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Increased Autoantibodies Against Ro/SS-A, CENP-B, and La/SS-B in Patients With Kidney Allograft Antibody-mediated Rejection

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Background. Antibody-mediated rejection (AMR) causes more than 50% of late kidney graft losses. In addition to anti-human leukocyte antigen (HLA) donor-specific antibodies, antibodies against non-HLA antigens are also linked to AMR. Identifying key non-HLA antibodies will improve our understanding of AMR. **Methods.** We analyzed non-HLA antibodies in sera from 80 kidney transplant patients with AMR, mixed rejection, acute cellular rejection (ACR), or acute tubular necrosis. IgM and IgG antibodies against 134 non-HLA antigens were measured in serum samples collected pretransplant or at the time of diagnosis. **Results.** Fifteen non-HLA antibodies were significantly increased ($P < 0.05$) in AMR and mixed rejection compared with ACR or acute tubular necrosis pretransplant, and 7 at diagnosis. AMR and mixed cases showed significantly increased pretransplant levels of IgG anti-Ro/Sjögren syndrome-antigen A (SS-A) and anti-major centromere autoantigen (CENP)-B, compared with ACR. Together with IgM anti-CENP-B and anti-La/SS-B, these antibodies were significantly increased in AMR/mixed rejection at diagnosis. Increased IgG anti-Ro/SS-A, IgG anti-CENP-B, and IgM anti-La/SS-B were associated with the presence of microvascular lesions and class-II donor-specific antibodies ($P < 0.05$). Significant increases in IgG anti-Ro/SS-A and IgM anti-CENP-B antibodies in AMR/mixed rejection compared with ACR were reproduced in an external cohort of 60 kidney transplant patients. **Conclusions.** This is the first study implicating autoantibodies anti-Ro/SS-A and anti-CENP-B in AMR. These antibodies may participate in the crosstalk between autoimmunity and alloimmunity in kidney AMR.

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A.K. conceived the study. S.C.-F., H.C., M.D., R.J., A.C., and A.K. participated in study design. S.C.-F., C.M.M., S.R.-R., and A.K. retrieved and curated clinical data from the discovery cohort. H.C., M.D., and M.-J.H. retrieved and curated clinical data from the validation cohort. S.C.-F. and A.C. performed experiments. S.C.-F., M.K., C.M.M., C.P., S.F., I.J., and A.K. analyzed the data. S.C.-F., M.K., C.P., and S.R.-R. made the figures. S.C.-F., S.R.-R., and A.K. drafted and revised the paper; and all authors approved the final version of the manuscript. I.J.

Antibody-mediated rejection (AMR) causes more than 50% of late graft failures in kidney transplantation.[1] AMR is usually caused by antibodies against human leukocyte antigens (HLA). Although histologic findings suggestive of AMR (ie, microvascular inflammation) are linked to anti-HLA–mediated injury, some patients develop these lesions in the absence of anti-HLA donor-specific antibodies (DSA).[2–6] In turn, not all transplant patients with anti-HLA DSA develop rejection,[7] suggesting the involvement of other mechanisms in AMR.

Non-HLA alloantibodies or autoantibodies may contribute to the pathogenesis of AMR. Antibodies against vimentin,[8,9] angiotensin II type-1 receptor,[2,10–15] collagen,[16,17] fibronectin,[16] perlecan/LG3,[18–21] and agrin,[5] as well as anti-apoptotic cell autoantibodies,[22–25] are associated with reduced survival and allograft rejection.[2,18,20,26] Non-HLA antibodies are not routinely monitored, and their contribution to kidney allograft injury is unclear. Moreover, their dynamic levels and their relationship with cellular rejection or other forms of graft injury remain unknown.

Production of autoantibodies may relate to viral infections, molecular mimicry, cryptic antigen exposure,[27–32] or as-yet unrecognized mechanisms. Autoantibodies produced post-transplant could result from immunotherapy-induced loss of regulatory T-cell proliferation and loss of tolerance to self-antigens.[33–35] Although several non-HLA autoantibodies recognized in systemic lupus erythematosus (SLE) and connective tissue disease have been extensively studied in autoimmunity,[36–39] their role in alloimmunity has not been examined. Yet autoimmune and alloimmune kidney injury share similarities, especially with regards to vascular injury.[40,41] Furthermore, both SLE and allograft rejection[42] are characterized by Th17 responses.[43,44] There is increasing recognition of the interplay between alloimmunity and autoimmunity,[17,23,45] and this crosstalk may perpetuate injury.[46]

Our aim was to identify non-HLA antibodies associated with AMR and to determine their evolution over time and their link to DSA and histopathology lesions. We describe herein a retrospective cohort of 80 kidney transplant patients with 134 non-HLA antibodies measured pretransplant and at the time of indication biopsy-based diagnosis. Antibodies previously implicated in solid organ transplant injury or autoimmunity were measured using protein arrays. We identified anti-Ro/Sjögren syndrome-antigen A (SS-A; 52 kDa), anti-major centromere autoantigen (CENP)-B, and anti-La/SS-B antibodies as significantly increased in kidney transplant recipients with AMR, compared with patients with acute cellular rejection (ACR) or acute tubular necrosis (ATN). These antibodies were associated with class-II DSA and microvascular lesions. Increased levels of these antibodies in AMR were reproduced in an external, independent cohort. This is the first study, to our knowledge, that links autoimmunity-related antibodies against Ro/SS-A and CENP-B to AMR after kidney transplantation.

MATERIALS AND METHODS

Study Population

We first studied a discovery cohort consisting of 80 patients. We identified kidney transplant recipients at the University Health Network in Toronto, with rejection diagnosed between 2008 and 2016, by searching the CoReTRIS registry.[47] We selected cases with histological diagnosis of rejection on a for-cause biopsy and at least 1 serum sample available in the HLA laboratory. Samples with insufficient volume and retrieved within 21 d after plasmapheresis and intravenous immunoglobulin (IVIG) were excluded. Patient exclusion criteria were no serum sample available or all serum samples affected by plasmapheresis and IVIG. Finally, we selected ATN cases graft age-matched to AMR and ACR cases. A renal pathologist (R.J.) scored the biopsies according to the Banff classification (2017).[3] Twenty-seven of these 80 patients were described in our recent study.[48] This study was approved by the University Health Network institutional research ethics board (CAPCR identifier: 13-7261).

Serum samples were collected pretransplant and at diagnosis. The median time difference between the at diagnosis serum sample collection and the biopsy date was 0 d, whereas the mean was −1.5 d. The presence of anti-HLA class-I and class-II antibodies in the sera was assessed using Luminex single-antigen bead assays, as part of standard clinical practice. To assess non-HLA antibody levels, we quantified IgG and IgM antibodies against 134 non-HLA antigens, using a VersArray Chipwriter Pro antigen microarray platform (Virtek, Canada). Antigen characteristics are described in Table S1 (**SDC**, http://links.lww.com/TXD/A365).

