunized Recipients

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Background: Presensitized patients awaiting a kidney transplant have a lower graft survival and a longer waiting time because of the limited number of potential donors and the higher risk of antibody-mediated rejection (AMR), particularly in the early posttransplant period, because of preformed donor-specific antibodies binding major histocompatibility complex (MHC) molecules expressed by the graft endothelium followed by the activation of the complement. Advances in kidney preservation techniques allow the development of ex vivo treatment of transplants. We hypothesized that masking MHC ex vivo before transplantation could help to prevent early AMR in presensitized recipients. We evaluated a strategy of MHC I masking by an antibody during ex vivo organ perfusion in a porcine model of kidney transplantation in alloimmunized recipients. **Methods:** Through the in vitro calcein-release assay and flow cytometry, we evaluated the protective effect of a monoclonal anti–swine leukocyte antigen class I antibody (clone JM1E3) against alloreactive IgG complement-dependent cytotoxicity toward donor endothelial cells. Kidneys perfused ex vivo with JM1E3 during hypothermic machine perfusion were transplanted to alloimmunized recipients. **Results:** In vitro incubation of endothelial cells with JM1E3 decreased alloreactive IgG cytotoxicity (mean complement-dependent cytotoxicity index [% of control condition] with 1 µg/mL 74.13% ± 35.26 [calcein assay] and 66.88% ± 33.46 [cytometry]), with high interindividual variability. After transplantation, acute AMR occurred in all recipients on day 1, with signs of complement activation (C5b-9 staining) as soon as 1 h after transplantation, despite effective JM1E3 binding on graft endothelium. **Conclusions:** Despite a partial protective effect of swine leukocyte antigen I masking with JM1E3 in vitro, ex vivo perfusion of the kidney with JM1E3 before transplantation was not sufficient alone at preventing or delaying AMR in highly sensitized recipients.

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Kidney transplantation is the best treatment for patients having end-stage renal disease.[1,2] Recipients with preformed anti–HLA antibodies (anti-HLA Ab) and more specifically, donor-specific antibodies (DSAs), display lower allograft survival and higher incidence of antibody-mediated rejection (AMR) than recipients without anti-HLA Ab or with non-DSA anti-HLA Ab.[3,4] Desensitization strategies have been developed to eliminate those preformed Ab, with mixed results. The most common strategy is to avoid a transplant bearing the HLAs targeted by the recipient preformed antibodies, thus increasing the recipient waiting time.[5]

The transplant endothelium constitutes the first interface between recipient blood and donor tissues. DSAs bind donor HLAs expressed on endothelial cells (ECs) and can induce endothelial damages leading to AMR.[6] Currently, there are no specific approved therapies for AMR, and most treatments aim to decrease circulating DSA and prevent DSA

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production.[7] Protecting the endothelium by making it resistant to DSA aggression could be a complementary approach.[8]

Improvement of organ preservation is currently a major issue in transplantation.[9] Hypothermic machine perfusion (HMP) consists of ex vivo organ perfusion with a preservation solution at 4 °C. The benefit of kidney HMP preservation over static cold storage (SCS) in terms of delayed graft function and allograft survival has been established, and HMP is now widely used.[10,11] New preservation techniques give us the opportunity to evaluate and treat the organ before transplantation. Ex vivo injection of reagents inside the graft presents the advantage of targeting the graft endothelium and circumvents the limitations associated with systemic drug delivery.

In the context of preformed DSA, a strategy to avoid early AMR could be to hinder DSA access to major histocompatibility complex (MHC) molecules on the graft endothelium. Masking or silencing allogeneic MHC may also decrease the activation of the alloimmune response. Few assays of this type of strategy have been performed so far. The addition of polyethylene glycol (PEG) to a preservation solution has been shown to improve organ function recovery and to reduce inflammation and fibrosis development, potentially because of the adsorption of PEG at the cell surface, allowing the "immunocamouflage" of the endothelium.[12-14] Silencing MHC class I in ECs in vitro protects them against allogeneic immune responses, abrogating T-cell responses and avoiding antibody-mediated complement-dependent cytotoxicity (CDC).[15] This strategy of MHC transcript silencing (both for class I and II) has been applied during ex vivo kidney perfusion in a rat model, successfully reducing MHC expression in the graft.[16] To our knowledge, the impact of such strategies of hiding or silencing MHC in the context of preformed DSA with a high risk of early AMR has not been described.

In this study, we hypothesized that masking MHC ex vivo before transplantation could help avoid early AMR in presensitized recipients. In a sensitized anti-MHC class I kidney allotransplantation porcine model, we evaluated the effects of anti–swine leukocyte antigen (SLA) class I monoclonal Ab (mAb) to prevent early AMR.

MATERIALS AND METHODS

Animals Alloimmunization

In vivo experiments were performed on nonsibling pigs (*Sus scrofa*) under general anesthesia. The study was reviewed by our regional ethical committee and authorized by the French Ministry of Research (APAFiS#6035).

Donor/recipient pairs were selected according to identical blood group and MHC mismatches assessed by mixed lymphocyte culture. Alloimmunization of recipients was performed by 3 intradermal challenges of donor peripheral blood mononuclear cells (PBMCs).

Cell Culture

Primary porcine aortic ECs (PAEC) were isolated from donor aortas after kidney procurement and cultured in Petri dishes coated with 1% porcine gelatin (Sigma-Aldrich, Saint-Quentin Fallavier, France) in DMEM (Thermo Fisher Scientific, Waltham, MS) supplemented with 10% fetal calf serum (Eurobio, Courtaboeuf, France), penicillin/streptomycin (Thermo Fisher Scientific) and glutamine (Sigma-Aldrich).[17]

An anti-SLA class I mAb (clone JM1E3, mouse IgG1, BIO-RAD, Hercule, CA) was used for in vitro and ex vivo experiments.

Alloimmunization Monitoring

The alloreactivity of recipients sera was assessed by flow cytometry crossmatch. Sera were incubated with donor PBMCs or PAECs, autologous recipient and third-party cells, then with fluorescein isothiocyanate (FITC)-goat anti-pig IgG Ab (BIO-RAD). Some recipients sera were absorbed on donor platelets before crossmatch. The alloreactivity of each serum was calculated in arbitrary units: % FITC-positive cells × Geo.Mean FITC intensity for live cells.

Recipient serum comCDC was assessed by flow cytometry cytotoxicity assay (FCCA) on donor PBMCs and PAECs. Cells were incubated with heat-inactivated sera, rabbit complement (Sigma-Aldrich), and finally propidium iodide (PI, Sigma-Aldrich). The percentage of specific CDC for each serum sample was calculated as follows:

[% PI-positive cells (serum + complement) − % PI-positive cells (serum)]/[100 − % PI-positive cells (serum)]

IgGs of recipients were purified from alloreactive sera (plateau of immunization) on protein A columns and concentrated to approximately 20 mg/mL. Binding of recipient IgG and mAb JM1E3 to donor PAECs was assessed by flow cytometry. After incubation with IgG of recipients or JM1E3 at various dilutions for 1 h, PAECs were incubated with FITC-goat anti-pig IgG Ab (BIO-RAD) or FITC-goat anti-mouse IgG H + L antibody (Jackson ImmunoResearch), respectively.

