Transfer of ADAMTS13 antibody-mediated thrombotic thrombocytopenic purpura via kidney transplantation

Thrombotic thrombocytopenic purpura (TTP) is a rare (annual prevalence ~10 cases per million people), relapsing and life-threatening thrombotic microangiopathy (TMA).[1,2] Acute TTP is defined by a mechanical hemolytic anemia, severe thrombocytopenia and systemic visceral ischemia causing multi-organ failure in the absence of urgent frontline therapeutic plasma exchange.[1,2] In rare cases, TTP may cause sudden cardiac death related to myocardial infarction and cardiac arrhythmia.[3] TTP is due to a severe functional deficiency (activity <10 IU/dL) of ADAMTS13 (A Disintegrin and Metalloprotease with ThromboSpondin type 1 repeats, member 13), the specific von Willebrand factor-cleaving protease.[4,5] ADAMTS13 deficiency causes the accumulation of hyper-adhesive ultralarge von Willebrand factor multimers in blood which induce the spontaneous formation of platelet-rich microthrombi within arterioles and capillaries.[1-5] So far, severe ADAMTS13 deficiency is the only highly sensitive and specific marker for acute TTP.[6] Moreover, an altered open conformation of ADAMTS13 was recently reported to be a hallmark of acute TTP.[7,8] In most cases, ADAMTS13 deficiency is acquired via specific auto-antibodies, increasing ADAMTS13 clearance and inhibiting its catalytic activity.[1-5] These ADAMTS13 antibodies cause the immune-mediated form of TTP (iTTP). Several animal models of iTTP have been developed by directly transferring ADAMTS13 antibodies, especially the baboon model in which the venous injection of isolated ADAMTS13 antibodies triggers iTTP.[9] To our knowledge, iTTP by passive transfer of ADAMTS13 antibodies has never been described in humans. In particular, in pregnancy-associated iTTP, no case of fetal TTP due to passive transfer of ADAMTS13 antibodies through the placenta has ever been described.[10] We report here the cases of two patients with iTTP transmitted through renal transplantation from a single donor who suffered a sudden death.

A 29-year old man with no medical history was admitted to hospital after sudden cardiac arrest of unknown cause (Maastricht 2 classification). His blood cell count showed a thrombocytopenia of 35x10^9/L. The patient died and his kidneys were subsequently procured for transplantation into two unrelated recipients. The demographic, clinical and biological features of both recipients are presented in Table 1. Three days after surgery, both recipients developed a TMA syndrome including a microangiopathic hemolytic anemia and severe thrombocytopenia. Recipient 1 had a rapid, spontaneous correction of hemolysis and thrombocytopenia but his renal function did not improve necessitating continuous hemodialysis and de-transplantation 1 month later. In contrast, recipient 2 needed therapeutic plasma exchange and prednisolone to recover from hemolysis and thrombocytopenia but his renal function improved allowing discharge from hospital 15 days after transplantation. The occurrence of a *de novo* TMA in the two recipients of a kidney transplant from a single donor, who died suddenly, prompted us to retrospectively investigate ADAMTS13 in historical serum samples from the three patients. Informed consent was obtained from the patients or their families according to the Declaration of

Table 1. Clinical and biological features of the two kidney recipients.

Clinical and biological data	Recipient 1						Recipient 2					
Sex/age (years)	male / 62						male / 66					
Cause of end-stage renal disease	hypertensive nephrosclerosis						IgA nephropathy					
Post-renal transplantation induction	thymoglobulin - glucocorticoid						basiliximab - glucocorticoid					
TMA treatment	none						TPE - prednisolone					
Kidney biopsy	patchy cortical necrosis due to arteriolar and glomerular thrombi						acute tubular necrosis with thrombi within glomerular arterioles					
Outcome	de-transplantation at month 1						recovery of renal function					
Kinetics (day)	D1	D3	D5	D7	D15	D30	D1	D3	D5	D7	D15	D30
Platelet count (x10^9/L)	222	40	53	151	328	152	201	48	49	128	218	225
Hemoglobin (g/dL)	12.9	8.8	8.9	11.5	9.6	9.9	12.1	12	12.7	11.3	9.7	9.6
Schistocytes	–	+++	+	+	–	–	–	+++	++	–	–	–
Haptoglobin (g/L)	1.3	<0.2	0.8	0.5	NA	NA	1.3	<0.2	0.2	0.35	0.5	NA
Lactate deshydrogenase (IU/L)	288	5460	3195	2468	1328	697	299	856	546	310	311	NA
Creatinine (µmol/L)	994 (HD)	697 (HD)	754 (HD)	571 (HD)	323 (HD)	938 (HD)	560 (HD)	534	525	354	239	111
ADAMTS13 activity (IU/dL)		<10				21		<10				30
ADAMTS13 antigen (ng/mL)		140				NA		480				NA
Anti-ADAMTS13 IgG titer (IU/mL)		61				<15		45				<15
ADAMTS13 conformation index		<0.5				<0.5		<0.5				<0.5

