MHC matching fails to prevent long-term rejection of iPSC-derived neurons in non-human primates

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Cell therapy products (CTP) derived from pluripotent stem cells (iPSCs) may constitute a renewable, specifically differentiated source of cells to potentially cure patients with neurodegenerative disorders. However, the immunogenicity of CTP remains a major issue for therapeutic approaches based on transplantation of non-autologous stem cell-derived neural grafts. Despite its considerable side-effects, long-term immunosuppression, appears indispensable to mitigate neuro-inflammation and prevent rejection of allogeneic CTP. Matching iPSC donors’ and patients’ HLA haplotypes has been proposed as a way to access CTP with enhanced immunological compatibility, ultimately reducing the need for immunosuppression. In the present work, we challenge this paradigm by grafting autologous, MHC-matched and mis-matched neuronal grafts in a primate model of Huntington’s disease. Unlike previous reports in unlesioned hosts, we show that in the absence of immunosuppression MHC matching alone is insufficient to grant long-term survival of neuronal grafts in the lesioned brain.
Clinical trials using fetal cells in Parkinson’s and Huntington’s diseases (HD) have paved the way for the development of human pluripotent stem cell (hPSC)-based replacement strategies in the brain. These pioneering trials have demonstrated frequent allo-immunisation to fetal donor antigens, sometimes associated with neuro-inflammation and rejection. Despite the increased risk of cancer, infection and cardiovascular diseases, long-term immunosuppression is still used to protect allogeneic neural grafts from rejection. Availability of induced-hPSCs (iPSCs) derived from the patient himself or from selected donors with some degree of HLA matching opens up opportunities to secure scalable sources of cell therapy products (CTP) with enhanced (e.g., HLA A, B, DR triple homozygous human iPSC or full immunological compatibility (e.g., autologous iPSC).

Some pre-clinical studies using autologous (AU) or syngeneic iPSC-derived grafts, the ideal immunological combinations, showed that such grafts can be well tolerated even in non-immune privileged sites in humanized mice and in the non-lesioned brain of non-human primates (NHPs). In contrast, others have reported that mouse and human iPSC derivatives can be immunogenic in syngeneic or AU recipients and in an AU humanized mouse model, respectively. Interestingly, it has also been shown that a host immune response (T-cell infiltration) associated with necrosis following transplantation of syngeneic iPSC, appears to be dependent on the antigenic profile of the transplant. Recently, the more economically sustainable “MHC-paradigm”, i.e., matching the major histocompatibility complex (MHC) of both donor and recipient, was tested in NHPs using retinal or dopaminergic transplants. Both studies showed no overt sign of humoral or cellular immune response directed against MHC-matched (MA) grafts and demonstrated improved engraftment of such transplants.

Among all living Macaca fascicularis (Mafa) populations, that of Mauritius island presents the lowest genetic diversity because of its genetic isolation since its foundation. In this study we take advantage of reduced genetic diversity in Mauritian macaques to challenge the “MHC-paradigm” by transplanting neuronal grafts in an excitotoxin-lesion model of HD in NHPs and by comparing the immunogenicity of AU, MA and fully mismatched (MI) grafts (Fig. 1a). The first step is to generate several iPSC lines with specific MHC haplotypes and the corresponding striatal neuronal grafts then to control their properties in vitro and in vivo in quinolinic acid-lesioned nude rats. The second step is to assess the immunogenicity of the three types of transplants in vivo in AU, MA, and MI NHP recipients at 3 and 6 months after transplantation in the lesioned brain, in the absence of peripheral immunosuppression. We observe a local infiltration of immune cells including CD8+ T cells in the core graft in allogenic MI and MA recipients that is significantly higher than that detected in AU recipients or in non-transplanted, QA-lesioned NHP. Our results suggest a delayed but ongoing sub-acute rejection in MA recipients. We thus conclude that MHC matching alone is insufficient to grant long-term survival of neuronal grafts in an excitotoxin-lesion model of HD in NHPs.