Cells were analyzed using a FACSCanto II or a FACSCelesta flow cytometer with DIVA (Becton Dickinson) and FlowJo software programs (Tree Star, Ashland, OR).

Calcein Cell Viability Assays

For CDC assessment, PAECs were seeded onto a 96-well flat-bottom plate and incubated with calcein solution (Thermo Fisher Scientific) for 30 min at 37 °C, then with JM1E3 or recipients IgG at various concentrations for 1 h, and finally with rabbit complement for 30 min. Cells incubated with JM1E3 or IgG without complement were considered negative controls. Cells incubated with Triton 2% were considered positive controls. The supernatant was transferred into a flat-bottom black polystyrene plate (Corning, NY), and the fluorescence intensity reflecting calcein release was measured with a Spark reader and Magellan software (excitation wavelength 470 nm, bandpass 509 nm). The percentage of CDC was calculated as follows: (test − negative control)/(positive control − negative control).

To assess the protective effect of JM1E3 against alloreactive IgG CDC (**Figure S1A, SDC**, http://links.lww.com/TXD/A532), PAECs were incubated with various concentrations of JM1E3 before calcein staining, followed by incubation with recipient IgG at a predetermined cytotoxic concentration and finally with rabbit complement. The percentage of CDC was calculated as mentioned above. To compare all individuals, CDC was normalized to the CDC of alloreactive IgG without JM1E3 preincubation for each recipient, considered to represent the maximal CDC of alloreactive IgG (CDC index).

Resistance of PAECs to CDC Assessed by Flow Cytometry

PAECs were seeded in 6-well plates and preincubated with various concentrations of JM1E3 (**Figure S1B, SDC**, http://

links.lww.com/TXD/A532). Cells were then incubated with alloreactive IgG at a predetermined cytotoxic concentration. FCCA was performed on PAECs and percentage of specific CDC was calculated as described here. CDC index was calculated as described with a calcein assay.

Some cells were collected after incubation with JM1E3 and alloreactive IgG were stained with anti-mouse IgG Ab or anti-porcine IgG Ab and then secondary Ab to assess JM1E3 and alloreactive IgG binding by flow cytometry. To compare all individuals, binding indexes were calculated, normalizing JM1E3 binding to saturating concentration (10 μg/mL) and alloreactive IgG binding to the condition without JM1E3 preincubation for each individual, respectively.

Kidney Ex Vivo Perfusion and Transplantation

For 2 donors (A and B), 1 kidney was preserved in SCS for 2 h and 1 in HMP on a Waves kidney perfusion system (Institut Georges Lopez, France) at a perfusion pressure of 25 mm Hg for 2 h (control groups SCS and HMP; Figure 1) in PERF-GEN solution (Institut Georges Lopez). Recipient native kidneys were used to test duration of perfusion and dose of JM1E3. A perfusion of JM1E3 of 3 h was set (time to significant decrease of JM1E3 concentration in perfusion solution followed by a plateau [data not shown]). A concentration of 1 μg/mL was set as it was associated with a saturation of MHC class I molecules at endothelial cell surface in vitro and when applied ex vivo during perfusion with a decreased concentration over the course of perfusion until a plateau, and regular staining at endothelium surface on kidney biopsies (data not shown). For the 2 next donors (C and D, group α-SLA I), 1 kidney was preserved for 3 h in HMP with JM1E3 at 1 μg/mL added to the perfusion solution. The second kidney was first preserved for 3 h in SCS and then perfused for 3 h in HMP with JM1E3 at 1 μg/mL. According to the guidelines of our ethical committee in animal experimental research, we proceeded this way to limit the number of animals included in the study. All animals receiving a kidney perfused with anti-SLA I Ab JM1E3 were included in the group α-SLA I.

Renal allotransplantation was performed on binephrectomized recipients as previously described.[18] Each recipient received a kidney from their donor at the plateau of immunization. One hour after transplant reperfusion, the transplant was macroscopically inspected, and biopsies were performed. In case of anuria at day 1 posttransplantation, the transplant was inspected macroscopically, and in the case of obvious rejection, a transplantectomy was performed before euthanasia.

Detection of JM1E3 mAb in the perfusion solution (α-SLA I group) was performed using an enzyme-linked immunosorbent assay. A 96-well Nunc MaxiSorp flat-bottom plate (Thermo Fisher Scientific) was coated with an anti-mouse

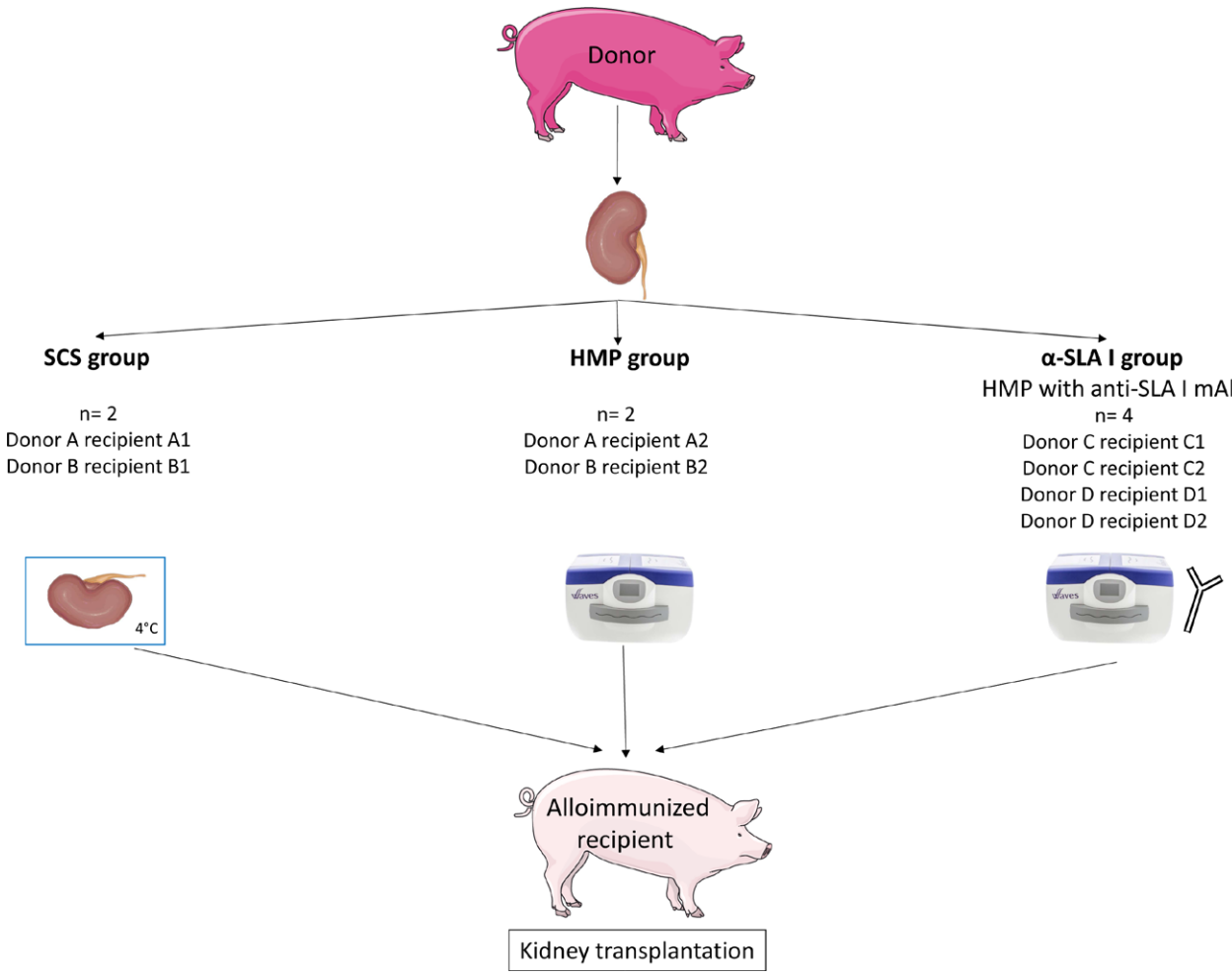


FIGURE 1. Ex vivo preservation and transplantation groups. HMP, hypothermic machine perfusion; mAb, monoclonal antibody; SCS, static cold storage; SLA, swine leukocyte antigen.

