



HAL
open science

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

Delphine Kervella, Julien Branchereau, Thomas Prudhomme, Véronique Nerrière-Daguin, Karine Renaudin, David Minault, Jérémy Hervouet, Bernard Martinet, Sarah Bruneau, Stéphanie Le Bas-Bernardet, et al.

► **To cite this version:**

Delphine Kervella, Julien Branchereau, Thomas Prudhomme, Véronique Nerrière-Daguin, Karine Renaudin, et al.. MHC Class I Masking to Prevent AMR in a Porcine Kidney Transplantation Model in Alloimmunized Recipients. *Transplantation Direct*, 2023, 9 (6), pp.e1490. 10.1097/TXD.0000000000001490 . inserm-04200411

HAL Id: inserm-04200411

<https://inserm.hal.science/inserm-04200411v1>

Submitted on 8 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

OPEN

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

Delphine Kervella, MD, PhD,^{1,4} Julien Branchereau, MD, PhD,^{1,2} Thomas Prudhomme, MD,¹ Véronique Nèrrière-Daguin, BSc,¹ Karine Renaudin, MD, PhD,³ David Minault,¹ Jérémy Hervouet,¹ Bernard Martinet,¹ Sarah Bruneau, PhD,¹ Stéphanie Le Bas-Bernardet, PhD,¹ and Gilles Blancho, MD, PhD^{1,4}

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.

(*Transplantation Direct* 2023;9: e1490; doi: 10.1097/TXD.0000000000001490.)

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

Received 17 March 2023. Accepted 30 March 2023.

¹ Center for Research in Transplantation and Translational Immunology, INSERM, Nantes Université, Centre Hospitalier Universitaire Nantes, UMR 1064, Institut Transplantation Urologie Néphrologie, Nantes, France.

² Service d'Urologie, Centre Hospitalier Universitaire Nantes, Nantes Université, Institut Transplantation Urologie Néphrologie, Nantes, France.

³ Service d'anatomopathologie, Centre Hospitalier Universitaire Nantes, Nantes Université, Nantes, France.

⁴ Service de Néphrologie et Immunologie clinique, Centre Hospitalier Universitaire Nantes, Nantes Université, Institut Transplantation Urologie Néphrologie, Nantes, France.

This study received the financial support of Novartis Pharma (Reuil-Malmaison, France). D.K. received a grant from Fondation Centaure (Nantes, France). J.B. received logistical support from Institut Georges Lopez (Lissieu, France).

The authors declare no conflicts of interest.

D.K., S.L.B.-B. and G.B. participated in research design, performance of the research, data analysis, and writing of the article. J.B., T.P., V.N.-D. participated

in performance of the research and data analysis. K.R. and S.B. participated in data analysis. D.M. participated in performance of the research. J.H. and B.M. participated in performance of the research.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

Correspondence: Gilles Blancho, MD, PhD, Institut de Transplantation-Urologie-Néphrologie (ITUN), Centre Hospitalier Universitaire de Nantes, 30 boulevard Jean Monnet, 44093 Nantes, France. (gilles.blancho@chu-nantes.fr).

Copyright © 2023 The Author(s). *Transplantation Direct*. Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001490

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 pre-sensitized 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, Courtabouef, France), penicillin/streptomycin (Thermo Fisher Scientific) and glutamine (Sigma-Aldrich).¹⁷

An anti-SLA class I mAb (clone JM1E3, mouse IgG1, BIO-RAD, Hercules, 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:

$$[\% \text{ PI-positive cells (serum + complement)} - \% \text{ PI-positive cells (serum)}] / [100 - \% \text{ PI-positive cells (serum)}]$$

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 $\mu\text{g/mL}$) 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 mmHg 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 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$. 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