We also evaluated if our key findings were reproduced in a second, external cohort of patients. For this purpose, we studied sera from 60 kidney transplant patients from Center Hospitalier de l'Université de Montréal that were retrieved at diagnosis (median time ≤4 d from the indication biopsy). In these samples, IgG and IgM levels against key non-HLA antibodies were measured using the same microarray platform. This cohort was previously described and consisted of 29 stable nonrejecting cases, 16 ACR cases, and 15 cases with acute vascular rejection (AVR).[49] Male sex was predominant in the ACR (66%) and control (60%) groups but not among the AVR patients (40%). Twelve patients were positive for class-I or class-II DSA (6 cases with AVR, 3 with ACR, and 3 with stable graft function). In comparison to the control cases, patients with AVR were younger. Cases with AVR also displayed a significant decrease in the estimated glomerular filtration rate (eGFR) at the time of serum collection, relative to the control group (median eGFR [AVR] = 5 [0–37] mL/min per 1.73 m^2; median eGFR [control] = 59 [52–72]

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mL/min per 1.73 m^2; $P < 0.05$). The immunosuppression regimen was comparable across groups and often included calcineurin inhibitors, steroids, and mycophenolate mofetil. At the histopathology level, 75% of patients with AVR were positive for C4d staining in peritubular capillaries, whereas all ACR cases were negative. Half of the AVR cases that were C4d positive were also positive for DSA before transplantation.[49] Upon review of the AVR cases, we were able to assign a diagnosis of AMR or mixed rejection (n = 5), ACR grade 2–3 (n = 3), and AVR with insufficient information to delineate between AMR and ACR (n = 7). The study was approved by the clinical research ethics committee at Center Hospitalier de l'Université de Montréal (Research Ethics Board number: 2008-2545, HD.07.034).

Histopathology

Indication biopsies were embedded in paraffin, and 3-µm sections were obtained in a microtome (Leica). Sections were deparaffinized through graded alcohols and subjected to hematoxylin/eosin, trichrome, periodic acid–Schiff, and periodic Schiff-methenamine stains and examined by light microscopy. Staining of C4d was performed on 4-µm frozen sections by immunofluorescence. Morphologic features were diagnosed and given a semiquantitative score (0–3) by R.J., according to the updated Banff classification.[3,48]

Non-HLA Antigen Microarrays

The 134 antigens, including proteins, peptides, and cell lysates, were diluted to 0.2 mg/mL in PBS and stored in aliquots at −80°C. These antigens were selected because of their importance in autoimmune diseases[50,51] or because they were linked to humoral rejection of several organs, including kidney,[52] lung,[53] or heart.[54] Antibody levels were measured using median fluorescence intensity (MFI). Values of MFI ≥200 in at least 1 sample were set as limit of detection to further study the antibody signal against a particular antigen. A signal with MFI ≥200 was >3 SD from the average MFI of the negative control, and >7 SD from the average MFI of the blanks. A similar criterion was previously used in other protein arrays of patient sera.[25] Setting the positivity cutoff at MFI ≥200 prevented us from detecting artifactual but significant differences involving marginal MFIs. Details on the generation of the microarrays, sample processing, and quantification of fluorescence intensity to determine antibody levels can be found in the Supplemental Information, **SDC**, http://links.lww.com/TXD/A365.

Statistical Analysis

Differential antibody levels analysis of both data sets (Toronto and Montreal cohorts) was performed in R. Two-sided Wilcoxon-Mann-Whitney nonparametric tests were used to assess differences in non-HLA antibody levels between groups in the first cohort (Toronto), whereas 1-sided Wilcoxon-Mann-Whitney nonparametric tests were used for the comparative analyses in the second cohort (Montreal). By definition, both AMR and mixed cases show DSA and histological signs of antibody-mediated injury. We thus combined the AMR and the mixed cases in 1 single AMR/mixed group in the differential antibody MFI analysis and compared this group with ACR and ATN. This enabled us to enhance the statistical power of the comparative analysis. Data are presented as medians and interquartile range, and $P < 0.05$ was considered significant.

Upon identification of key antibodies differentially altered in AMR/mixed rejection patients in the Toronto cohort, we conducted a sample size calculation analysis based on the levels of IgG anti-CENP-B, IgG anti-Ro/SS-A, and IgM anti-La/SS-B, to estimate the sample size required to achieve adequate statistical power in the second cohort. This analysis was done with R package WMWssp[55] version 0.4.0, using the WMWssp function.

Bioinformatics

We performed hierarchical clustering analyses of the antibodies differentially altered between study groups in the Toronto cohort. We also assessed their changes over time, and their association with clinical variables. Finally, we built a protein–protein interaction network of key non-HLA antibody targets (Supplemental Information, **SDC**, http://links.lww.com/TXD/A365).

RESULTS

Study Population

We studied sera from 32 patients with AMR, 20 patients with mixed rejection, 16 patients with ACR, and 12 patients with ATN (Table 1; Figure 1A). Most patients were males, except for the AMR cases, who had ≈50% males. Patients with ATN were older than other groups. The median time between transplantation and diagnostic biopsy was similar in patients with AMR, ACR, and ATN (9.5–15.5 d) but higher among mixed cases (174 days). Most patients with AMR (78%) or mixed rejection (90%) had class-I and class-II DSA (Table 1). Seven patients with AMR or mixed rejection had no DSA. None of the ACR cases had DSA. Although 4/12 ATN patients had DSA, their biopsies did not show signs of rejection (Table 2). Glomerulitis and C4d deposition were detected exclusively among AMR and mixed cases. These 2 groups showed the highest scores for peritubular capillaritis. The highest interstitial inflammation, tubulitis, and total inflammation were observed in mixed rejection and ACR (Table 2). All 52 patients with AMR or mixed rejection had active rejection. Of the 32 patients with AMR, 29 were classified as acute/active AMR cases, and only 3 of them showed signs of chronic glomerulopathy and were classified as patients with chronic active AMR. Similarly, 19/20 patients with mixed rejection were classified as acute/active rejection cases, and only 1 of them showed histopathologic signs of chronic active rejection (Table 2).

Antibodies Against Ro/SS-A, CENP-B, and La/SS-B Are Increased in Kidney AMR and Mixed Rejection

The workflow of the study is shown in Figure 1B. We analyzed 112 serum samples using protein arrays against 134 non-HLA antigens (Table S1, **SDC**, http://links.lww.com/TXD/A365). We focused on 119 IgG and 120 IgM antibodies against non-HLA antigens detected with MFI ≥200 in ≥1 sample. We were mainly interested in non-HLA antibodies associated with antibody-mediated injury. Among the studied non-HLA antibodies, 20 were significantly altered ($P < 0.05$) in AMR/mixed rejection compared with ACR and ATN, at one or more time points (Table 3), and 19 (95%) of them were increased in AMR/mixed rejection. IgG anti-Ro/SS-A (52 kDa) and IgG anti-CENP-B were significantly increased in AMR/mixed patients compared with ACR, both pretransplant ($P = 0.011$ and $P = 0.009$, respectively) and at diagnosis

TABLE 1.