IgGL (Jackson ImmunoResearch, Philadelphia, PA) and saturated with PBS-bovine serum albumin 5% (BSA, Sigma-Aldrich). Perfusion solution samples were incubated for 2 h at 37 °C. Detection was performed with a peroxidase-coupled goat anti-mouse Fc antibody (Jackson ImmunoResearch) revealed with TMB substrate reagent (Becton Dickinson, San Diego, CA). Optical density was measured with a Spark reader and Magellan software (Tecan Trading AG, Switzerland, excitation wavelength 450 nm, bandpass 630 nm).

Histological Analyses

Biopsy samples fixed in 10% buffered formalin were paraffin-embedded, sectioned at 4 µm, stained with Masson's trichrome, periodic acid–Schiff, and hematoxylin–eosin–saffron, and examined by a pathologist that assessed Banff score.[19]

Immunofluorescence staining was performed on 8 µm sections of frozen tissue fixed in acetone. JM1E3 binding to the transplant was assessed by incubating the sections with an Alexa Fluor 568-goat anti-mouse IgG (H+L) antibody (Invitrogen, Carlsbad, CA) for 1 h at room temperature (RT). C5b-9 staining was performed with mouse IgG2 anti-human C5b-9 Ab (clone aE11, DIATEC, Norway) overnight at 4 °C followed by an Alexa Fluor 488–anti-mouse IgG2 Ab (Invitrogen) incubated for 1 h at room temperature (RT). For CD3 and CD68 staining, sections were incubated with Fab goat anti-mouse IgG (H+L) (Jackson ImmunoResearch) 2 h at RT, then incubated overnight at 4 °C with FITC-mouse anti-porcine CD3ε (clone PPT3, Southern Biotech, Birmingham, AL) or with mouse anti-rat CD68 (clone ED1, BIO-RAD) and finally with FITC-donkey anti-mouse IgG (H+L; Jackson ImmunoResearch). Sections were analyzed using a Nikon microscope and ACT-1 software (Nikon, Tokyo, Japan).

Statistical Analyses

Statistical analyses were performed using Prism software (GraphPad Software, San Diego, CA). Normalized CDC percentages and normalized recipient IgG binding on donor PAECs after preincubation with JM1E3 were all compared with the values under the condition without JM1E3

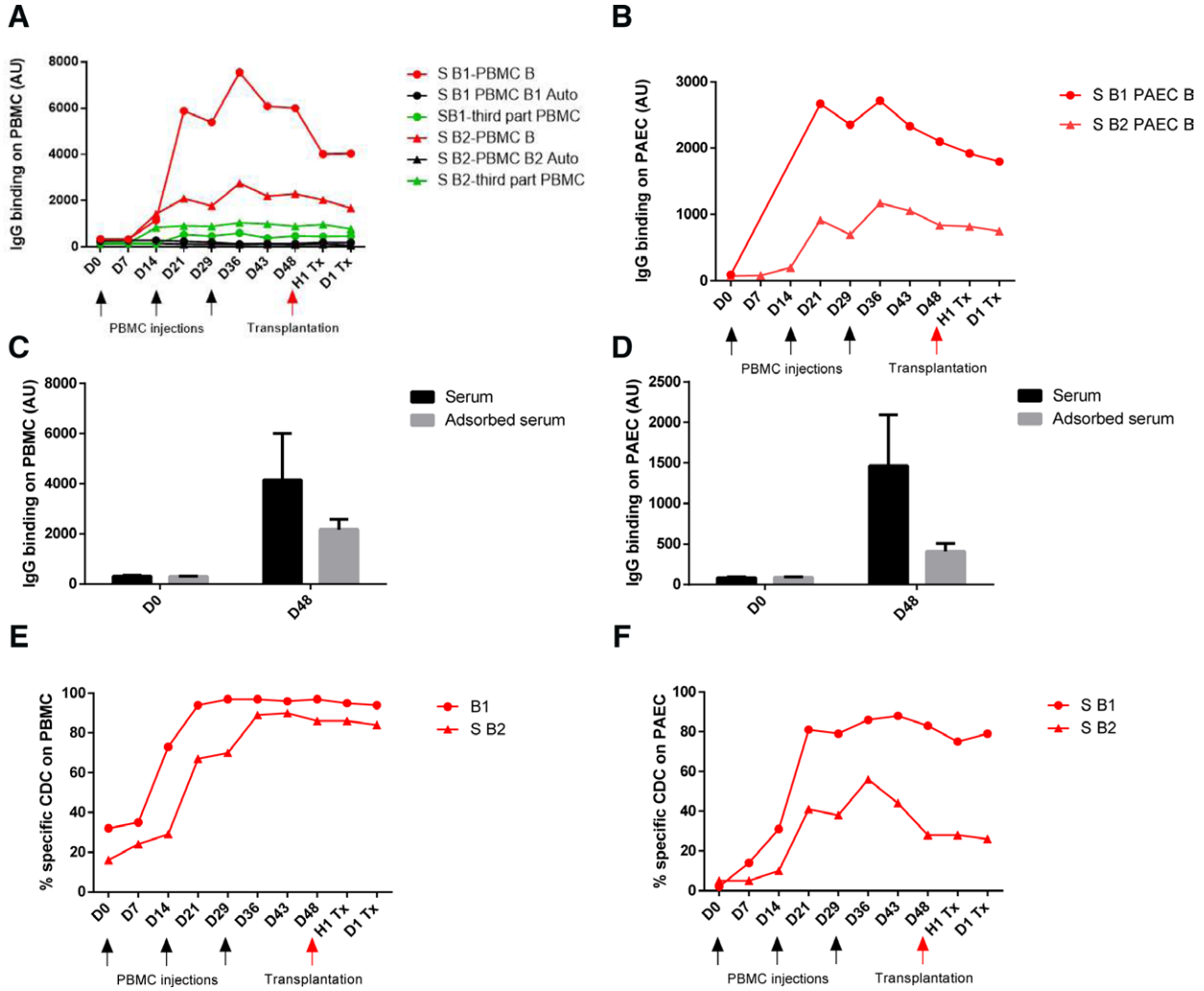


FIGURE 2. Representative recipient immunization against donors confirmed by flow cytometry. A, IgG CM of recipient sera with donor PBMCs (red lines), autologous PBMCs (black lines), and third-party PBMCs (green lines). B, IgG CM of recipient sera with donor PAECs. C and D, IgG CM with donor PBMCs (C) and donor PAECs (D) of recipient untreated sera (black) and recipient sera adsorbed on donor platelets (gray; (mean AU+SEM for 2 recipients [A1 and A2] with the same donor). E and F, CDC of recipient sera to donor PBMC (E) and donor PAECs (F). AU, arbitrary unit; CDC, complement-dependent cytotoxicity; CM, crossmatch; PAEC, porcine aortic endothelial cells; PBMC, peripheral blood mononuclear cell; SEM, standard error of the mean.