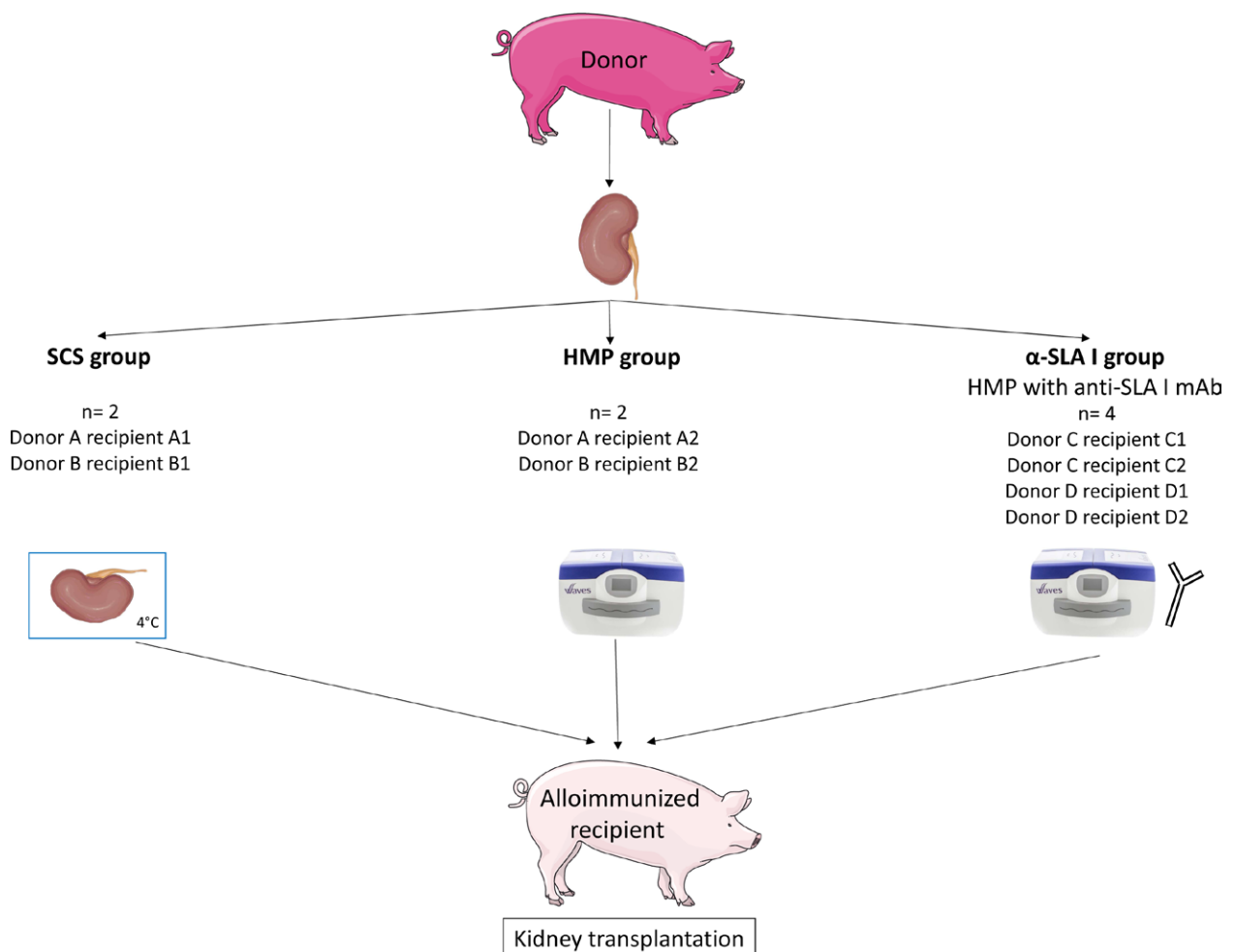


FIGURE 1. Ex vivo preservation and transplantation groups. HMP, hypothermic machine perfusion; mAb, monoclonal antibody; SCS, static cold storage; SLA, swine leukocyte antigen.

IgG (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 μ m, stained with Masson's trichrome, periodic acid–Schiff, and hematoxylin–eosin–safran, and examined by a pathologist that assessed Banff score.¹⁹

Immunofluorescence staining was performed on 8 μ m 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 CD3 ϵ (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