Patient characteristics

	Antibody-mediated rejection	Mixed rejection	Acute cellular rejection	Acute tubular necrosis
Number of patients	32	20	16	12
Sex, number of males, n (%)	17 (53)	13 (65)	12 (75)	9 (75)
Patient age at biopsy, median, y (IQR)	51.5 (36.7–57)	48 (36.7–56)	44 (36.2–51.5)	64.5 (62.2–69.7)
Time posttransplant, median, d (IQR)	10 (8–25.7)	174 (72–737)	15.5 (12–29.2)	9.5 (7.7–12.2)
Cause of ESKD				
Diabetic nephropathy, n (%)	7 (22)	3 (15)	1 (6.2)	4 (33.3)
IgA nephropathy, n (%)	2 (6.2)	2 (10)	5 (31.2)	1 (8.3)
PCKD, n (%)	3 (9.4)	5 (25)	3 (18.7)	2 (16.6)
Vasculitis, n (%)	3 (9.4)	0 (0)	1 (6.2)	0 (0)
FSGS, n (%)	2 (6.2)	2 (10)	1 (6.2)	1 (8.3)
Hypertension, n (%)	1 (3)	1 (5)	0 (0)	1 (8.3)
SLE, n (%)	2 (6.2)	1 (5)	0 (0)	0 (0)
TMA, n (%)	2 (6.2)	0 (0)	0 (0)	0 (0)
Unknown, n (%)	3 (9.4)	4 (20)	0 (0)	2 (16.6)
Other, n (%)	7 (22)	3 (15)	5 (31.2)	1 (8.3)
Preexisting autoimmune,[a] n (%)	10 (31)	3 (15)	3 (19)	0 (0)
Renal replacement therapy	31 (97)	16 (80)	14 (87.5)	12 (100)
Intermittent hemodialysis, n (%)	29 (90.6)	11 (55)	10 (62.5)	11 (91.6)
Peritoneal dialysis, n (%)	2 (6.2)	5 (25)	4 (25)	1 (8.3)
Preemptive, n (%)	1 (3)	4 (20)	2 (12.5)	0 (0)
Prior desensitization, n (%)	12 (37.5)	3 (15)	0 (0)	0 (0)
Rituximab, n (%)	2 (6.2)	2 (10)	0 (0)	0 (0)
Kidney transplant, donor type				
Living donor, n (%)	14 (44)	9 (45)	9 (56.2)	3 (25)
Deceased donor, n (%)	18 (56.2)	11 (55)	7 (43.7)	9 (75)
Induction therapy				
Thymoglobulin, n (%)	30 (94)	16 (80)	11 (68.8)	12 (100)
Basiliximab, n (%)	2 (6.2)	3 (15)	5 (31.2)	0 (0)
Unknown, n (%)	1 (3)	1 (5)	0 (0)	0 (0)
Maintenance therapy, n (%)				
Prednisone	32 (100)	20 (100)	16 (100)	12 (100)
Antiproliferative	31 (97)	20 (100)	16 (100)	12 (100)
Calcineurin Inhibitor	32 (100)	20 (100)	16 (100)	12 (100)
ABO incompatible, n (%)	1 (3)	0 (0)	0 (0)	0 (0)
Delayed graft function, n (%)	14 (44)	3 (15)	4 (25)	2 (16.6)
Primary nonfunction, n (%)	2 (6.2)	1 (5)	0 (0)	0 (0)
DSA, current or historic				
Any DSA	25 (78)	18 (90)	0 (0)	4 (33.3)
Class I, n (%)	16 (50)	8 (40)	0 (0)	4 (33.3)
Class II, n (%)	21 (66)	17 (85)	0 (0)	0 (0)
Unknown, n (%)	1 (3)	1 (5)	1 (6.2)	1 (8.3)

[a]Autoimmune conditions included: primary sclerosing cholangitis (n = 1), inflammatory bowel disease (n = 4), ANCA-vasculitis (n = 4), SLE (n = 3), hypo/hyperthyroidism (n = 6), and diabetes type I (n = 3).
DSA, donor-specific antibodies; ESKD, end-stage kidney disease; FSGS, focal and segmental glomerulosclerosis; IQR, interquartile range; n, number; PCKD, polycystic kidney disease; SLE, systemic lupus erythematosus; TMA, thrombotic microangiopathy.

(P = 0.032 and P = 0.012, respectively). Together with these 2 antibodies, IgM anti-CENP-B and IgM anti-La/SS-B were significantly increased at diagnosis in AMR/mixed patients, compared with ACR (P = 0.044 and P = 0.030, respectively).

As shown by the violin plots, the distributions of IgG anti-Ro/SS-A (52 kDa), IgG and IgM anti-CENP-B, and IgM anti-La/SS-B were remarkably similar between patients with AMR and mixed rejection but different from ACR and ATN, and remained consistent across time (Figure 2A). Hierarchical clustering analysis showed that levels of IgG anti-Ro/SS-A (52 kDa) pretransplant and at diagnosis clustered with IgG and IgM anti-CENP-B and were highest in patients with class-II DSA (Figure S1A and C, **SDC**, http://links.lww.com/TXD/A365). Anti-mitochondrial antibodies against components of the pyruvate dehydrogenase (PDH) complex, namely IgM anti-PDH (P = 0.047) and IgG anti-M2 (PCD-E2, OGDC-E2, BCOADC-E2 antigens) (P = 0.031), were also significantly increased in AMR/mixed compared with ACR and ATN, respectively, at diagnosis (Figure 2A and B; Table 3).

In addition to IgG anti-Ro/SS-A and anti-CENP-B, we found 14 non-HLA antibodies significantly altered before transplant. Thirteen of them were increased in AMR/mixed rejection (Figure S2, **SDC**, http://links.lww.com/TXD/A365; Table 3). Pretransplant levels of 4 IgG antibodies were significantly higher in AMR/mixed rejection compared with ACR, including anti-mitochondrial IgG antibodies against M2 (P = 0.005) and PDH (P = 0.023). Compared with ATN, AMR and mixed cases displayed significantly increased pretransplant