preincubation using the Wilcoxon matched-pairs signed rank test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Immunization of Recipient Pigs

The donor/recipient pairs are described in Figure 1. Antidonor IgGs were detected in immunized recipient sera from day 14 with a plateau from day 21 (Figures 2A and B displaying a representative combination, other combinations displayed in **Figures S2–S4** (**SDC**, http://links.lww.com/TXD/A532). Sera bound to third-part PBMCs to a much lesser extent. Sera adsorbed on donor platelets displayed much less IgG binding on donor PBMCs and PAECs (Figure 2C and D) than nonadsorbed sera, suggesting that allo-Ab were mainly directed against donor SLA class I antigens. Recipient serum samples exhibited a strong CDC toward donor PBMCs (Figure 2E) and PAECs (Figure 2F). Despite interindividual variability in IgG binding between donor/recipient pairs, anti-donor immunization was effective and specific in all recipients, with a specific CDC on PBMCs >60 % in all combinations.

Alloreactivity of JM1E3 and Alloreactive IgG

Anti-SLA class I JM1E3 mAb bound to all donor PAECs with a constant plateau of binding >1 µg/mL (Figure 3A). JM1E3 plus complement did not lead to CDC, as expected as mouse IgG1 did not bind complement (data not shown). Binding of purified recipient IgG to donor PAECs at various

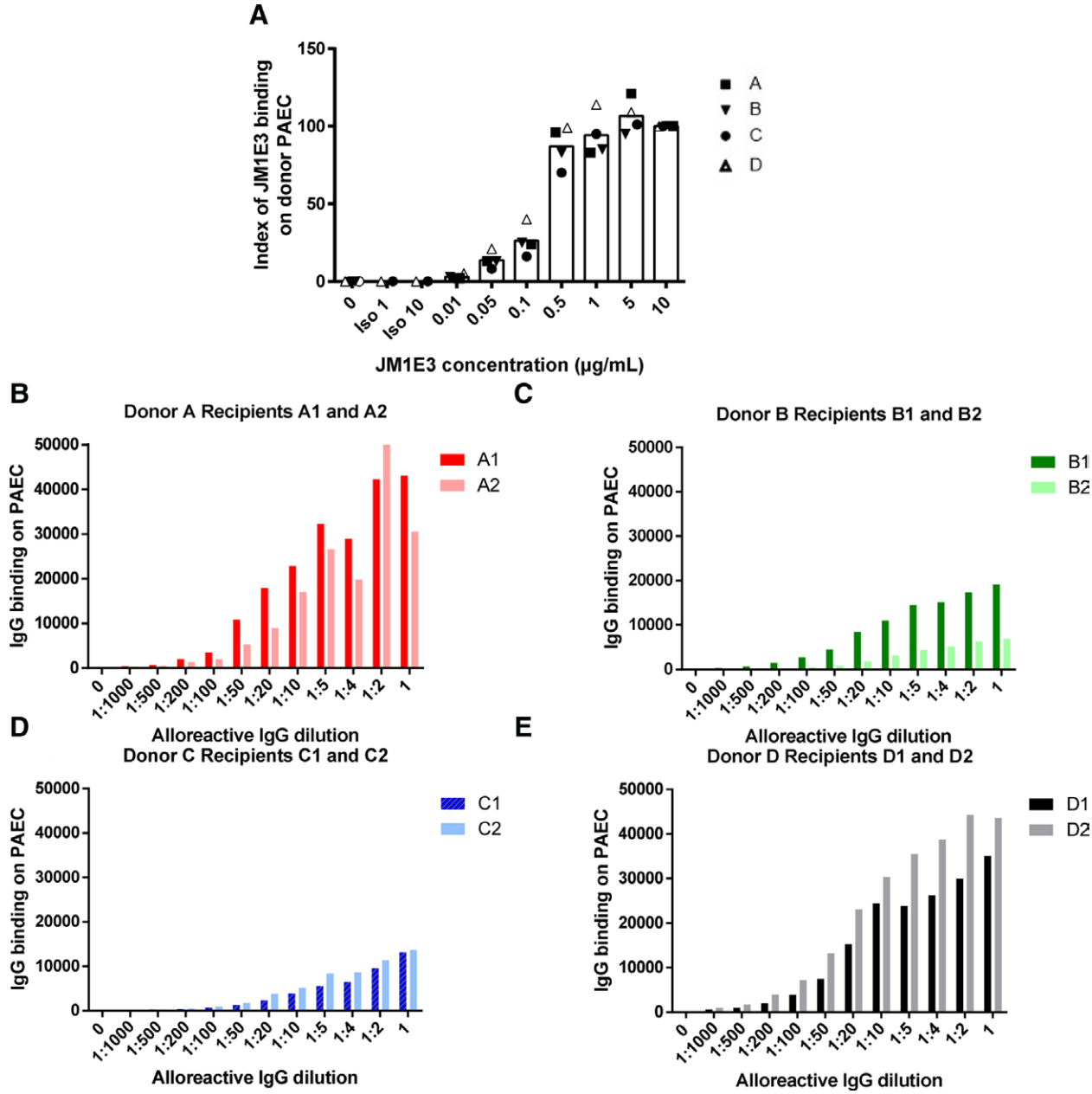
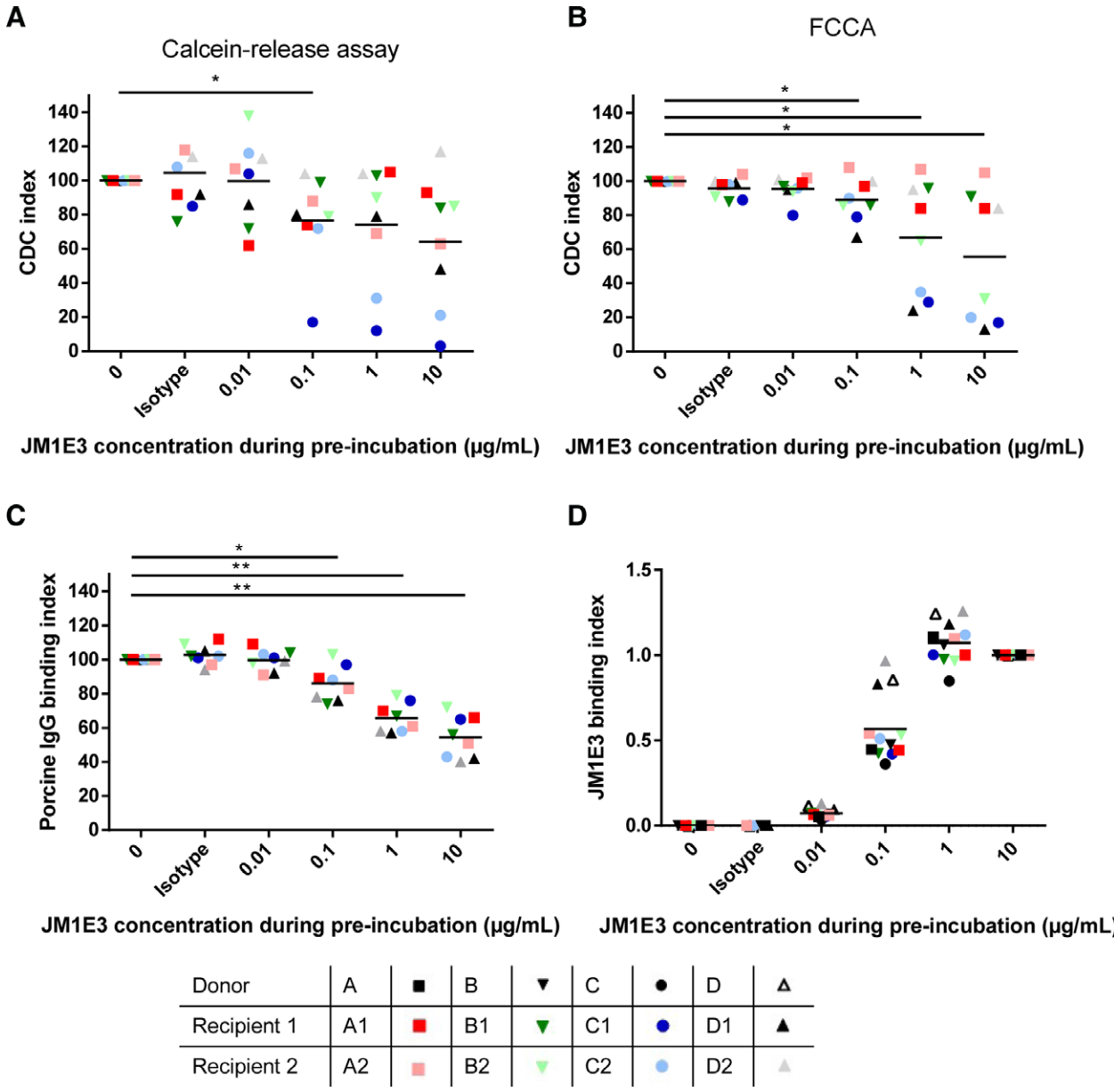