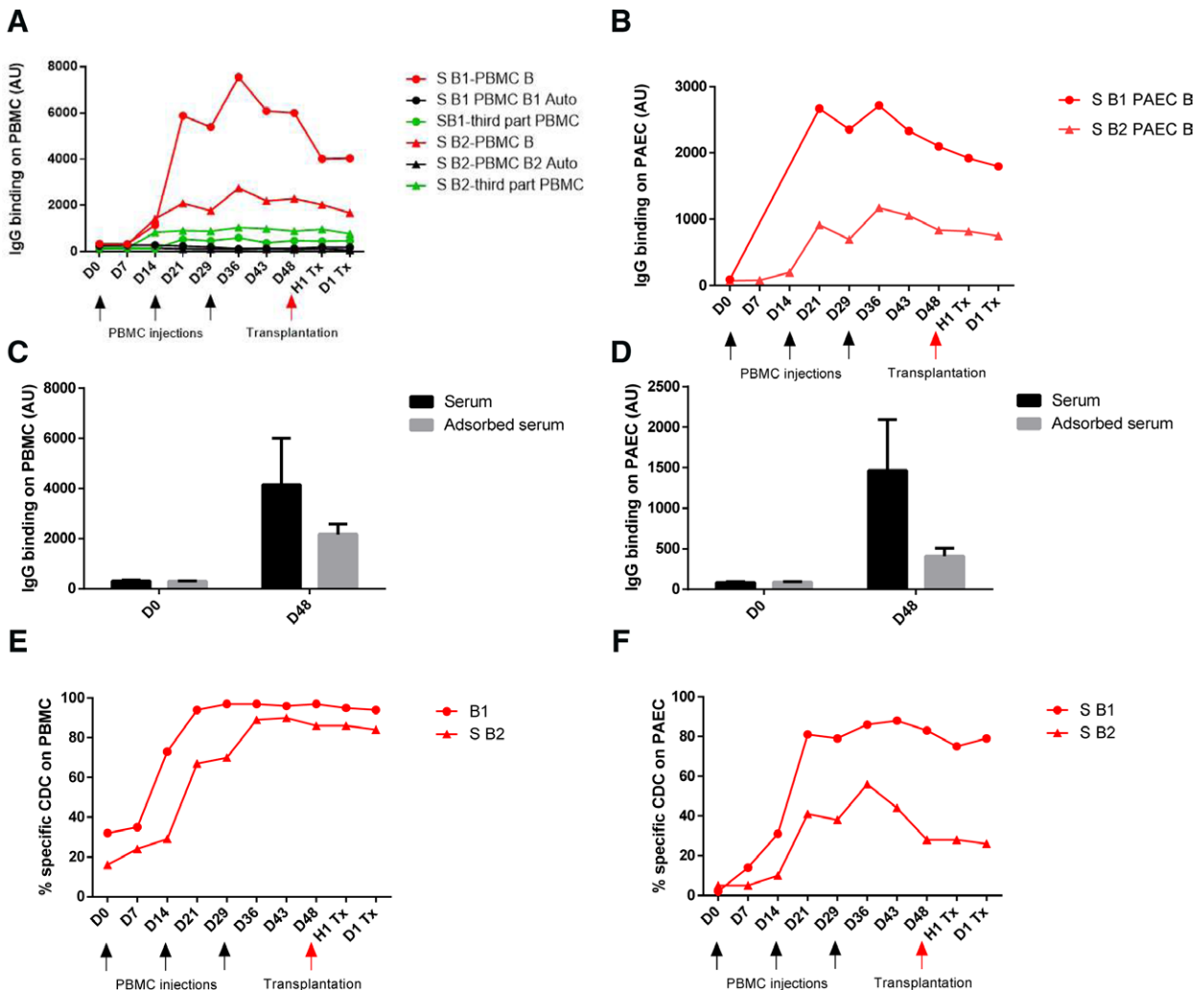


FIGURE 2. Representative recipient immunization against donors confirmed by flow cytometry. A, IgG CM of recipient sera with donor PBMCs (red lines), autologous PBMCs (black lines), and third-party PBMCs (green lines). B, IgG CM of recipient sera with donor PAECs. C and D, IgG CM with donor PBMCs (C) and donor PAECs (D) of recipient untreated sera (black) and recipient sera adsorbed on donor platelets (gray; mean AU \pm 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