Results
Production of MHC-typed striatal grafts. In order to generate MA striatal grafts, we genotyped NHP recipients and identified AU, MA or fully MI recipients with specific haplotypes. We identified two MHC homozygous individuals (M1/M1 and M3/M3, respectively) and derived two iPSC lines from peripheral blood mononuclear cells (PBMCs) (Fig. 1a). A third line, derived from PBMCs of a MHC heterozygous individual (M2/M5) was used as a control (Supplementary Table 1; Supplementary Fig. 1). All three lines expressed the pluripotency marker OCT4 (Fig. 1b) and were differentiated into striatal neuron precursors as previously described. The differentiation potential into striatal neurons (Calbindin+ or glia (GFAP+)) of each CTP batch was first controlled in vitro (Fig. 1c). The viability and in vivo differentiation potential into telencephalic neurons (FOXG1+, MAP2+) and more specifically, striatal projection neurons (Calbindin+, DARPP32+) or interneurons (CalRetinin+) of each batch of CTP transplanted in NHPs was also controlled in quinolinic acid-lesioned nude rats (Fig. 2).

To assess the potential immunogenicity of CTPs at the time of grafting into immunocompetent NHPs, we measured the level of expression of MHC class I & II antigens, of T-cell co-stimulatory molecules, and of two key immunosuppressive cytokines, IL-10 and TGFb1, as previously described (Fig. 3 and Supplementary Fig. 2). Our results showed that CTPs weakly expressed MHC-I antigens (Mafa Class-I A, B measured by cross reacting anti-HLA-A, B, C antibodies) and did not express MHC-II antigens (Mafa Class-II DR measured by cross-reacting anti-HLA-DR antibodies) or any of the three major co-stimulatory proteins we analyzed (CD40, CD80 and CD86) (Fig. 3a, b). As expected, interferon gamma (IFN-γ) stimulation only increased MHC-I expression (Fig. 3a). Elevated levels of IL10 and TGFb1, that have been previously linked to tolerogenic immune response to autologous-iPSC derivatives in mice, were not observed in Mac_iPSC-derived striatal CTPs (Supplementary Fig. 2).

Graft localization, survival, and neuronal identity in NHPs. Next, we assessed the immunogenicity of our three CTPs in vivo in AU, MA, and MI NHP recipients at 3 and/or 6 months after quinolinic acid-lesion and transplantation in the absence of immunosuppression. Group 1 (n = 4) consisted of paired AU and MI recipients analyzed at 6 months post-grafting (PG). Group 2 (1 AU and 2 MA recipients) was analyzed at 6 months PG (Fig. 1a). Group 3 consisted of non-transplanted QA-lesioned NHPs (n = 4). Magnetic resonance imaging (MRI) analysis revealed that QA lesions were detected only transiently (up to 1 month post-QA) as blurred-edged hyper-intensity signals on T2-weighted images and as dark hypo-intense regions at 3 and 6 months post-QA. CTPs were detected as sharp-edged hyper-intense areas from 2 months PG onwards (Fig. 4a). This allowed an accurate longitudinal follow-up of graft size and location that was ultimately validated by post mortem analysis (Fig. 4b). Fully MI recipients showed a variable intensity of MRI signals including hyper-intense liquid-filled cavities in the core graft detected early (2 months PG) (Figs. 4b and 5).

Histological analyses at 3 and 6 months PG confirmed the survival, location and telencephalic (FOXG1+, neuronal (NeuN+, MAP2+, HuC/D+) and striatal (Calretinin: CalRet+, Calbindin: CalB+) identity of graft-derived cells in all recipients (Fig. 5 and Supplementary Figs. 3–5). All grafts were DARPP32-negative (Supplementary Figs. 4 and 5). Large cavities were only detected in MI recipients (Fig. 5). The pattern of FOXG1+, CalRet+, and NeuN+ staining appeared more homogeneous in AU grafts and more heterogeneous in MA recipients both at 3 and 6 months PG (Fig. 5 and Supplementary Figs. 4 and 5). The proliferation potential of the different grafts assessed with the Phospho Histone H3 (PHH3) antibody revealed that the number of dividing cells at 3 months PG was greater than that observed at 6 months PG regardless of the donor-recipient combination (T²-test, p = 0.02), and that their density was always inferior to 1% of the graft (Fig. 5 and Supplementary Fig. 3).