FIGURE 3. Binding of monoclonal anti-SLA I Ab (JM1E3) and recipient IgG on donor PAECs assessed by flow cytometry. A, Binding of JM1E3 on donor PAECs (geometric mean of fluorescence intensity, normalized to JM1E3 10 µg/mL, mean + SEM, n = 4). B–E, Binding of recipient purified IgG to donor PAECs for each combination of donor and recipients (geometric mean of fluorescence intensity). Ab, antibody; PAEC, porcine aortic endothelial cells; SEM, standard error of the mean; SLA, swine leukocyte antigen.



Donor	A	■	B	▼	C	●	D	△
Recipient 1	A1	■	B1	▼	C1	●	D1	▲
Recipient 2	A2	■	B2	▼	C2	●	D2	△

FIGURE 4. PAEC resistance to the CDC of alloreactive IgG (isolated at the plateau of immunization) after incubation with monoclonal anti-SLA I Ab (JM1E3). A and B, Index of specific CDC of alloreactive IgG on donor PAECs after 3 h incubation with JM1E3 measured by the calcein-release assay (A, mean of triplicate for each individual) and by FCCA (B). C, Index of porcine IgG binding on donor PAECs after JM1E3 incubation followed by incubation with alloreactive IgG. D, Index of JM1E3 binding on donor PAECs after JM1E3 incubation followed by incubation with alloreactive IgG. *$P<0.05$, **$P<0.01$ (Wilcoxon matched-pairs signed-rank test). Specific CDC and porcine IgG binding were normalized to the conditions without JM1E3 preincubation (considered to represent the maximal CDC of alloreactive IgG). The results for JM1E3 binding were normalized to the condition with the higher dose of JM1E3 (10 µg/mL) for each donor/recipient pair. Specific CDC by FCCA (B), porcine IgG binding (C), and JM1E3 binding (D) were assessed in the same experiment for each donor. Ab, antibody; CDC, complement-dependent cytotoxicity; FCCA, flow cytometry cytotoxicity assay; PAEC, porcine aortic endothelial cells; SLA, swine leukocyte antigen.

dilutions was variable between recipients (Figure 3B–E). CDC of recipient IgG toward donor PAECs was assessed, and the dilution exhibiting the highest CDC for each of them was selected for further tests (data not shown).

In Vitro Pretreatment With JM1E3

After PAEC preincubation with JM1E3, alloreactive IgG CDC was assessed by calcein-release assay (Figure 4A) and FCCA (Figure 4B). With the calcein assay, CDC of alloreactive IgG to donor PAECs was decreased after preincubation with saturating doses of JM1E3 (mean CDC index after 0.1, 1, and 10 µg/mL JM1E3 76.63% ± 26.67 [$P=0.023$ versus no JM1E3], 74.13% ± 35.26 [ns], and 64.25% ± 38.39 [ns], respectively). With FCCA, CDC was significantly lower after PAEC preincubation with a saturating dose of JM1E3 compared with no preincubation (mean CDC index 89.13% ± 12.81 [($P=0.047$ versus no JM1E3], 66.88% ± 33.46 ($P=0.039$), and 55.63% ± 38.70 [$P=0.016$] for 0.1, 1, and 10 µg/mL JM1E3 preincubation, respectively), suggesting an in vitro protective effect of JM1E3 preincubation against recipient IgG CDC, despite important interindividual variations observed.

There was a significant decrease in recipient IgG binding on PAECs after preincubation with 0.1, 1, and 10 µg/mL JM1E3 (IgG binding index 86% ± 10%, 66% ± 9%, and 54% ± 12%, respectively, $P = 0.0234$, $P = 0.0078$, and $P = 0.0078$; Figure 4C), suggesting that PAEC preincubation with JM1E3 impaired alloreactive IgG binding. JM1E3 binding on donor PAECs was not impaired by further incubation with recipient IgG (Figure 4D). Similarly, MHC class I expression was not altered by incubation of PAECs with JM1E3 and recipient IgG (data not shown). The combination in which we observed the strongest protection against DSA CDC is donor C/recipients C1 and C2, the combination displaying lower IgG binding both in crossmatches (**Figure S3, SDC**, http://links.lww.com/TXD/A532) and when assessing recipient IgG binding on PAECs (Figure 3D). Altogether, PAEC incubation with saturating doses of JM1E3 partially prevented binding of alloreactive IgG, resulting in a decreased CDC of alloreactive IgG in some individuals.