The main demographic, clinical and biological features of both kidney recipients are summarized. Specific ADAMTS13 studies showed that, at the early stage of acute TMA (day 3 after transplantation), ADAMTS13 activity was <10 IU/dL, ADAMTS13 antigen was decreased or subnormal (140 and 480 ng/mL, respectively) and anti-ADAMTS13 IgG were positive (61 and 45 IU/mL, respectively), supporting the diagnosis of immune-mediated thrombotic thrombocytopenic purpura (TTP). In contrast to the donor, the ADAMTS13 conformation of the recipients was found to be closed (CI <0.5). One month later, after TTP remission, both recipients recovered detectable ADAMTS13 activity (21 and 30 IU/dL) and their ADAMTS13 conformation remained closed. TMA: thrombotic microangiopathy; TPE: therapeutic plasma exchange; D: day; NA: not available; HD: hemodialysis.

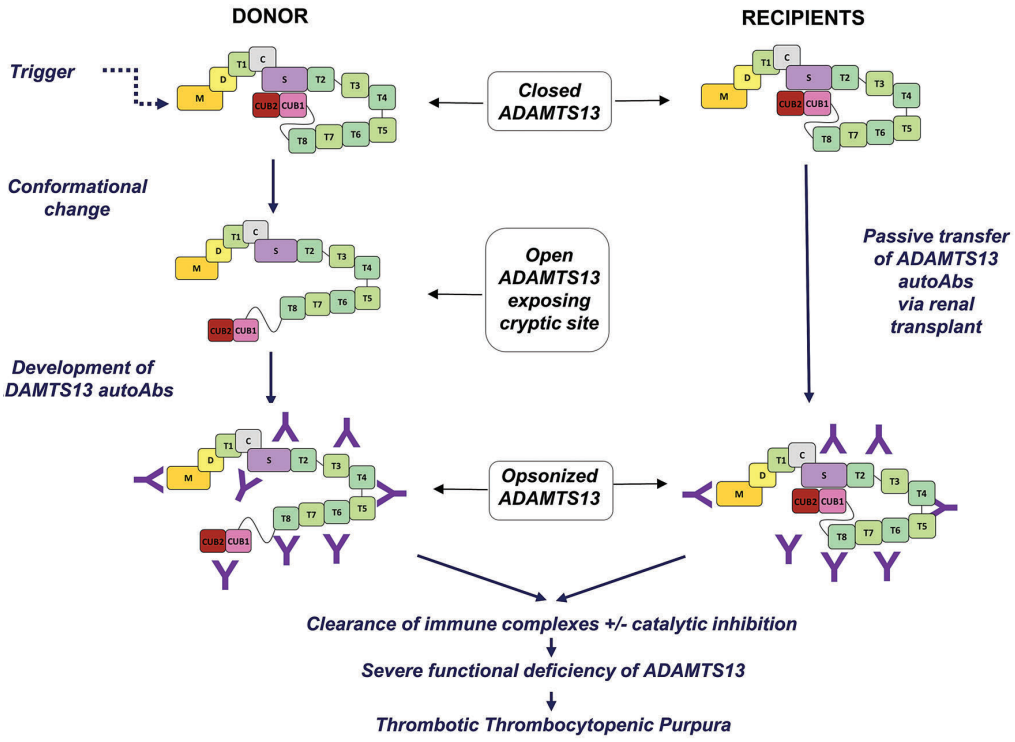

Figure 1. Comparative pathophysiological model for immune thrombotic thrombocytopenic purpura in the donor and recipients. The pathophysiology of immune thrombotic thrombocytopenic purpura (iTTP) is different between the donor and the recipients. In the donor (left), the unfolding/opening of ADAMTS13 observed during iTTP is hypothesized to proceed in two steps. First, a still unknown trigger may initiate the conformational change of ADAMTS13 consisting in a switch from a closed to an open conformation that allows the exposure of a cryptic site leading to the development of ADAMTS13 auto-antibodies (autoAbs). These auto-antibodies are polyclonal and include a constant epitope to the ADAMTS13 cryptic site. Secondly, the ADAMTS13 antibodies may not only act as enhancers of ADAMTS13 opening by binding to its exposed cryptic site but they may also opsonize ADAMTS13 leading to its clearance with or without its catalytic inhibition. In contrast, in both recipients (right), as the initial trigger is absent, ADAMTS13 remains closed. However, the passive transfer of ADAMTS13 antibodies via the transplant allows the opsonization of ADAMTS13 leading to its clearance with or without its catalytic inhibition. In the donor and the recipients, both pathways have in common the occurrence of a severe ADAMTS13 functional deficiency causing symptoms of TTP.