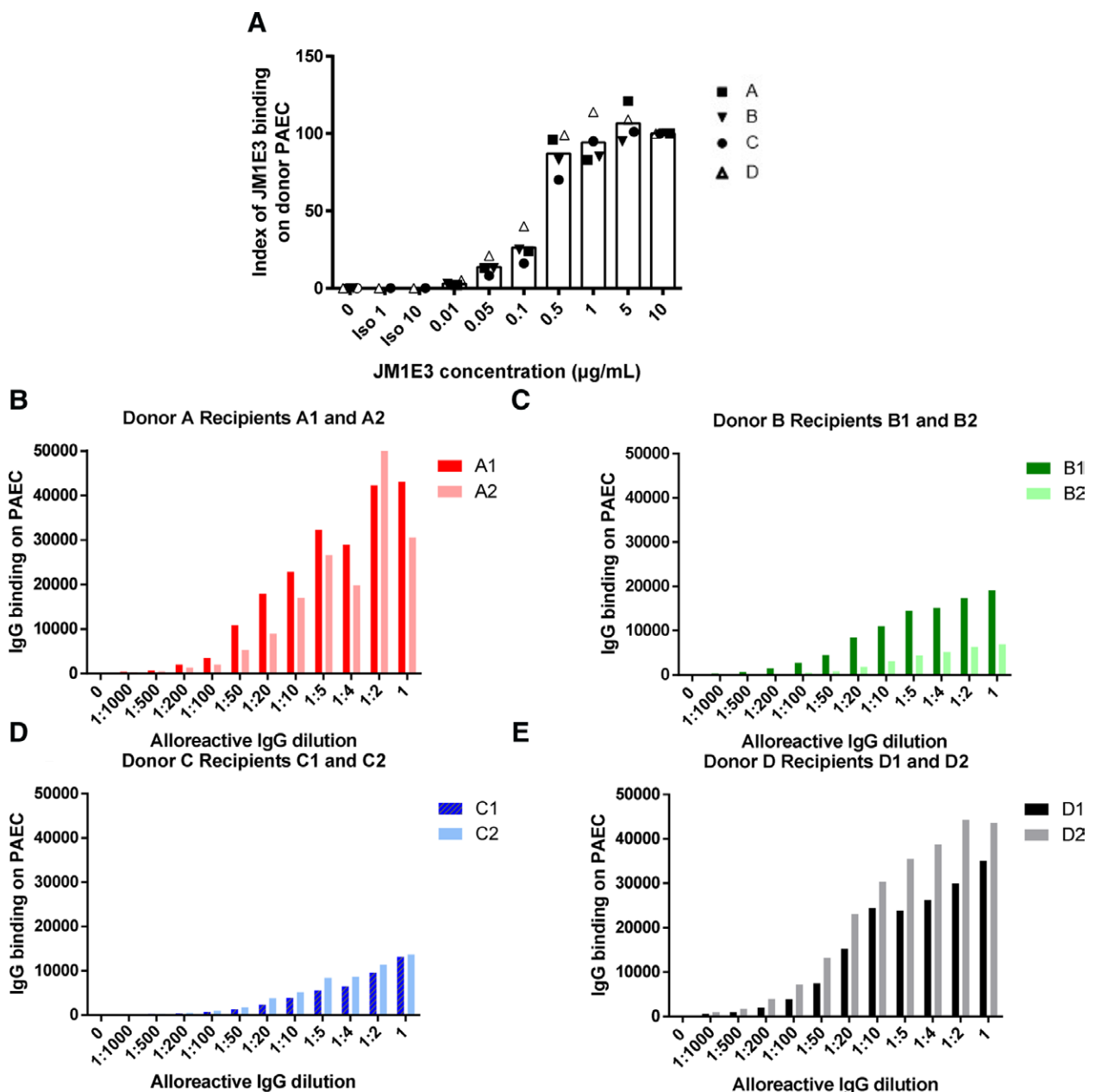


FIGURE 3. Binding of monoclonal anti-SLA I Ab (JM1E3) and recipient IgG on donor PAECs assessed by flow cytometry. A, Binding of JM1E3 on donor PAECs (geometric mean of fluorescence intensity, normalized to JM1E3 10 µg/mL, mean+SEM, n=4). B–E, Binding of recipient purified IgG to donor PAECs for each 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.