Transplantations Following Ex Vivo JM1E3 Perfusion

JM1E3 detection in the perfusion solution by enzyme-linked immunosorbent assay showed a decrease in JM1E3 concentration over time, from a mean of 0.972 ± 0.064 µg/mL at T0 to 0.303 ± 0.0263 at 3 h (Figure 5A). Immunofluorescence staining of kidney biopsies showed positive staining for JM1E3 in peritubular capillaries and glomeruli at the end of perfusion (Figure 5B–D). Colocalization of JM1E3 and CD31 staining showed JM1E3 binding in

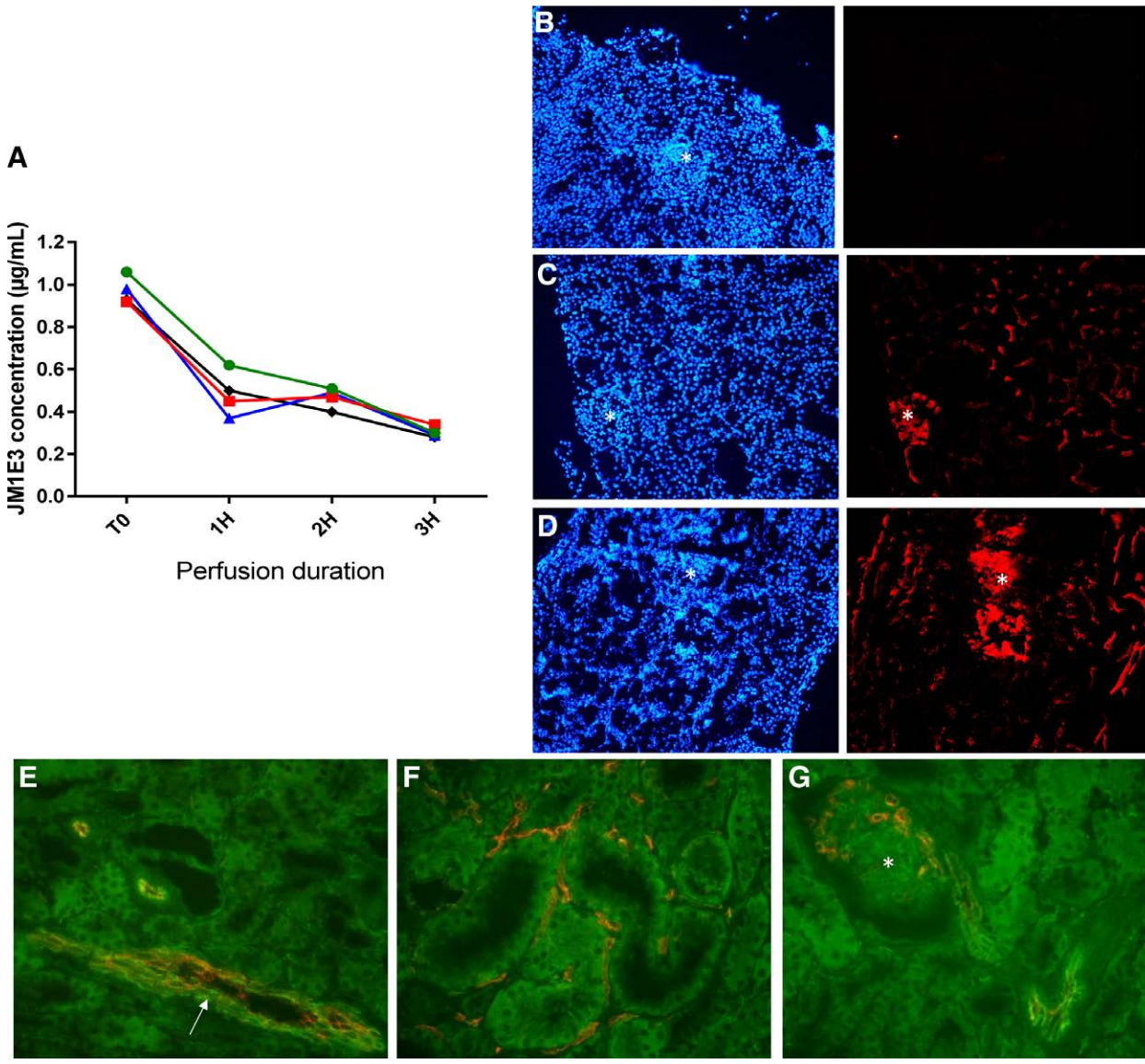


FIGURE 5. Detection of monoclonal anti-SLA I Ab (JM1E3) in perfusion solution and on kidney biopsies during ex vivo HMP of the kidney. A, JM1E3 measurement in perfusion solution (ELISA). B–D, Immunofluorescence staining on kidney core-needle biopsies during perfusion, DAPI staining (left panels) and JM1E3 detection (right panel) with anti-mouse IgG Ab on a nonperfused control (B), after 3 h HMP with JM1E3 (C, donor C, for recipient C1) and after 3 h SCS and 3 h HMP with JM1E3 (D, donor C, for recipient C2) (×200). E–G, Costaining for JM1E3 (red) and CD31 (green) after 1 h of HMP with JM1E3 (recipient B2 native kidney used to test JM1E3 perfusion, ×400). Stars identify glomeruli and arrow artery sections. Ab, antibody; CD, cluster of differentiation; DAPI, 4′,6-diamidino-2-phenylindole; ELISA, enzyme-linked immunosorbent assay; HMP, hypothermic machine perfusion; IgG, immunoglobulin G; SCS, static cold storage; SLA, swine leukocyte antigen.

TABLE 1.

Outcomes and histology of kidney transplantations in alloimmunized pigs

Group	Donor ID	Recipient ID	Macroscopic aspect at H+1	Histology H+1	Banff score H+1	C5b-9 staining at H+1	Time of transplant failure	Macroscopic aspect at failure	Histology at transplant failure	Banff score at transplant failure	C5b-9 staining at failure
SCS	A	A1	Patchy	Mild ATN, congestion	g0 i0 t0 ptc2 focal v0	+	Day 1 (anuria)	Infarcted, no thrombosis	Hemorrhagic infarction	NA	+++
SCS	B	B1	Patchy	ATN, congestion	g0 i0 t0 ptc0 v0	+++	Day 1 (anuria)	Infarcted, no thrombosis	Hemorrhagic infarction	v3	+++
HMP	A	A2	Patchy	Mild ATN, congestion	g2 i0 t0 ptc1 v2	+++	H+5 (death)	Patchy	Mild ATN congestion	G3 i0 t0 ptc2 focal v1	+++
HMP	B	B2	Patchy	Mild ATN, focal congestion	g0 i0 t0 ptc0 v0	+++	Day 1 (anuria)	Infarcted, arterial thrombosis	Hemorrhagic infarction	v0	+++
α-SLA I	C	C1	Normal	ATN, glomerular ischemia	g3 i0 t0 ptc3 v0	+++	H+6 (death)	Patchy	ATN, congestion	g3 i0 t0 ptc1 v0	++
α-SLA I	C	C2	Normal	ATN	g2 i0 t0 ptc2 v0	++	Day 1 (anuria)	Infarcted, no thrombosis	Hemorrhagic infarction	NA	++
α-SLA I	D	D1	Patchy	ATN, congestion	g0 i0 t0 ptc0 v0	++	Day 1 (anuria)	Infarcted, arterial thrombosis	Hemorrhagic infarction	NA	++
α-SLA I	D	D2	Patchy	ATN	g0 i0 t0 ptc0 v0	++	Day 1 (anuria)	Infarcted, arterial thrombosis	Hemorrhagic infarction	NA	+++

ATN, acute tubular necrosis; g, glomerulitis; HMP, hypothermic machine perfusion; i, interstitial infiltrate; NA, not available; ptc, peritubular capillaritis; SCS, static cold storage; SLA, swine leukocyte antigen; t, tubulitis; v, vasculitis.

glomeruli, peritubular capillaries, and arteries, consistent with binding to the graft endothelium (Figure 5E–G). Histological examination of biopsy samples at the end of preservation was normal for all individuals.