Infiltration of immune cells in MI and matched grafts. Post mortem analysis at 6 months post-QA or PG revealed the presence of host-derived immune cells expressing Iba-1, MHC-class
**Fig. 1** Generation and in vitro characterization of cell therapy products (CTPs) with specific MHC haplotypes. 

**a** Schematic representation of the experimental groups for intra-striatal cell transplantation in HD macaques (Mac) showing autologous (white, Mac 1–3), MHC-matched (gray, Mac 4–5) and MHC mismatched (black, Mac 6–7) recipients of CTPs derived from iPSC lines generated from PBMCs drawn from MHC homozygous (M1/M1; M3/M3) or heterozygous (M2/M5) cell donors (white). Survival time post-transplantation is indicated on each subject as 3 or 6 months (mo).

**b** Immunohistochemistry results showing OCT4/DAPI double staining of each of the 3 iPSCs lines derived from PBMC NHP donors (Mac 1, 2, and 3) at passage (P) 14, 13, and 12 respectively.

**c** Staining of CTPs derived from each of the iPSC batches at in vitro differentiation day (D.I.V.) 48 showing staining for Calbindin (CalB) and astrocytes (GFAP). Scale bar: 100 µm.
II (revealed by anti-HLA-DR monoclonal antibodies), CD68, CD45, CD8 and CD4 (Fig. 6 and Supplementary Figs. 4–6). Non-transplanted QA-lesioned controls presented an activation of macrophages (CD68+) and little to no activation of infiltrating immune cells (CD45+, CD8+, CD4+)\(^1\). In this context, AU grafts elicited little to no additional activation of these infiltrating immune cells. In contrast, MI recipients presented a considerable infiltration and marked staining for Iba1, MHC-class II, CD68, CD8, and CD4 around the graft and cavities (Fig. 6 and Supplementary Fig. 6b). These findings suggest an ongoing rejection
process of the allogeneic graft in MI recipients and are in agreement with clinical observations of allo-immunisation leading to rejection in HD patients transplanted with fetal grafts.

More interestingly, the staining in MA recipients at 6 months PG was closer to that of MI recipients at 3 months PG than to the staining of AU grafts at 3 and 6 months PG. Accordingly, a clear infiltration and a strong staining for most markers (Iba1, MHC-class II, CD45, CD8, CD4) could be observed, suggesting a delayed but ongoing sub-acute rejection.

Absence of humoral response to autologous and allogenic grafts. Finally, the humoral response against all three types of grafts and their ability to trigger complement-dependent cytotoxicity (CDC) was assessed in vitro monthly. Unexpectedly, before transplantation or QA lesion, pre-existing anti-graft antibodies were observed even in the context of AU combinations, and such antibodies had a cytotoxic activity primarily mediated by IgM. However, no transplant combination elicited a significant increase in the serum levels of anti-graft antibodies or CDC activity at any time-point following transplantation (Fig. 7 and Supplementary Fig. 7). Unlike a recent report using allogeneic retinal transplants in NHPs, our data shows the absence of transplant-elicited humoral immune response against the three CTPs used in this study. On the other hand, a longitudinal measurement of six cytokines in the serum and CSF did not reveal a cytokine profile compatible with a T cell-based rejection of the iPSC-derived grafts (Supplementary Fig. 8).