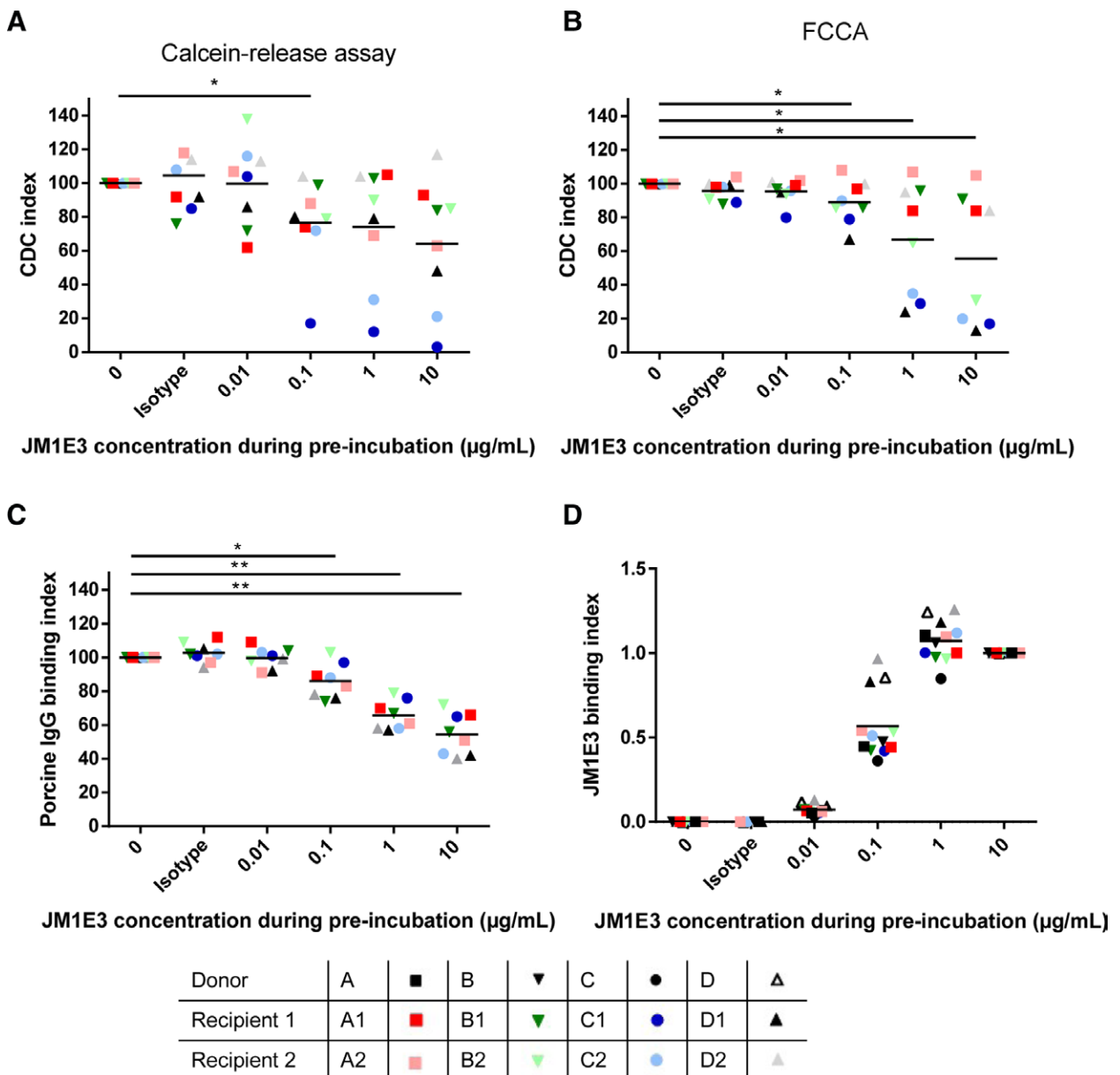


FIGURE 4. PAEC resistance to the CDC of alloreactive IgG (isolated at the plateau of immunization) after incubation with monoclonal anti-SLA I Ab (JM1E3). A and B, Index of specific CDC of alloreactive IgG on donor PAECs after 3h incubation with JM1E3 measured by the calcein-release assay (A, mean of 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 $\mu\text{g}/\text{mL}$) 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 $\mu\text{g}/\text{mL}$ JM1E3 $76.63\% \pm 26.67$ [$P = 0.023$ versus no JM1E3], $74.13\% \pm 35.26$ [ns], and $64.25\% \pm 38.39$ [ns], respectively). With FCCA, CDC was significantly lower after PAEC preincubation with a saturating dose of JM1E3 compared with no preincubation (mean CDC index $89.13\% \pm 12.81$ [$P = 0.047$ versus no JM1E3], $66.88\% \pm 33.46$ ($P = 0.039$), and $55.63\% \pm 38.70$ [$P = 0.016$] for 0.1, 1, and 10 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$ JM1E3 (IgG binding index $86\% \pm 10\%$, $66\% \pm 9\%$, and $54\% \pm 12\%$, 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 $\mu\text{g}/\text{mL}$ 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