After transplantation, in both control groups, SCS and HMP, kidneys showed a patchy aspect 1 h after reperfusion, with preserved arterial and venous permeability (Table 1). Histological examination of biopsy samples at 1 h posttransplant showed features of mild acute tubular necrosis and congestion (Figure 6). Banff score was normal for 2 recipients at 1 h (B1 and B2, Table 1), and features of AMR were present for 2 recipients (A1 and A2). C5b-9 immunofluorescence was positive in peritubular capillaries in all kidney biopsies at 1 h posttransplant (Figure 7A–F). One recipient (A2) died 5 h after transplantation from technical failure. Three out of these 4 control recipients were anuric at day 1 posttransplant, with a macroscopic aspect of infarction for all 3 and arterial thrombosis for 1 (B1). Histology showed hemorrhagic infarction in all 3 kidneys.

In the experimental group with anti-SLA I Ab perfusion, 2 kidneys had a normal aspect, whereas 2 kidneys had a patchy aspect 1 h after reperfusion (Table 1). Histology at 1 h posttransplant showed acute tubular necrosis in all kidneys, with microvascular inflammation in 2 recipients (C1 and C2). In 2 individuals (D1 and D2), no histological features of AMR were observed at 1 h. In all recipients, C5b-9 staining in peritubular capillaries was positive at 1 h, suggesting that some DSA bound to the graft (Figure 7G–L). One recipient (C1) died 6 h after transplantation from technical failure. The 3 other recipients were anuric at day 1, with infarcted macroscopic aspect and arterial thrombosis in 2 recipients. Histology at transplant failure showed hemorrhagic infarction. CD3 staining was negative in all kidneys. CD68 staining was slightly positive focally in biopsy samples before transplantation, without changes after transplantation. JM1E3 staining in peritubular capillaries and glomeruli, already described as positive at the end of perfusion, was also positive at 1 h posttransplant and at day 1 (Figure 5).

DISCUSSION

We describe a strategy of MHC class I masking during ex vivo perfusion in a porcine model of kidney transplantation in alloimmunized recipients. Despite a strong binding of anti-SLA class I mAb to kidney endothelium during ex vivo perfusion, this was not sufficient to prevent acute AMR. DSAs of recipients were directed against MHC class I because the crossmatch was negative after serum adsorption on platelets.

In vitro incubation of PAECs with anti-SLA class I mAb partially protected donor cells against alloimmunized recipients IgG CDC. This protection was variable from one individual to another. In all individuals, PAEC preincubation with JM1E3 at saturating concentrations significantly decreased the binding of alloimmunized recipient IgG. Some individuals displayed diminished cytotoxicity of recipient IgG after JM1E3 preincubation, whereas in others, no protection was exhibited. The individuals in which the best protection against DSA CDC was observed were the ones with the weaker binding of DSA (both in crossmatch with sera and assessment of IgG binding to PAECs). This protection of PAECs against DSA CDC could partly rely on the steric hindrance of SLA I. JM1E3 binds to a monomorphic epitope of SLA class I, probably leaving some polymorphic epitopes of MHC accessible to DSA. The interindividual variations may be explained by differences in DSA level and affinity from one individual to another.

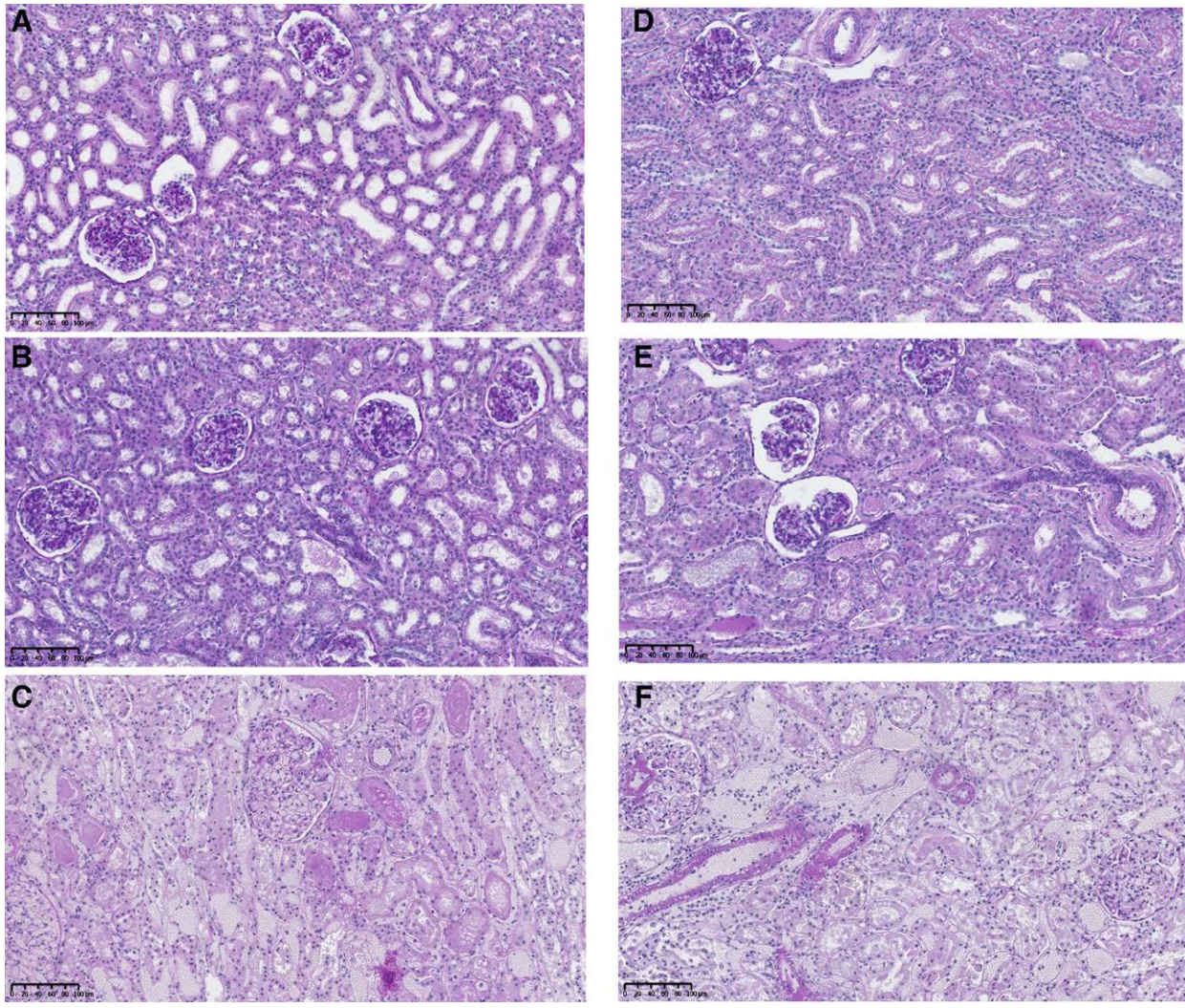


FIGURE 6. Histological examination of kidneys. Periodic acid-Schiff at ×20 magnification. A–C, Transplantation without JM1E3 perfusion (static cold storage 2 h): biopsy sample pretransplant (A), 1 h after transplantation (B) and day 1 posttransplant (C). D–F Transplantation after JM1E3 perfusion (static cold storage for 3 h followed by hypothermic machine perfusion with JM1E3 perfusion for 3 h): biopsy sample pretransplant (D), 1 h after transplantation (E) and day 1 posttransplant (F).