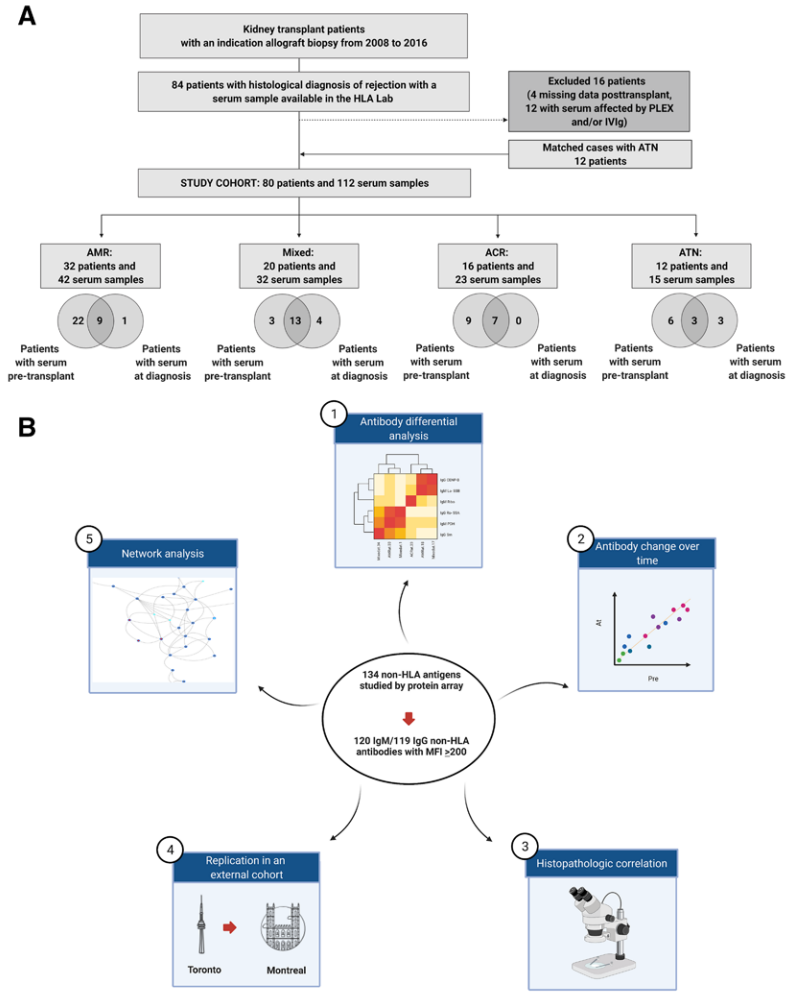

FIGURE 1. Experimental design and study workflow. In the discovery phase, we identified kidney transplant recipients with rejection diagnosed between 2008 and 2016 (A). Patient exclusion criteria were no serum sample available or all serum samples collected within 21 d after plasmapheresis (PLEX) and intravenous immunoglobulin (IVIG) administration. Graft age-matched cases with acute tubular necrosis (ATN) were also included. A total of 112 sera were selected from 80 kidney transplant patients, with antibody-mediated rejection (AMR, n = 32), mixed antibody-mediated and cellular rejection (n = 20), acute cellular rejection (ACR, n = 16), or ATN (n = 12). Our study workflow is shown in panel B. We subjected all non-human leukocyte antigen (HLA) antibodies to statistical analyses to assess differences between groups. We also performed clustering analyses to assess how the antibodies clustered in relation to the diagnoses and the anti-HLA donor-specific antibodies (DSA). Changes over time of key non-HLA antibodies were studied by plotting the median fluorescence intensity (MFI) at diagnosis compared to MFI pretransplant, in patients who had both samples available for the analysis. We next studied the association between the levels of each non-HLA antibody and the presence of histopathology lesions and anti-HLA DSA. We also interrogated our top antibodies of interest in an independent external cohort. Finally, we built a protein–protein interaction network that integrates our top non-HLA antibody targets with our previous proteomics data sets of the AMR glomeruli and tubulointerstitium.

levels of 9 non-HLA antibodies, including anti-mitochondrial antibodies IgM anti-OGDC-E2 (P = 0.029) and IgG anti-PDH (P = 0.049).[56,57] The distributions of these antibodies were similar between AMR and mixed cases (Figure S2, **SDC**, http://links.lww.com/TXD/A365). Pretransplant levels of 6 antibodies differed between sexes. Although IgM against 3 ssDNA/dsDNA antigens was increased in women, men showed increased levels of IgG against Grp78/BiP, HGMEC lysate, and Asparaginyl-tRNA Synthetase (Table S2, **SDC**, http://links.lww.com/TXD/A365).

Intraindividual Variability of Non-HLA Antibodies Over Time

We next evaluated antibody changes over time. We focused on the subset of patients that had serum samples collected at both time points (before transplant and at diagnosis). We examined intraindividual changes in the levels of the 9 IgG and 10 IgM antibodies altered in AMR/mixed rejection pretransplant and at diagnosis. For each antibody, we compared the levels at diagnosis to pretransplant levels. Most IgG and IgM antibodies did not fluctuate over time (Figure S3A and B, **SDC**, http://links.lww.com/TXD/A365). Nonetheless, several AMR and mixed rejection patients displayed an increase in antibodies at diagnosis, including IgG against Ro/SS-A (52 kDa), human IgA, and M2, and IgM against La/SS-B, and PDH (Figure 2C).

Non-HLA Antibodies Are Associated With Histopathology Features and DSA

We examined whether levels of non-HLA antibodies were associated with the presence of histopathological lesions and anti-HLA DSA. We focused on 26 non-HLA antibodies

TABLE 2.

Biopsy findings of the patient cohort

	AMR	Mixed	ACR	ATN
Histopathology classification (no. of cases)	32	20	16	12
Acute active rejection	29	19	16	0
Chronic active rejection	3	1	0	0
Chronic inactive rejection	0	0	0	0
Histopathology features, median (IQR)				
i	0 (0–0.75)	2 (1.7–3)	2 (1.7–2)	0 (0–0)
t	0 (0–0)	2 (2–3)	3 (2.7–3)	0 (0–0)
ti	0 (0–1)	2 (2–3)	2 (2–3)	0 (0–0)
g	1 (0–2)	0 (0–2)	0 (0–0)	0 (0–0)
ptc	0.5 (0–1.75)	2 (1–2)	1 (0–1)	0 (0–0)
cg	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
mm	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
v	0 (0–0)	1 (0–1)	0 (0–0)	0 (0–0)
ci	0 (0–0)	1 (0–1)	1(0–1)	0 (0–0)
ct	0 (0–0)	1 (0–1)	1 (0.7–1)	0 (0–1)
ah	0 (0–1)	1 (0–1)	0 (0–1)	0 (0–0)
cv	0 (0–2)	1 (0–1.5)	1 (0–1.7)	1 (0–1)
C4d	3 (3–3)	3 (2–3)	0 (0–0)	0 (0–0)
Globally sclerosed glomeruli (%)	3.8 (0–7.5)	5 (0–11.3)	4 (0–10.8)	4 (0–6.3)

Histopathology lesions were evaluated according to the most updated Banff classification.
ACR, acute cellular rejection; ah, arteriolar hyalinosis; AMR, antibody-mediated rejection; ATN, acute tubular necrosis; c4d, c4 deposition; cg, chronic glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy; cv, vascular fibrous intimal thickening; g, glomerulitis; i, interstitial inflammation; IQR, interquartile range; mm, mesangial matrix expansion; ptc, peritubular capillaritis; t, tubulitis; ti, total inflammation; v, intimal arteritis.

pretransplant and 34 antibodies at diagnosis that was significantly and more strongly associated with at least 1 feature ($P < 0.05$; Figure 3; Table S3, **SDC**, http://links.lww.com/TXD/A365).

Increased levels of IgG anti-Ro/SS-A (52 kDa) and anti-CENP-B, both pretransplant and at diagnosis, were significantly associated with the presence of peritubular capillaritis, glomerulitis, intimal arteritis, C4d deposition, and chronic glomerulopathy but not with tubulitis or interstitial/total inflammation (Figure 3). Increased pretransplant levels of IgG and IgM anti-CENP-B were significantly associated with the presence of class-I and, more strongly, class-II DSA, whereas elevated IgG anti-Ro/SS-A (52 kDa) was associated predominantly with the presence of class-II DSA (Figure 3A). At diagnosis, increased levels of IgG anti-Ro/SS-A (52 kDa) and IgG anti-CENP-B were significantly associated with the presence of class-I/II DSA (Figure 3B). Accordingly, higher levels of antibodies pretransplant and at diagnosis tended to cocluster with the presence of class-II DSA (Figure S1, **SDC**, http://links.lww.com/TXD/A365). Two other antibodies significantly increased in AMR/mixed rejection at diagnosis, namely IgM anti-La/SS-B and IgG anti-M2, were significantly associated with the presence of glomerulitis, C4d deposition, chronic glomerulopathy, and DSA (Figure 3B). Interestingly, we found that relevant clinical features were significantly associated with IgG against molecular chaperones: although higher IgG anti-HSP90 was linked to the presence of chronic glomerulopathy, increased IgG anti-HSP27 and anti-HSP60 levels were associated with C4d deposition and DSA (Figure 3B). Few antibodies (6/26 pretransplant and 13/34 at diagnosis), including IgG anti-dsDNA, IgG anti-HSP60, and IgG anti-Tropomyosin, were significantly and positively associated with tubulitis and total inflammation (Figure 3A).

Given the association between increased levels of key non-HLA antibodies and the presence of class-I and class-II DSA, we next examined if any non-HLA antibodies were significantly altered between AMR/mixed patients that were DSA positive (n = 43), and those that were DSA negative (n = 7). Of note, 2 patients did not have adequate donor HLA typing to establish the presence or absence of DSA. Interestingly, IgG antibody levels against 3 different DNA-related antigens (ssDNA, dsDNA, and dsDNA [plasmid]) were significantly increased before transplant in DSA positive AMR/mixed patients, compared with DSA-negative cases. In addition, IgG anti-ssDNA and IgG anti-dsDNA were still significantly increased in the presence of DSA at diagnosis (Table S4, **SDC**, http://links.lww.com/TXD/A365). The levels of IgG anti-CENP-B were remarkably similar between DSA-positive and DSA-negative AMR/mixed patients before transplant and at diagnosis (Table S4, **SDC**, http://links.lww.com/TXD/A365). However, we observed significantly increased levels of IgG anti-CENP-B in AMR/mixed rejection compared with ACR, even when considering only the DSA negative cases, both pretransplant and at diagnosis (Table S5, **SDC**, http://links.lww.com/TXD/A365).