Helsinki. This study was approved by the ethical committees of Saint Antoine and La Pitié-Salpêtrière hospitals and registered at *www.clinicaltrials.gov as NCT00426686*.

Blood was collected from the donor prior to his death. The recipients' blood for ADAMTS13 analysis was collected 3 days after transplantation (at the time of acute TMA, before therapeutic plasma exchange) and 1 month later (TMA remission). ADAMTS13 activity (normal range, 50-100 IU/dL), antigen (normal range, 350-730 ng/mL), IgG autoantibodies (positivity >15 IU/mL) and conformation [normal closed, conformation index (CI) <0.5] were measured in serum as previously described.[7,11] The ADAMTS13 analysis supported the diagnosis of TTP in the three patients. The donor had a severe ADAMTS13 deficiency (ADAMTS13 activity <10 IU/dL and antigen 240 ng/mL), positive anti-ADAMTS13 IgG (32 IU/mL) and an open ADAMTS13 conformation (CI of 4.2), supporting a diagnosis of iTTP. The results of the ADAMTS13 analysis in both recipients, summarized in Table 1, also supported the disgnosis of iTTP, whereas their ADAMTS13 conformation was closed (CI < 0.5).

After kidney transplantation, TTP is very much less common than atypical hemolytic uremic syndrome.[12] Indeed, post-transplantation TTP is extremely rare, occurs at least 1 week after transplantation and is usually not related to ADAMTS13 antibodies.[1-5,11] The TTP observed in our two kidney recipients did not, therefore, fit the classical framework. Moreover, the fact that both recipients shared the same donor suggested that their TMA was exceptionally related to an agent transmitted by the kidney transplants. A classical iTTP linked to endogenous ADAMTS13 auto-antibodies was likely responsible for the sudden death of the young and previously healthy male donor.[3] In contrast, the recipients' iTTP was most likely related to a kidney-mediated passive transfer of ADAMTS13 antibodies. Several arguments support this hypothesis. Firstly, the short period between the occurrence of TMA after transplantation likely reflects a fast release in blood of kidney-stocked, preformed ADAMTS13 antibodies and therefore excludes both allo-immunization against the donor's ADAMTS13 and a transfer of ADAMTS13 antibody-producing B-lymphocytes from the donor. Secondly, the rapid improvement of hematologic signs, either spontaneously or after a short course of treatment with therapeutic plasma exchange, is compatible with a progressive clearance of exogenous ADAMTS13 antibodies. However, the irreversibility of the renal ischemia in one recipient and the persistence of a partial ADAMTS13 deficiency (activity ~30%) 1 month after acute TTP in both recipients suggest that ADAMTS13 antibodies clustered in the donor's kidneys were still being released at low levels into the recipients' blood several weeks after the acute TTP. Thirdly, our results on the conformation of ADAMTS13 may also indirectly argue in favor of passive transfer of ADAMTS13 antibodies (Figure 1). ADAMTS13 opening in iTTP is hypothesized to be initiated by a still unknown trigger and then enhanced by