Discussion

Our results suggest that, unlike AU neuronal grafts, allogenic (MI and MA) grafts elicit a local infiltration of CD8+ T cells and an increase in local HLA-DR staining, far beyond the effect observed in the QA-lesioned non-transplanted NHPs. In our NHP model of HD, in the absence of immunosuppression, haplotype-matching was insufficient to grant long-term survival of neuronal grafts and appeared to trigger a delayed/attenuated immune response in the host. Our results are not in agreement with the main claim of Morizane and colleagues who showed that, in the absence of immunosuppression, MHC matching improved engraftment of iPSC-derived TH+ neurons in the non-lesioned brain of NHPs and reduced the immune response by suppressing the accumulation of microglia (Iba-1+) and...
lymphocytes (CD45+) into the grafts. It is noteworthy that the extent of MHC matching in both studies are similar. At least four hypotheses may explain the discrepancies between our findings and those previously published. Firstly, transplantation into intact versus lesioned animals might not yield the same results in terms of cell maturation and/or rejection. Indeed, even though acute excitotoxic lesions may bear only limited resemblance to the chronic and progressive inflammation observed in HD patients, transplanting into a lesioned brain may be more pertinent as it more closely mimics the inflammatory milieu associated with neurodegenerative disorders in man. Secondly, the shorter duration of the follow-up in the dopaminergic neuronal MA grafts study may have affected the timely identification of an ongoing immune process. Thirdly, the antigenic profile, closely related to the maturation time indispensable to generate fully differentiated striatal iPSC-derived neurons, may substantially differ from that of dopaminergic neurons. This may ultimately result in a different, possibly more aggressive, anti-graft immune response. In all cases, our post mortem data suggest that grafts are largely composed of post-mitotic striatal neurons and that maturation is time-dependent rather than dependent on the recipient-donor MHC matching combination (AU, MA, MI). Finally, the extent of cell division and/or the presence of immature cells may give rise to areas with immature or leaky BBB and thus be more prone to trigger the host’s immune response.

In conclusion, in the specific context of brain transplantation, our pre-clinical data suggest that a better immunological match is not necessarily associated with significantly improved survival and maturation of the grafts. Alternative strategies allowing CTPs to escape altogether the host cell-mediated immune response or a combination of MHC-matching and peripheral immunosuppression should be further investigated in NHPs to allow stem cell therapy to achieve its full therapeutic potential.

Methods

NHPs: ethics and housing. All animal studies were conducted according to European regulations (EU Directive 2010/63) and in compliance with Standards for Humane Care and Use of Laboratory Animals of the Office of Laboratory Animal Welfare (OLAW—no. #AS826–01) in a facility authorized by local authorities (authorization no. #B92-032-02). The experimental protocol was reviewed and approved (authorization no.14_019) by the local ethics committee (CETEA No.44). All efforts were made to minimize animal suffering and animal care was supervised by veterinarians and animal technicians skilled in the healthcare and housing of NHPs. All primates were housed under standard environmental conditions (12-h light-dark cycle, temperature: 22 ± 1 °C and humidity: 50%) with ad libitum access to food and water. Experiments were conducted on a total of 11 male cynomolgus monkeys (Macaca fascicularis, supplied by Noveprim, Mauritius Island) of a mean age of 5.4 ± 0.2 years and a mean weight of 5.4 ± 0.3 kg.