Antibodies Against Ro/SS-A, CENP-B, and La/SS-B Are Increased in AMR/Mixed Rejection Compared With ACR in an External Cohort

We next investigated if the observed significant increases in non-HLA antibodies in AMR/mixed rejection identified in the discovery group (Toronto) could be reproduced in an independent cohort. We leveraged a second, external cohort to interrogate the antibodies against 4 antigens found to be significantly increased in AMR/mixed cases at diagnosis, namely anti-Ro/SS-A (52 kDa), anti-CENP-B, anti-La/SS-B, and anti-PDH. We also interrogated their corresponding IgG or IgM levels (Table 4). In particular, we analyzed serum samples from a previously described cohort of 60 kidney transplant patients (Montreal),[49] including patients with AVR, ACR, and stable

TABLE 3.

Antibodies against non-HLA antigens significantly altered in AMR/mixed rejection, compared, ACR and ATN

		Antibody levels		
		Before transplant		
Antibody name	**Antigen specificity**	**AMR/mixed, median (IQR)**	**ACR, median (IQR)**	***P***
IgG M2	M2 (PDC-E2 + OGDC-E2 + BCOADC-E2)	267 (0–513)	0 (0–0)	0.0051
IgG CENP-B	Major centromere autoantigen B	312.5 (0–877.2)	0 (0–235.2)	0.0097
IgG Ro/SS-A (52 kDa)	Ro/SS-A (52 kDa)	315 (0–915.7)	0 (0–257.5)	0.0112
IgG gliadin	Gliadin	3209 (1485.8–5474)	1618.5 (793–3110.6)	0.0157
IgG PDH	Pyruvate dehydrogenase	238 (0–507)	0 (0–226)	0.0232
IgG smooth muscle	Smooth muscle actin	382.5 (304.5–532.8)	310.8 (230.5–374.1)	0.0457
		AMR/mixed, median (IQR)	**ATN, median (IQR)**	***P***
IgM PL-12	Alanyl-tRNA synthetase	1049.5 (544.2–1672.8)	352 (236.5–601)	0.0074
IgM HGMEC lysate	Glomerular endothelial cells	1393.5 (881–2234.5)	752 (522–1131.5)	0.0224
IgM PM/Scl-100	PM/Scl-100	317.5 (0–451.5)	0 (0–0)	0.0234
IgM OGDC-E2	M2 (OGDC)	426 (104.8–766)	0 (0–307.5)	0.0298
IgG HCEC cytoplasm	Cardiac endothelial cells	380.5 (229.2–532.7)	222 (0–294.5)	0.0309
IgM LG3	Basement membrane-specific heparan sulfate proteoglycan core protein	402.5 (238.3–607.8)	0 (0–401)	0.0365
IgM LKM 1 hp	LKM 1 hp antigen of cytochrome P450 2D6	210.5 (0–314)	0 (0–0)	0.0393
IgG Ro/SS-A (60 kDa, R)	Ro/SS-A (60 kDa)	0 (0–233.5)	296 (0–559.5)	0.0403
IgM Sm (NR, B)	Small nuclear ribonucleoprotein Sm	311 (0–495.7)	0 (0–299.5)	0.0470
IgG PDH	Pyruvate dehydrogenase	238 (0–507)	0 (0–0)	0.0494
		Antibody levels		
		At diagnosis		
Antibody name	**Antigen specificity**	**AMR/mixed, median (IQR)**	**ACR, median (IQR)**	***P***
IgG CENP-B	Major centromere autoantigen B	510 (0–877.8)	0 (0–0)	0.0126
IgG Ro/SS-A (52 kDa)	Ro/SS-A (52 kDa)	353.5 (0–1494)	0 (0–0)	0.0325
IgM La/SS-B	La/SS-B	735.5 (390.8–1843)	296 (139.5–411)	0.0261
IgM CENP-B	Major centromere autoantigen B	666.5 (313.5–1889.5)	262 (100.2–429)	0.0447
IgM PDH	PDH	251 (0–684.5)	0 (0–0)	0.0472
		AMR/mixed, median (IQR)	**ATN, median (IQR)**	***P***
IgG M2	M2 (PDC-E2 + OGDC-E2 + BCOADC-E2)	219 (0–596.5)	0 (0–0)	0.0313
IgG human IgA	Human IgA	750.5 (229.5–1322)	0 (0–337.5)	0.0495

Significantly altered ($P < 0.05$) IgG and IgM antibodies before transplant, at diagnosis, and postdiagnosis are shown.
ACR, acute cellular rejection; AMR, antibody-mediated rejection; ATN, acute tubular necrosis; B, bovine; HLA, human leukocyte antigen; hp, high purity; Ig, immunoglobulin; IQR, interquartile range; NR, nonrecombinant; R, recombinant.

recipients. We were able to reclassify a subgroup of AVR cases as having AMR or mixed rejection or ACR grade 2–3.

Concordantly with our findings in the discovery cohort, we observed significantly increased levels of IgG anti-Ro/SS-A (52 kDa, $P = 0.004$), IgG anti-PDH ($P = 0.008$), and IgM anti-CENP-B ($P = 0.029$) in AMR/mixed compared with ACR patients. AMR/mixed cases also displayed increased levels of IgG anti-La/SS-B, relative to the ACR cases ($P = 0.030$; Figure 4, Table 4). When compared with stable controls, AMR/mixed patients showed significantly increased levels of IgG anti-Ro/SS-A (52 kDa, $P = 0.002$), IgG anti-La/SS-B ($P = 0.009$), IgM anti-CENP-B ($P = 0.027$), and higher levels of IgG anti-PDH ($P = 0.066$). Reassuringly, IgG anti-Ro/SS-A (52 kDa) and IgM anti-CENP-B were significantly increased when comparing all cases with AVR with stable controls ($P = 0.008$ and $P = 0.007$, respectively), and remained elevated when comparing AVR to ACR (Table S6, **SDC**, http://links.lww.com/TXD/A365).

Network Analysis Identifies Interactions Between Antibody Targets and Differentially Expressed Proteins in AMR

Antibodies against HLA and non-HLA antigens interact with proteins expressed by parenchymal cells, including endothelial and epithelial cells.[58–62] We leveraged our recent proteomics study of glomeruli and tubulointerstitium in grafts with AMR compared to ACR and ATN[48] and built a protein–protein interaction network to study the connections between proteins significantly dysregulated in AMR kidneys, and protein targets of key antibodies identified in this study. We focused on targets of antibodies increased in AMR/mixed rejection and externally validated: tripartite motif-containing protein 21 (TRIM21; target of anti-Ro/SS-A [52 kDa]), CENP-B (target of anti-CENP-B), SS-B (target of anti-La/SS-B), and PDHA1/PDHB (targets of anti-PDH) (Figure 5). We found direct interactions between TRIM21, HSP90AB1 (increased in AMR glomeruli), and HLADRB1 (increased in AMR tubulointerstitium).[48] The molecular chaperone HSP90AA1 and the proliferation marker PCNA connected with HLA class-I antigens (increased in AMR)[48] and with antibody targets including centromeric proteins (CENP-A and CENP-B), metabolic enzymes (PDHA1, PDHB, DLAT, DBST, and DBT), and the ribosome-related protein SS-B. The high connectivity between these proteins suggests biological relevance of both the proteins and antibodies directed against them in antibody-mediated injury.