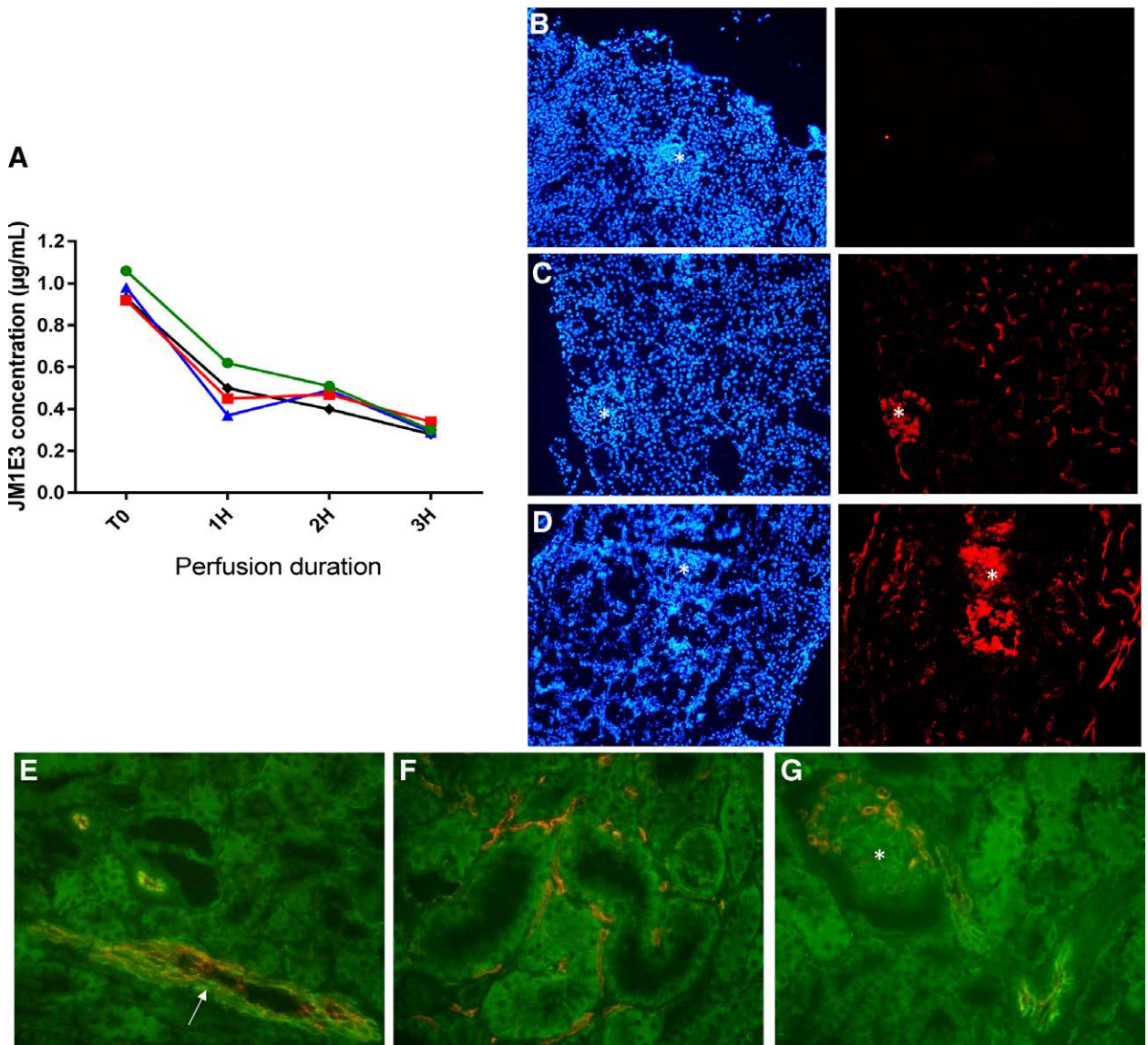


FIGURE 5. Detection of monoclonal anti-SLA I Ab (JM1E3) in perfusion solution and on kidney biopsies during ex vivo HMP of the kidney. A, JM1E3 measurement in perfusion solution (ELISA). B–D, Immunofluorescence staining on kidney core-needle biopsies during perfusion, DAPI staining (left panels) and 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) ($\times 200$). 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, $\times 400$). 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	Recipi- ent ID	Macroscopic	Histology	Banff score	C5b-9	Time of	Macroscopic	Histology at	Banff score	C5b-9
			aspect at H+1	H+1	H+1	staining at H+1	transplant failure	aspect at failure	transplant failure	at transplant failure	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 conges- tion	g0 i0 t0 ptc0 v0	+++	Day 1 (anuria)	Infarcted, arterial thrombosis	Hemorrhagic infarction	v0	+++
α-SLA I	C	C1	Normal	ATN, glomeru- lar 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.