Fig. 4 Graft size, location in QA-lesioned NHPs analyzed in vivo with MRI. a Representative coronal images of the longitudinal MRI follow-up of CTP derivatives after intra-striatal transplantation in autologous (white), MHC-matched (gray) and MHC mis-matched (black) recipients showing areas of hyper- (red arrows) and hypo-intensity in the caudate (bilateral) and putamen (unilateral, left) corresponding to the transplanted CTPs at 1, 2, 3 and, where applicable, 6 months post-surgery. b Graft MRI images prior to euthanasia and on post mortem sections stained with CalRet in autologous (white), MHC-matched (gray) and MHC mismatched (black) recipients at end-point (3 months PG for Mac 1, 3, 6, 7; 6 months PG for Mac 2, 4, 5).
Genotyping of NHPs. Genomic DNA for all NHP considered was extracted from peripheral blood sample using QIAamp Blood Kit (QIAGEN, Courtaboeuf, France). The animal MHC genotypes were determined by means of genotyping 17 microsatellites scattered across the MHC region\textsuperscript{14}. Genotypes were determined with DNA Size Standard-kit-600 (Beckman Coulter, Villepinte, France) after denaturation and separation of the amplification products by capillary electrophoresis with a CEQ8000 analyzer and scored with the software CEQ8000 Genetic Analysis System v8.0 (Beckman Coulter, Villepinte, France). Primer used to amplify the 18 MHC microsatellites were the following: D6S2972 AAATGTGAGAATAAAGGAGA and GATAAAGGGGAACTACTACA; D6S2970 TCCCATGGTCAAGTTCTCAG and TCATGGATCTTATCAGCCTC; D6S2854 TCATGAGCGTGGCACTGCAC and CCGTATGTTGCAACCAGGAG; D6S2704 TTTTTGCCACTCTGGAGGATGGG and GAGCATAATATCTGGTCTACTGC; D6S2847 TATTGGACAGCACTGCTCTGG and TGCCATTCAGATTGGTTTCTCTG; C4-2-25 ATGTTAGTTTTAGAAGATAACACTC and TCTTCTGTGCAAGCAAGCACTGTAC; D6S2691 GTAGCTGTGGAAACAGTGTCCATG and CTTGACTTGAAACTCAGAGACC; MICA CCTTTTTTTCAGGGAAAGTGC and CCTTACCATCTCCAGAAACTGC; D6S2793 CTACCTCCTTGCCAAACTTGCTATTTGT and AATAGCCATGAGAAGCTATGTGGGGGA; D6S2782 TTTACCTGCTCTACTGCTACG and GGAAGACATTAACGTGTTTAGCA; D6S2669 TGCCTTCCGTAAGCCTCAGTCT and TTAAGGACAGCAAAGCCAGCAGCA; D6S2892 TGCATGTCCTGTGAGGTAAG and ACTCAACCCTGCTGTTGTAG; DRACA TGGAATCTCATCAAGGTCAG and ACATTTGTATGCTTCAGATG; D6S2876 GGTAAAATTCCTGACTGGCC and GACAGCTCTTCTTAACCTGC; D6S2747 AGGAATCTAGTGCTCTCTCC and CTCTAGCAAAAGGAAGAGCC; D6S2745 CCTAGAGATTCCTCCACTATTA and CCAATGTTTGATAGCAGACTGGGGT; D6S2741 AGACTAGTAGGAGCTGAGTAAGGAGG and CTGACAGTTGCTGTTATCTCAGAC; D6S2771 ATTCCTTTCACTAGTTCTGG and CCACCTTAAAGAAATTAGAAAAG from refs.\textsuperscript{14,21,22}

Fig. 5 Histological assessment of graft localization, survival and neuronal identity of the grafts in mismatched (3 months PG), autologous (6 months PG) and matched (6 months PG) NHP recipients. a, b Immunohistological staining of coronal sections of the brain at the level of graft with anti-FOXG1 (a) and Calretinin (b) antibodies. Red arrows indicate the location of the grafted cells. c Brain slices were stained with post-mitotic neuronal markers NeuN (cell nuclei), MAP2 (soma and neuritic extensions), HuC/D (peri-nuclear soma), SOX1 (immature neural cells), and PHH3 (proliferative cells) compared to Mafa-DR (MHC II). Red dotted lines represent graft contour. Scale bar: 100 µm
Fig. 6 Post mortem analysis of the immune markers in QA-lesioned NHPs after CTP transplantation. 

**a** Representative coronal sections of the NHP brain at the level of the graft (commissural) showing staining for microglia/macrophages (Iba1; CD68), MHC Class II positive cells (revealed by an anti HLA-DR antibody cross-reacting with Mafa-DR antigens), and cytotoxic T-cells (CD8, cartography,) in quinolinic acid lesioned (QA) untransplanted controls (green) and in autologous (AU, white), MHC-matched (MA, gray), and mismatched (MI, black) CTP recipients at 3 or 6 months post-grafting (PG).