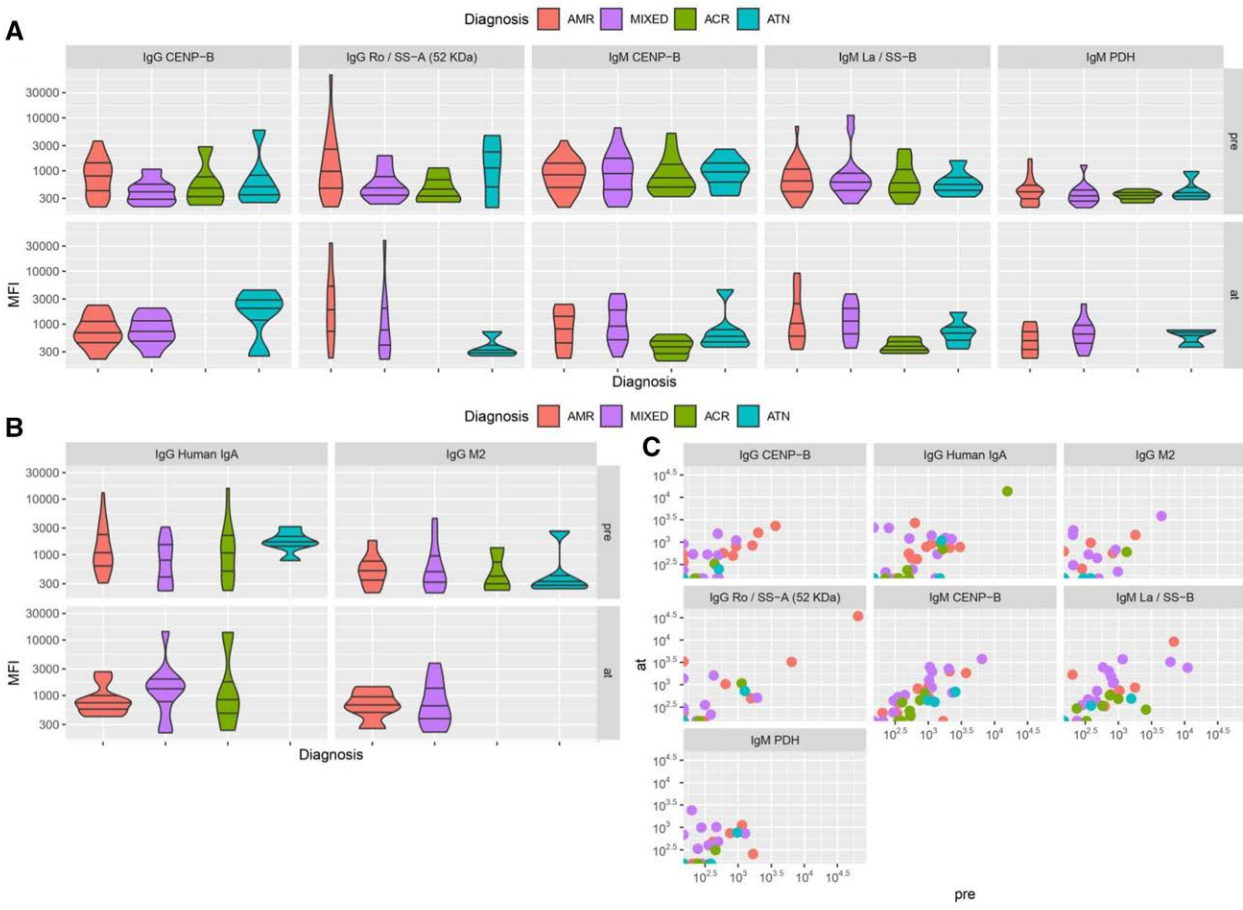

FIGURE 2. The evolution of top non-human leukocyte antigen (HLA) antibodies increased in antibody-mediated rejection (AMR) and mixed rejection over time. The violin plots depict the distributions of the median fluorescence intensity (MFI) values of the 5 antibodies significantly increased in AMR/mixed vs acute cellular rejection (ACR; A) and the 2 antibodies significantly increased in AMR/mixed vs acute tubular necrosis (ATN; B) at the time of diagnosis. The 3 horizontal lines within each violin represent the median (central line) and the interquartile range. Changes in the levels of these 7 antibodies over time were assessed by visualizing scatter plots of antibody MFI at diagnosis (y-axis) vs antibody MFI before transplant (x-axis), in patients who had both serum samples (C).

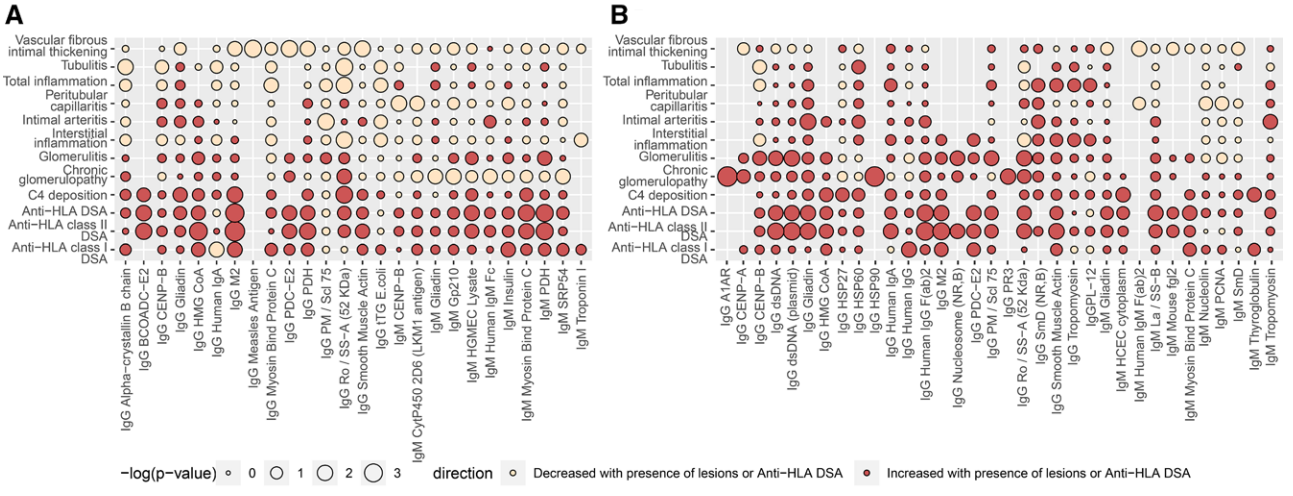

FIGURE 3. Association between levels of non-human leukocyte antigen (HLA) antibodies and relevant histologic and clinical parameters. The bubble plot represents an association matrix between the presence of histopathology and serology features important in antibody-mediated rejection (AMR) and the median fluorescence intensity (MFI) values of non-HLA antibodies before transplant (A) and at the time of diagnosis (B). The non-HLA antibodies that were more strongly associated with at least 1 clinical variable (according to *P* value) are represented. The size of the nodes is inversely proportional to the *P* value of the association. In turn, the color of the nodes indicates the direction of the association: increased antibody levels with the presence of histopathology lesions and presence of donor-specific antibodies (DSA) are colored in red, while decreased antibody levels with the presence of histopathology lesions and presence of DSA are colored in beige. ACR, acute cellular rejection; ATN, acute tubular necrosis; Ig, immunoglobulin.

TABLE 4.