We showed the feasibility of mAb perfusion during HMP. However, the binding of the anti-SLA I mAb on the transplant endothelium was not sufficient to prevent acute AMR at day 1, with already features of AMR from 1 h postreperfusion, without any sign of cellular rejection. Thus, a partial effect in vitro turned out to be insufficient in vivo. Our experimental model of AMR is very stringent because recipient immunization is very strong (stronger than clinical situations) and grafts displayed signs of AMR as early as 1 h after reperfusion in half of them and at day 1 in all of them, potentially explaining the absence of in vivo protection. Organ ischemia–reperfusion, leading to kidney endothelium activation, may also induce upregulation of SLA class I expression in the graft endothelium.[20] In that case, only SLAs that were expressed at steady state would have been masked by JM1E3 during ex vivo perfusion, allowing free access of circulating DSA to newly expressed SLA I molecules in vivo once grafted. In contrast, in in vitro tests, JM1E3 and recipient IgGs were both incubated with resting PAECs expressing constitutive levels of SLA I molecules. Moreover, our in vitro experiments were performed on aortic macrovascular ECs, which exhibit phenotypical and physiological differences from microvascular cells that are targeted in vivo by DSAs.[21]

However, masking MHC at the endothelium surface of the transplant remains a strategy that could be beneficial to avoid AMR as a complementary tool to transplant in sensitized patients across the MHC barrier. It could be tested in a less stringent model associated with complement inhibition that has been shown in a similar model to prevent early acute AMR in sensitized nonhuman primates.[22] It could be part of a strategy to induce accommodation of the transplant.[8] Finally, the activation of the alloimmune response in the recipient is influenced by endothelial activation following ischemia-reperfusion injury, and masking MHC during organ preservation might help limit the antigen presentation by ECs.

Altogether, our study shows that MHC class I masking with a mAb partially reduces DSA CDC but is not, by itself, sufficient to prevent acute AMR in a model of kidney transplantation to hyperimmunized recipients, although it could be added to strategies to transplant them, given an appropriate setting. However, this study is, a proof of concept of successful mAb perfusion during HMP that could be optimized by

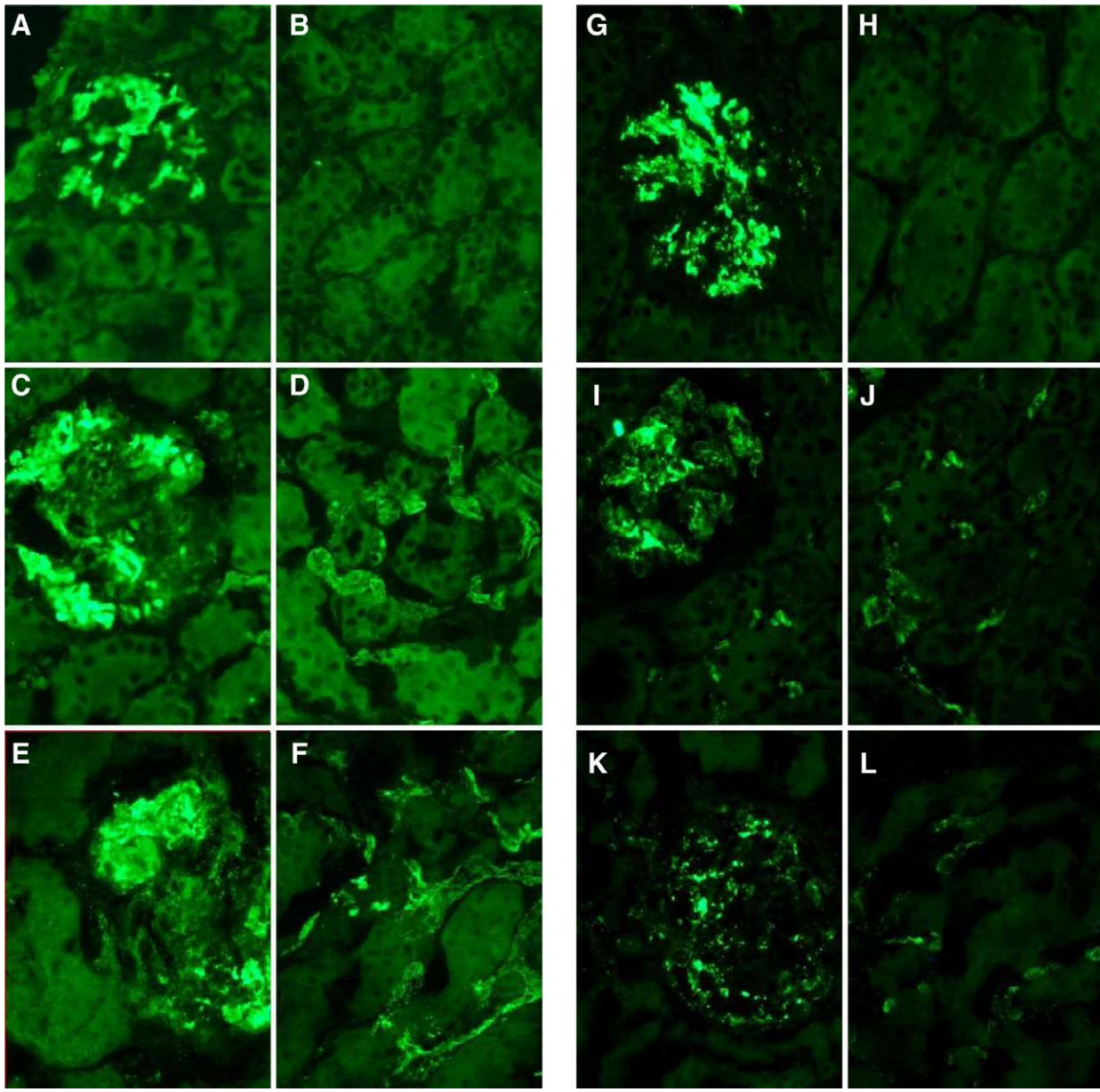


FIGURE 7. Ex vivo perfusion of monoclonal anti-SLA I Ab does not prevent complement cascade activation after transplantation in an alloimmunized recipient. C5b9 staining on a kidney biopsy in a pair without JM1E3 monoclonal anti-SLA I Ab perfusion: before transplantation (A, B), 1 h after transplantation (C, D), and 1 d after transplantation (E, F). C5b9 staining on a kidney biopsy in a pair with ex vivo JM1E3 perfusion: before transplantation (G, H), 1 h after transplantation (I, J), and 1 d after transplantation (K, L). Ab, antibody; SLA, swine leukocyte antigen.

combining several approaches of masking, such as a cocktail of Ab binding different epitopes and PEG.

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