**b–e** Quantification of Iba1 (**b**), Mafa-DR (**c**), CD68 (**d**) and CD8 (**e**) immunoreactivity in AU, MA, MI CTP recipients at 3 or 6 months post-grafting (PG) and in untransplanted controls. Bar graphs represent mean value of the three regions of interest considered (left and right caudate, and left putamen) for each animal; gray dots represent individual values for each region (n = 3 biologically independent cell deposit per animal).
From the genotypes obtained for the 17 microsatellites, the most probable haplotypes were deduced for each animal\textsuperscript{14}. As reported previously, seven founder MHC haplotypes (M1 to M7) were identified in the Mauritian macaque population as well as recombinant haplotypes derived from these seven haplotypes\textsuperscript{14,23}. The genotypes of the animals are shown in Supplementary Fig. 1 and Table 1.

**Imaging in NHPs.** Magnetic resonance imaging (MRI) was performed on all NHPs at baseline in order to determine the stereotactic coordinates for surgery and was repeated monthly to monitor grafts.

Primates were induced with ketamine (1 mg kg\textsuperscript{-1}) and xylazine (0.5 mg kg\textsuperscript{-1}), maintained anesthetized with an intravenous (i.v.) infusion of propofol (1 ml kg\textsuperscript{-1} h\textsuperscript{-1}) and placed in the magnet in a sphinx position with the head fixed in a stereotactic MRI-compatible frame (M2E, France). NHPs were heated by a hot air flux and their temperature and respiration parameters monitored remotely.

All acquisitions were performed on a horizontal 7 T Varian scanner (Palo Alto, CA, USA) equipped with a gradient coil reaching 100 mT m\textsuperscript{-1}. A surface coil (RAPID Biomedical GmbH, Rimpar, Germany) was used for transmission and reception. T2-weighted images were acquired using a high-resolution 2D fast spin-echo sequence (469 × 469 μm\textsuperscript{2} in-plane resolution, 1 mm slice thickness, 70 slices), with echo time TE/ Repetition time TR = 20/8000 ms, 5 echoes, effective TE = 52.5 ms and acquisition time Tacq = 43 min. For T2* - weighted images the parameters used were: 469 × 469 μm\textsuperscript{2} in-plane resolution, 1 mm slice thickness, 40 slices, 5 TE (from 5.5 to 30 ms), repetition time TR = 2 ms and acquisition time Tacq = 8 min.

**Fig. 7** Detection of hemolytic CTP-specific antibodies in the serum of transplanted macaques. The sera of AU, MA, MI recipients were collected before and at different time-points following transplantation. a The presence of anti-CTP IgG was detected by cell-based ELISA. b The capability of anti-graft antibodies to trigger the complement-dependent cytotoxicity was also evaluated by measuring the fluorescence signal of the Cell-Tox Green probe (for (a) and (b) data are expressed as mean ± s.e.m of n = 3 replicates, one-way ANOVA followed by Dunnett’s multiple comparisons post hoc test using day 0 as control; for b positive and negative controls: individual replicates shown) or c by measuring the percentage of the dead cells (propidium iodide-positive cells) by flow cytometry (n = 2 technical replicate of single biological samples, mean per time point).
Post mortem analysis in NHPs.

1 track equidistant of the two lesion sites in the caudate nuclei and 1 track 1.5 mm apart D/V: 1 track 1 mm anterior of the most anterior QA lesion site in the putamen, 1 track equidistant of the two lesion sites in the putamen and 1 track 1 mm posterior of the least posterior QA lesion site in the putamen.

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M cells were delivered into the left putamen (3 tracks, 2 × 5 μL deposits track−1)