Study of key non-HLA antibodies in a second, external cohort

		Antibody levels			Differential levels analysis	
		At diagnosis			AMR/mixed vs ACR	AMR/mixed vs control
Antibody name	Antigen specificity	ACR, median (IQR)	AMR/mixed, median (IQR)	Control, median (IQR)	*P*	*P*
IgG Ro/SS-A (52 kDa)	Ro/SS-A (52 kDa)	383 (129–740.5)	9002 (1714–10527)	228 (0–652)	0.0041	0.0022
IgG La/SS-B	La/SS-B	282 (0–492)	557 (551–748)	206 (0–506)	0.0299	0.0094
IgG CENP-B	Major centromere autoantigen B	240 (0–539)	440 (411–828)	0 (0–554)	0.1367	0.087
IgG PDH	PDH	0 (0–0)	292 (0–339)	0 (0–0)	0.0082	0.0664
IgM Ro/SS-A (52 kDa)	Ro/SS-A (52 kDa)	404 (116–793.5)	304 (236–330)	224 (0–557)	0.6264	0.2983
IgM La/SS-B	La/SS-B	0 (0–327)	254 (0–380)	0 (0–326)	0.2310	0.2111
IgM CENP-B	Major centromere autoantigen B	266 (0–476.5)	972 (646–1404)	200 (0–330)	0.0289	0.0267
IgM PDH	PDH	0 (0–0)	0 (0–0)	0 (0–0)	0.4134	0.3011

The levels of IgG and IgM non-HLA antibodies significantly increased at diagnosis in AMR/mixed rejection in the discovery cohort (UHN, Toronto) were interrogated in a second, independent cohort (CHUM, Montreal). $P < 0.05$ was considered significant.

ACR, acute cellular rejection; AMR, antibody-mediated rejection; HLA, human leukocyte antigen; Ig, immunoglobulin; IQR, interquartile range.

DISCUSSION

Although autoantibodies against Ro/SS-A (52 kDa), CENP-B and La/SS-B are elevated and pathogenic in several autoimmune diseases,[37,63–65] their role in kidney allograft rejection has never been reported. Here, we show that (1) antibodies against Ro/SS-A (52 kDa), CENP-B and La/SS-B were significantly higher in patients with AMR/mixed rejection compared to ACR at diagnosis; (2) antibodies anti-Ro/SS-A (52 kDa) and anti-CENP-B preceded transplantation and increased at the time of AMR/mixed diagnosis, in both early and late rejections; and (3) these antibodies were associated with class-II DSA and microvascular lesions (Figure 6). Our findings suggest that these autoantibodies could participate in kidney allograft injury in AMR.

Our approach pinpointed specific antibodies significantly altered among different forms of allograft injury. In 2 independent cohorts, we demonstrated that autoantibodies against Ro/SS-A (52 kDa), CENP-B, and La/SS-B were increased in patients with AMR/mixed rejection compared with ACR at diagnosis. Ro/SS-A (52 kDa) antigen, also known as Ro52 or TRIM21, is recognized as the SS-A,[66] together with Ro60. Anti-Ro/SS-A antibodies have been described in autoimmune conditions including SS, SLE, and systemic sclerosis and proposed as markers of disease activity.[67] TRIM21 is an Fc receptor that neutralizes opsonized viral particles entering cells.[68] TRIM21 can be upregulated and translocated to the nucleus under proinflammatory conditions and modulate type-I interferon expression.[69] TRIM21 can also be expressed on the surface of apoptotic cells and become an immune target.[70] Monocyte surface TRIM21 expression was increased in patients with SS and upregulated by interferon-gamma.[66] Anti-TRIM21 antibodies specifically suppress the anti-inflammatory functions of this protein while leaving type-I interferon production uncontrolled.[71] Anti-Ro antibodies could thus facilitate and enhance cytokine- and antibody-induced inflammation in AMR.

Anti-La/SS-B antibodies were elevated at diagnosis in AMR/mixed rejection. La/SS-B regulates cell cycle and binds to RNA polymerase-III transcripts, protecting them from exonucleases.[72] Like TRIM21, La can be exposed on the surface of apoptotic cells, although it typically resides in the nucleus.[70] Positivity for both anti-Ro/SS-A and anti-La/SS-B antibodies has been observed in SLE and SS.[37,63,73,74] How these autoantibodies are generated is unknown. In mice, immunization with recombinant TRIM21 or La/SS-B resulted in loss of T-cell tolerance towards these antigens, and subsequent activation of B-cells to produce anti-Ro/SS-A and anti-La/SS-B antibodies.[63] The concomitant increase in anti-Ro/SS-A and anti-La/SS-B antibodies in our patients with AMR/mixed rejection is consistent with their similarities and associated phenotypes.

We also found significantly increased antibodies against CENP-B in AMR/mixed rejection compared with ACR. At diagnosis, both IgG and IgM anti-CENP-B were elevated in these patients. CENP-B is key to maintain chromosome segregation during mitosis.[75] CENP-B also binds to vascular cells stimulating proliferation, migration, and cytokine release.[76,77] Anti-centromere antibodies have been described in several autoimmune and inflammatory diseases.[39,65,78–80] Senecal's group demonstrated that anti-CENP-B antibodies inhibited proliferation and interleukin-8 production in vascular cells. Aberrant vascular repair and progressive arterial occlusion were observed in the presence of these antibodies.[76] We speculate that these antibodies may have similar effects in AMR.

Antibodies against Ro/SS-A (52 kDa) and CENP-B preceded transplantation and increased at the time of AMR/mixed diagnosis, in both early and late rejections. Of note, only 3 patients in the AMR/mixed rejection groups had SLE; thus, our observations are not related to pretransplant autoimmune disease. Although these antibodies were virtually absent in ACR, they were detectable in AMR/mixed cases, even before transplantation. Longitudinal sera enabled us to note that although all antibodies showed little variability between pretransplant and at diagnosis measurements, several antibodies including anti-Ro/SS-A (52 kDa), anti-CENP-B and anti-La/SS-B increased at diagnosis compared with pretransplant. This suggests that these antibodies, similar to anti-angiotensin