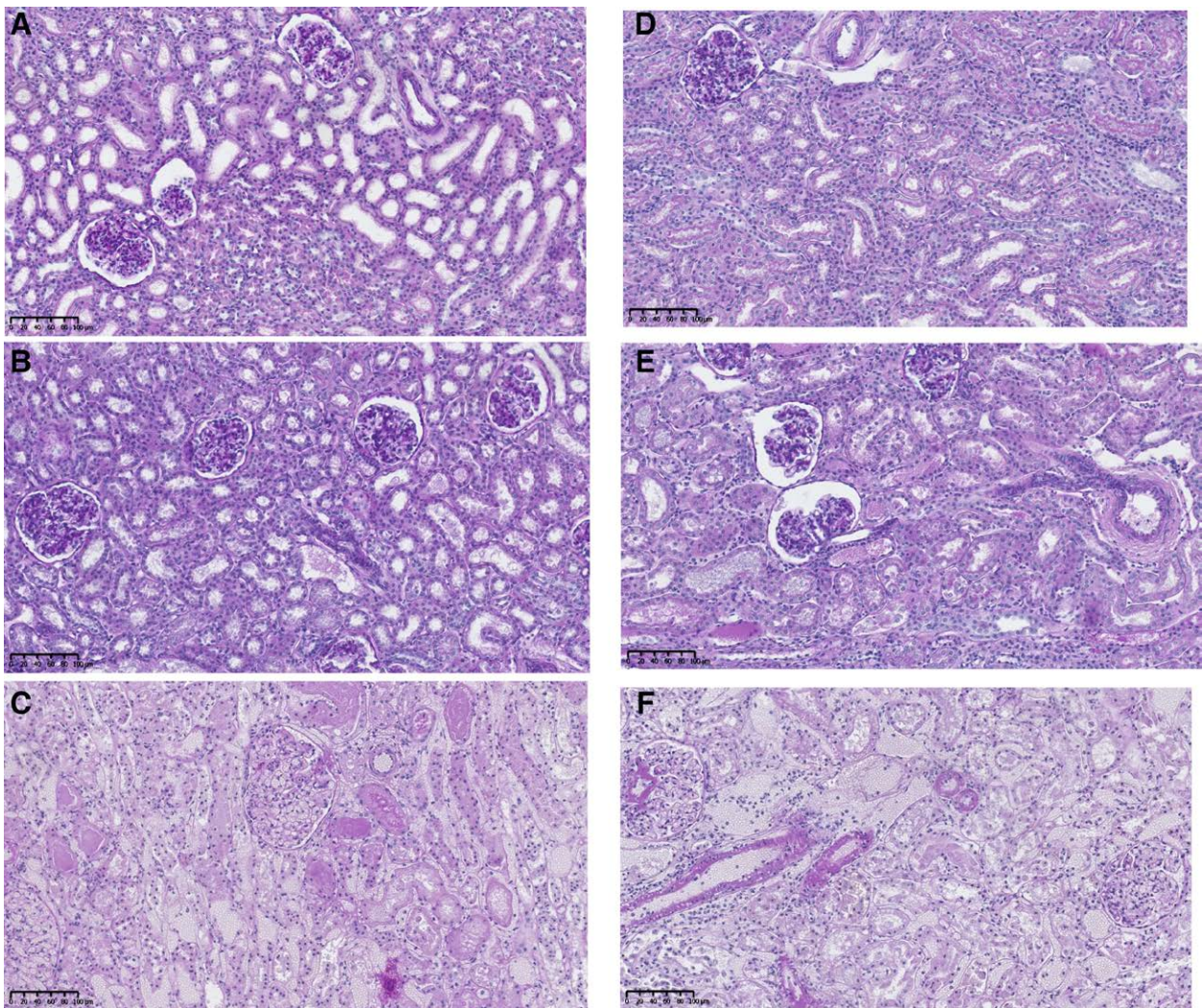


FIGURE 6. Histological examination of kidneys. Periodic acid-Schiff at $\times 20$ magnification. A–C, Transplantation without JM1E3 perfusion (static cold storage 2h): biopsy sample pretransplant (A), 1h after transplantation (B) and day 1 posttransplant (C). D–F Transplantation after JM1E3 perfusion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