and

...measurements, outlines of the striatum were first delineated using Mercator software (Explotrona, La Rochelle, France). For inflammatory reaction measurements, outlines of the striatum were first delineated using Mercator software and then the inflammatory area defined using GFAP or Maf-DR staining. RNA levels and quality were checked using the..
well (MFI) was recorded by FACScalibur cytometer (BD Bioscience) acquisition and was revealed using FITC-labeled anti-human IgG (Jackson ImmunoResearch) for 30 min at 37 °C. After washing with PBS-BSA-sodium azide, antibody binding terminally anesthetized with 1 g kg−1 solution (H2SO4 1 M, Sigma Aldrich, St. Louis, MO USA) and the plates were washed four times, tetramethyl benzidine (TMB) solution (Scytec, West PA, USA) were added to each well and incubated at room temperature for 1 h. Washes with PBS-0.1% Tween, horseradish peroxidase-conjugated goat anti-human antibody (Promega, Madison, WI, USA) was added at the end of complement exposure and incubated at 4 °C for 1 h. Following a R1 region. Control histograms (isotype controls) were overlaid onto the stained positive dataset allowing positive cells to be accurately identified on single parameter histograms (PE-FL2 or FITC-FL1 fluorescence) using a R1 gating strategy is illustrated in Supplementary Fig. 10a. Cell viability was assessed on the same wells by addition of 10 μl of Resazurin (Celltiterblue reagent, Promega) in 100 μl of complete medium; fluorescence (485/em590ex, Victor3TM) was recorded after a 2 h incubation period. Serum cytotoxicity was also measured by Flow Cytometry Complement-Cytotoxicity Assay23. Briefly, 50 pl of 1:4 diluted HI sera were incubated for 30 min at 37 °C with detached CTPs. Thereafter, the cells were washed twice and incubated for 30 min at 37 °C with 1:10 diluted baby rabbit complement (Gibco). Then, cells were washed and incubated in complete medium (10% thymidine (1 mg ml−1) was added to detect dead cells. Percentage of PI-positive cells was recorded by FACS calibur cytometer (BD Bioscience) acquisition and analyzed by CELLQUEST software (BD bioscience). The data were evaluated by plotting FSC versus SSC, and the barograms with PI positive cells were analyzed. Experiments where PI-positive cells in negative control samples (i.e., untreated cells or cells treated with complement only) exceeded 12% of the R1-gated population were repeated. This gating strategy is illustrated in Supplementary Fig. 10b.

Immunophenotyping of CTPs. The CTP lines examined in these studies were cultured in N2B27 media on poly-ornithine laminin-coated dishes at 100,000 cells cm−2. Culture medium with or without INF-y (100 ng ml−1) was added for 48 h prior to cell harvest with Accutase (STEMPro Accutase, Thermo Scientific, Waltham, MA USA). Cell-surface markers were analyzed by flow cytometry using antibodies targeting human epitopes that are shared with Macaca fascicularis (Maca). The monoclonal antibodies used were directed against the following specificities: the anti-HLA-A, -B, -C clone G46-2.6, (BD-Pharmingen, San Diego, CA), the anti-HLA-DR, the anti-CD56 (NCAM) (clone MEM-188) (Biolegend, San Diego, CA), the anti-CD80 (clone L307.4), anti-CD86 (clone FUN-1) (all from BD-Pharmingen). Isotype-matched immunoglobulins were used as controls. Briefly, 1 × 10^6 cells were incubated with PE-labeled antibodies for 30 min at 4 °C, washed with phosphate-buffered saline (PBS), 0.5% bovine serum albumin (BSA), and 0.1% Na Azide. Flow cytometric analysis was performed as a FACS calibur flow cytometer (Becton Dickenson, San Jose, CA) using the CELL-Quest program (Becton Dickinson). The data were analyzed by plotting forward scatter (FSC) versus side scatter (SSC) and by defining a R1 region. Control histograms (isotype controls) were overlaid onto the stained positive dataset allowing positive cells to be accurately identified on single parameter histograms (PE-FL2 or FITC-FL1 fluorescence) using a R1 gating strategy is illustrated in Supplementary Fig. 10a. Cell viability was assessed on the same wells by addition of 10 μl of Resazurin (Celltiterblue reagent, Promega) in 100 μl of complete medium; fluorescence (485/em590ex, Victor3TM) was recorded after a 2 h incubation period. Serum cytotoxicity was also measured by Flow Cytometry Complement-Cytotoxicity Assay23. Briefly, 50 pl of 1:4 diluted HI sera were incubated for 30 min at 37 °C with detached CTPs. Thereafter, the cells were washed twice and incubated for 30 min at 37 °C with 1:10 diluted baby rabbit complement (Gibco). Then, cells were washed and incubated in complete medium (10% thymidine (1 mg ml−1) was added to detect dead cells. Percentage of PI-positive cells was recorded by FACS calibur cytometer (BD Bioscience) acquisition and analyzed by CELLQUEST software (BD bioscience). The data were evaluated by plotting FSC versus SSC, and the barograms with PI positive cells were analyzed. Experiments where PI-positive cells in negative control samples (i.e., untreated cells or cells treated with complement only) exceeded 12% of the R1-gated population were repeated. This gating strategy is illustrated in Supplementary Fig. 10b.