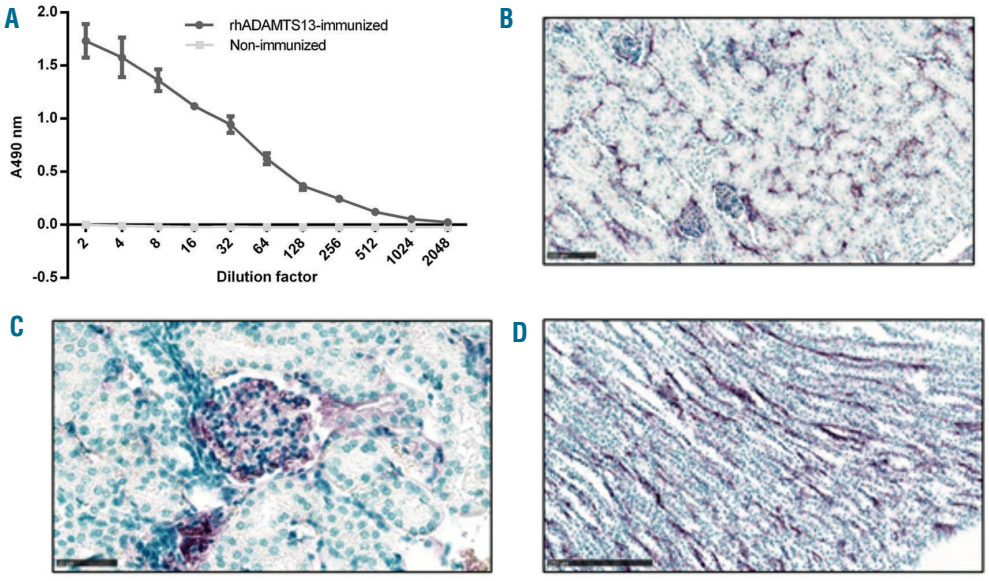

Figure 2. The presence of anti-ADAMTS13 antibodies in perfused mouse kidneys after immunization with recombinant human ADAMTS13. All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the KU Leuven, Belgium (study approved under project number P055/2015). BALB/c mice were immunized with two injections of 10 μg recombinant human (rh) ADAMTS13, one subcutaneous injection on day 1 and one intraperitoneal injection 2 weeks later. Age-matched BALB/c mice not given rhADAMTS13 injections were used as negative controls. Blood was collected via retro-orbital venipuncture on day 33 (n=2) or day 54 (n=1) and mice were then sacrificed and perfused through the heart. Immediately after perfusion, one kidney per mouse was washed, frozen and crushed on dry ice for cell lysate production. The other kidney was washed, fixed in 4% paraformaldehyde, processed and embedded in paraffin for immunohistochemical analysis. (A) The presence of anti-rhADAMTS13 antibodies in kidney lysates was determined by an enzyme-linked immunosorbent assay developed in-house. Specific anti-rhADAMTS13 antibodies were present in kidney lysates from immunized mice (n=3), but not in those from non-immunized mice (n=3). Absorbance values (mean ± SD) are depicted. (B-D) Five-micron kidney sections (RM2125 RTS manual microtome, Leica, Wetzlar, Germany) were embedded in paraffin. Sections were de-paraffinized in Sub-X clearing agent (Leica, Richmond, IL, USA) and rehydrated with distilled water using graded alcohol series. The sections were steamed and cooled in sodium citrate (pH 6.0) as the heat-induced antigen retrieval technique. After washing in tris-buffered saline with 0.1% Tween 20 (TBS-T), sections were blocked with 10% normal rabbit serum and 1% bovine serum albumin in TBS-T, followed by endogenous biotin blocking with the Avidin/Biotin System (Vector Laboratories, Burlingame, CA, USA). After blocking endogenous peroxidase activity with a 0.3% hydrogen peroxide solution and washing in TBS-T, sections were incubated for 1 h with biotinylated polyclonal rabbit-anti-mouse immunoglobulins (1/500, Dako, Glostrup, Denmark), followed by the addition of the Avidin/Biotinylated enzyme Complex (VECTASTAIN ABC kit, Vector Laboratories). The VIP Peroxidase substrate Kit (Vector Laboratories) was used to visualize the murine antibodies present in the kidney (purple color) and sections were counterstained with methyl green (Vector Laboratories) at 60°C. Murine antibodies were mainly localized in the glomeruli (B, C) and medullary peritubular capillary network (D). Pictures were taken at 40x magnification.

ADAMTS13 antibodies produced after exposure of a cryptic site.[7,8] Interestingly, the ADAMTS13 conformation was open in the donor, which further supports the diagnosis of classical iTTP. In contrast, the ADAMTS13 conformation was closed in both recipients during acute iTTP suggesting that the ADAMTS13 antibodies detected in their serum were insufficient to unfold ADAMTS13, likely because an initial trigger was absent. Thus, in both recipients, the presence of ADAMTS13 antibodies can be explained by exogenous transfer but not by endogenous autoimmune synthesis.

As the patients' kidney tissue samples were not workable for further histology, we immunized BALB/c mice with recombinant human ADAMTS13 (rhADAMTS13) produced as previously described,[13] in order to provide evidence that kidneys may stock ADAMTS13 antibodies in iTTP. The presence of rhADAMTS13 antibodies in both serum and kidney lysates was determined using an enzyme-linked immunosorbent assay developed in-house as described previously.[13] Murine antibodies in paraffin-embedded kidney sections were detected with a polyclonal rabbit-anti-murine immunoglobulin antibody (details in the legend to Figure 2). The rhADAMTS13 antibody titer in mice serum was above 1:10^5. Interestingly, rhADAMTS13 antibodies were detected in kidney lysates (Figure 2A). The presence of antibodies in the kidney was further visualized via immunohistochemical analysis with anti-mouse immunoglobulins (Figure 2B-D). Specific rhADAMTS13 antibodies, however, could not be visualized in the kidney sections due to technical limitations. These findings show that ADAMTS13 antibodies can be captured in kidney tissue, and suggest that ADAMTS13 auto-antibodies can be transferred via kidney transplantation.

In conclusion, to our knowledge, this is the first case of iTTP likely induced by passive transfer of ADAMTS13 antibodies via an organ transplant. Other immune-mediated diseases, namely peanut allergy and an autoimmune thrombocytopenic purpura, were exceptionally reported to be transferred from a donor to a recipient by either combined liver and kidney transplantation[14] or liver transplantation,[15] respectively. This study highlights potential specific features of ADAMTS13 antibodies.

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