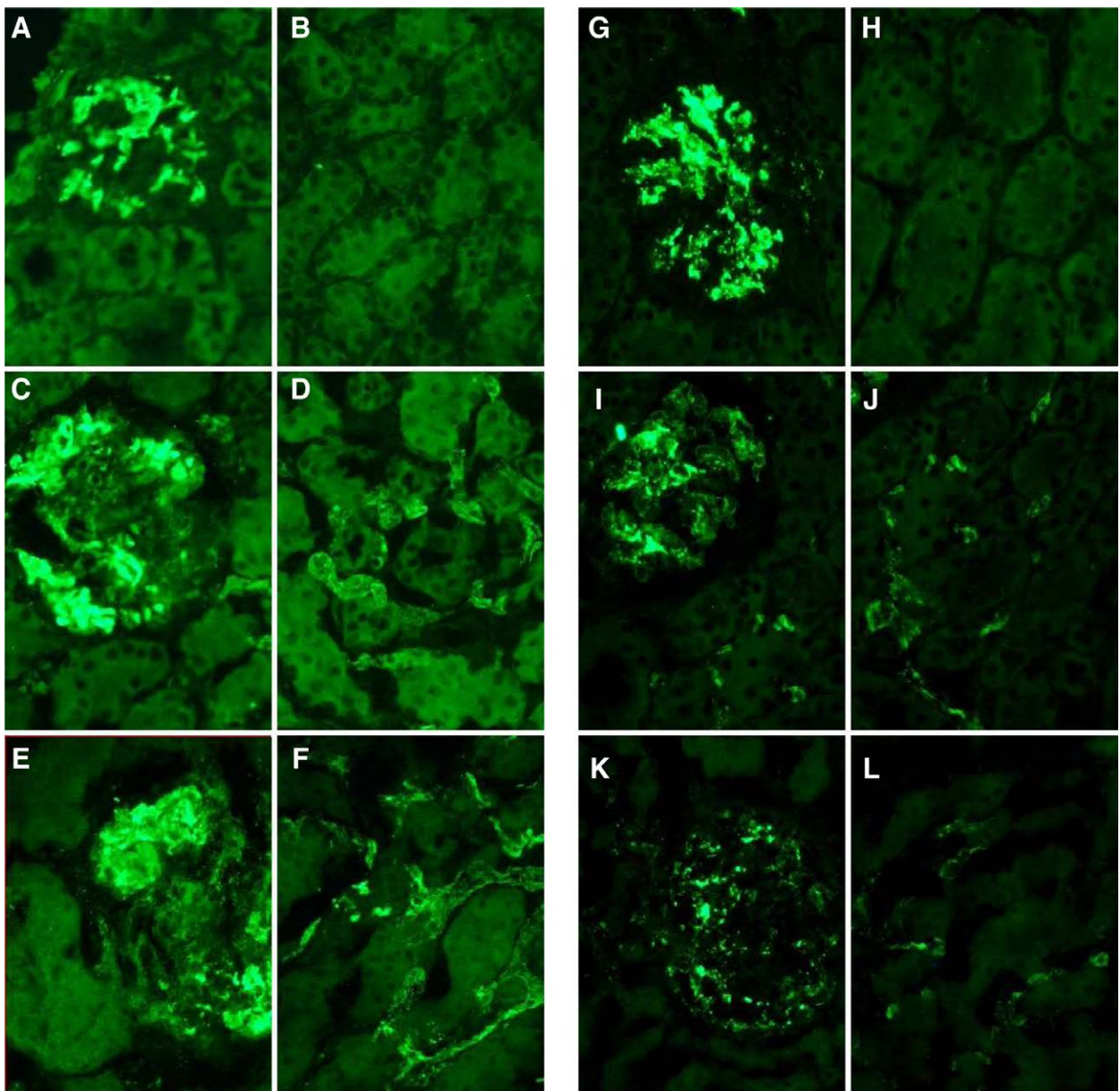


FIGURE 7. Ex vivo perfusion of monoclonal anti-SLA I Ab does not prevent complement cascade activation after transplantation in an alloimmunized recipient. C5b9 staining on a kidney biopsy in a pair without JM1E3 monoclonal anti-SLA I Ab perfusion: before transplantation (A, B), 1 h after transplantation (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.

ACKNOWLEDGMENTS

The authors would like to thank the MicroPICell platform from SFR Bonamy (Nantes, France) for the preparation of tissue biopsies sections and colorations.

REFERENCES

1. Tonelli M, Wiebe N, Knoll G, et al. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. *Am J Transplant.* 2011;11:2093–2109.
2. Wong G, Howard K, Chapman JR, et al. Comparative survival and economic benefits of deceased donor kidney transplantation and dialysis in people with varying ages and co-morbidities. *PLoS One.* 2012;7:e29591.
3. Hung SY, Lin TM, Chang MY, et al. Risk factors of sensitization to human leukocyte antigen in end-stage renal disease patients. *Hum Immunol.* 2014;75:531–535.
4. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *JASN.* 2010;21:1398–1406.
5. Keith DS, Vranic GM. Approach to the highly sensitized kidney transplant candidate. *CJASN.* 2016;11:684–693.
6. Loupy A, Lefaucheur C. Antibody-mediated rejection of solid-organ allografts. *N Engl J Med.* 2018;379:1150–1160.
7. Schinstock CA, Mannon RB, Budde K, et al. Recommended treatment for antibody-mediated rejection after kidney transplantation: the 2019 expert consensus from the Transplantation Society working group. *Transplantation.* 2020;104:911–922.
8. Kervella D, Le Bas-Bernardet S, Bruneau S, et al. Protection of transplants against antibody-mediated injuries: from xenotransplantation

- to allogeneic transplantation, mechanisms and therapeutic insights. *Front Immunol.* 2022;13:932242.
9. Weissenbacher A, Vrakas G, Nasralla D, et al. The future of organ perfusion and re-conditioning. *Transpl Int.* 2019;32:586–597.
 10. Moers C, van Gelder F, Napieralski BP, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2009;360:7–19.
 11. Moers C, Pirenne J, Paul A, et al; Machine Preservation Trial Study Group. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2012;366:770–771.
 12. Hauet T, Goujon JM, Baumert H, et al. Polyethylene glycol reduces the inflammatory injury due to cold ischemia/reperfusion in autotransplanted pig kidneys. *Kidney Int.* 2002;62:654–667.
 13. Thuillier R, Renard C, Rogel-Gaillard C, et al. Effect of polyethylene glycol-based preservation solutions on graft injury in experimental kidney transplantation. *Br J Surg.* 2011;98:368–378.
 14. Hauet T, Eugene M. A new approach in organ preservation: potential role of new polymers. *Kidney Int.* 2008;74:998–1003.
 15. Wiegmann B, Figueiredo C, Gras C, et al. Prevention of rejection of allogeneic endothelial cells in a biohybrid lung by silencing HLA-class I expression. *Biomaterials.* 2014;35:8123–8133.
 16. Yuzefovych Y, Valdivia E, Rong S, et al. Genetic engineering of the kidney to permanently silence MHC transcripts during ex vivo organ perfusion. *Front Immunol.* 2020;11:265.
 17. Chatelais M, Devallière J, Galli C, et al. Gene transfer of the adaptor Lnk (SH2B3) prevents porcine endothelial cell activation and apoptosis: implication for xenograft's cytoprotection: Lnk protects pig endothelial cells from activation and apoptosis. *Xenotransplantation.* 2011;18:108–120.
 18. Poirier N, Maillat F, Barussaud ML, et al. Acute humoral rejection of renal transplants in alloimmunized pigs. *J Surg Res.* 2007;139:261–268.
 19. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* 2018;18:293–307.
 20. Jang HR, Rabb H. Immune cells in experimental acute kidney injury. *Nat Rev Nephrol.* 2015;11:88–101.
 21. Murakami S, Morioka T, Nakagawa Y, et al. Expression of adhesion molecules by cultured human glomerular endothelial cells in response to cytokines: comparison to human umbilical vein and dermal microvascular endothelial cells. *Microvasc Res.* 2001;62:383–391.
 22. Tillou X, Poirier N, Le Bas-Bernardet S, et al. Recombinant human C1-inhibitor prevents acute antibody-mediated rejection in alloimmunized baboons. *Kidney Int.* 2010;78:152–159.