Anti-graft antibody response. To assess the extent of the antibody response induced by CTP grafts, serum was obtained from each recipient prior to and at 7, 30, 60, 90, 180 days following implantation. Donor CTP were seeded into Poly-L-ornithine/Laminin coated 96-well ELISA plates (Polsypor, NUNC™ Thermo Scientific, Waltham, MA USA). Briefly, 32,000 cells per well were incubated at 37 °C in 5% CO2 for 48 h, washed twice with PBS and fixed 30 min with 20% formalin (Sigma Aldrich). Plates were blocked overnight at 4 °C with PBS-5% BSA. In parallel, cell-free background plates were prepared and treated similarly. After washing twice with PBS-0.1% Tween, heat-inactivated (HI) recipient sera (in triplicate) were added at a dilution of 1:100, incubated at 4 °C for 1 h. Following four washes with PBS-0.1% Tween, horseradish peroxidase-conjugated goat anti-human IgG immunoglobulins (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) were added to each well and incubated at room temperature for 1 h. After washing four times, tetramethyl benzidine (TMB) solution (Scytek, West Logan, UT, USA), working solution was added to each well in a lytic solution. A stop solution (H2SO4 1 M, Sigma Aldrich, St. Louis, MO USA) and the plates were analyzed at 450 nm with an ELISA reader (Bio-Rad, Hercules, CA, USA). The absorbance was calculated as the average value of the triplicate determination. The anti-graft antibody response was further confirmed by flow cytometry. Briefly, cultured donor CTPs were detached and incubated with HI-recipient sera for 30 min at 37 °C. After washing with PBS-BSA-sodium azide, antibody binding was revealed using FITC-labeled anti-human IgG (Jackson ImmunoResearch Laboratories) or IgM (Dako, Milan, Italy) antibodies. Secondary antibody without serum was added to each well in a lytic solution. Median fluorescence intensity (MFI) was recorded by FACS calibur cytometer (BD Bioscience) acquisition and analyzed by CELLQUEST software (BD Bioscience).

Complement-dependent cytotoxicity (CDC). Donor CTPs were seeded into 96-well flat plates at a cell density of 32,000 cells well−1 in 100 μl of complete medium. After 48 h, the supernatant was discarded and cells were incubated for 30 min at 37 °C with 50 μl of 1:4 diluted HI-sera collected from each recipient at baseline and following CTP transplantation. Negative control wells were incubated with culture medium without any serum. After incubation, supernatants were discarded and cells were further incubated with 150 μl of baby rabbit complement (Cederlane, Burlington, Ontario, Canada) diluted 1:10 at 37 °C for 1 h. Negative control wells received complement only or HI-serum without the addition of complement. Positive control wells consisted of cells exposed to a lytic solution. Median fluorescence intensity (MFI) was recorded by FACS calibur cytometer (BD Bioscience) acquisition and analyzed by CELLQUEST software (BD Bioscience).

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Author contributions**

A.L.P., P.H., R.A.B., and E.C. conceived the idea and supervised the research. R.A.B., A. Bugi, S.W., M.V., M.M., C.J., A.N., S.L., A. Blancher, and A.L.P. performed the experiments and analyzed the data. The manuscript was written by R.A.B. and A.L.P., with input from E.C., A. Blancher and P.H. and comments from all other authors.

**Additional information**

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