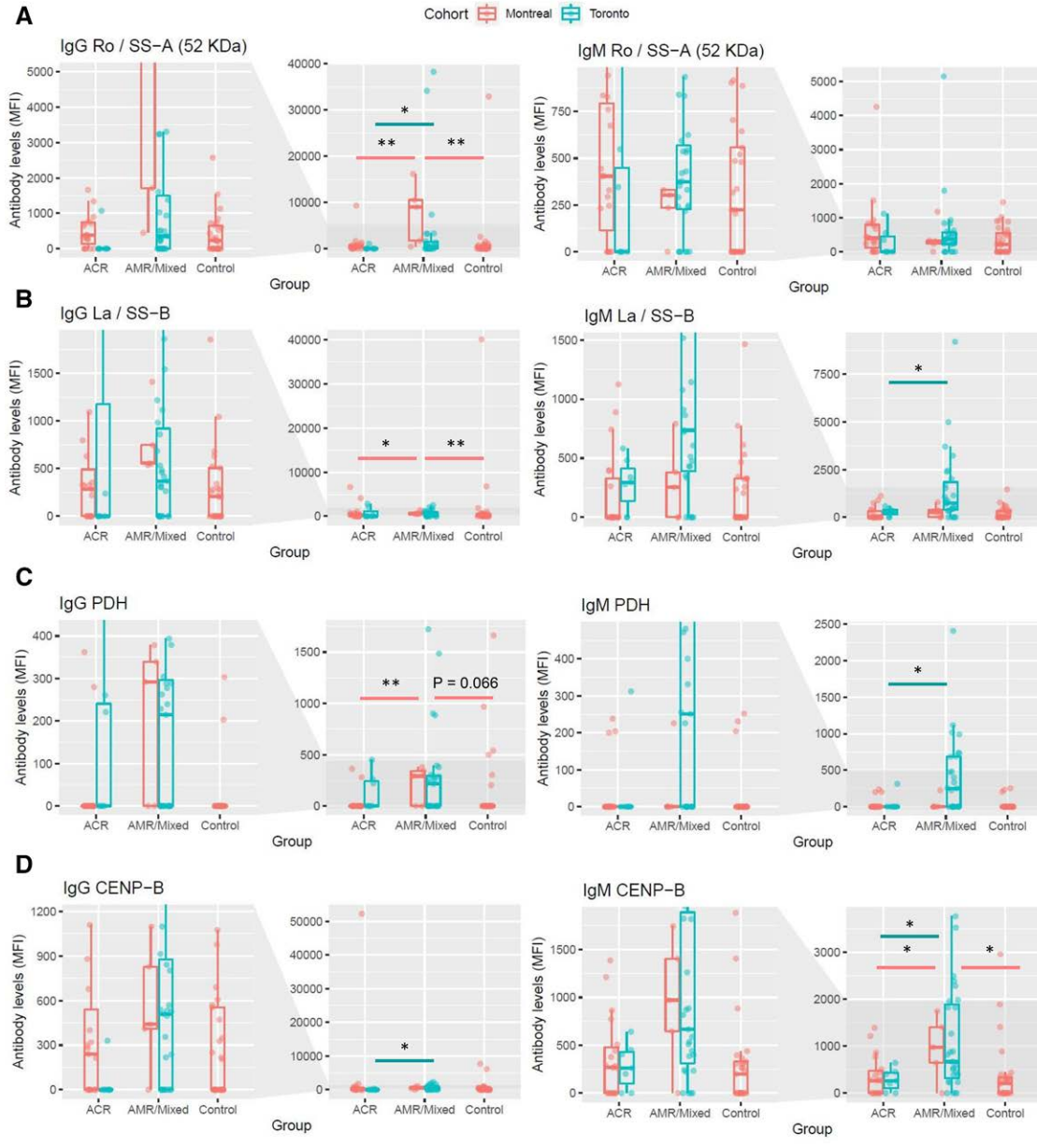

FIGURE 4. Study of key non-human leukocyte antigen (HLA) antibodies in an external cohort. Differences between groups in the levels of antibodies that were significantly increased in antibody-mediated rejection (AMR)/mixed rejection at the time of diagnosis in the discovery cohort (Toronto), were significantly associated with the presence of microvascular lesions, and were interrogated in a second, external cohort (Montreal). For each antibody, levels from kidney transplant patients with AMR/mixed rejection were compared to the levels from patients with acute cellular rejection (ACR) or stable nonrejecting kidney grafts (control) and plotted next to their corresponding levels in the Toronto cohort. Levels of IgG and IgM against Ro/SS-A (52 kDa) (A), La/SS-B (B), pyruvate dehydrogenase (PDH) (C), and major centromere autoantigen (CENP)-B (D) are shown. Data are represented as median ± interquartile range (IQR, box). *P < 0.05 vs ACR or vs control; **P < 0.01 vs ACR or vs control. MFI, median fluorescence intensity.

II type-1 receptor antibodies,[11] predated transplant and were formed by yet unrecognized mechanisms.

AMR and mixed rejection cases were diagnosed after distinct time intervals posttransplant. Although pure AMR cases were biopsied within a month posttransplant, mixed rejection cases were diagnosed after a median of 174 d posttransplant. Despite this large difference in the time of diagnosis, the distribution of the anti-Ro/SS-A (52 kDa), anti-CENP-B, and anti-La/SS-B antibodies was remarkably similar in the 2 groups. This suggests that similar mechanisms are at play in AMR and mixed rejection regarding the formation of these antibodies and their plausible influence on graft pathology.

Our third major observation was that increased levels of IgG anti-Ro/SS-A (52 kDa), anti-CENP-B, and IgM anti-La/SS-B were strongly associated with the presence of microvascular lesions and anti-HLA class-II DSA. Class-II DSA is more strongly associated with transplant glomerulopathy and are considered to be more pathogenic than class-I.[81–83] Levels of IgG anti-Ro/SS-A (52 kDa), anti-CENP-B, and IgM anti-La/SS-B were associated with glomerulitis, C4d deposition, and chronic glomerulopathy. As proposed for other non-HLA antibodies,[12,23] antibodies against Ro/SS-A (52 kDa) and La/SS-B may act in synergy with anti-HLA DSA, enhancing allograft injury. Anti-CENP-B and anti-La/SS-B were also

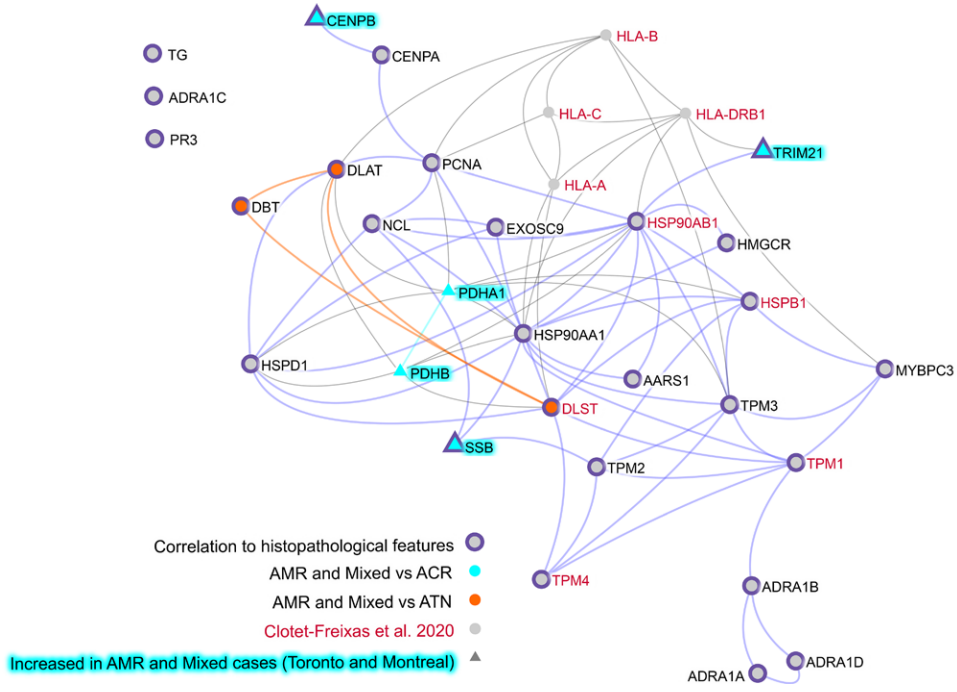

FIGURE 5. Network analysis of key antibody targets and proteins differentially expressed in kidney antibody-mediated rejection (AMR). Physical protein–protein interactions of key non-human leukocyte antigen (HLA) and HLA antibody targets were identified using the Integrated Interactions Database and visualized using NAViGaTOR 3.0.13. The selected targets were of relevance because their corresponding antibody was significantly increased in AMR/mixed patients at diagnosis and significantly associated with the presence of AMR-related histopathology lesions or anti-HLA donor-specific antibodies (DSA). Turquoise and orange nodes represent the targets of non-HLA antibodies differentially increased in AMR/mixed patients compared with acute cellular rejection (ACR) and acute tubular necrosis (ATN), respectively. The nodes with purple highlight reflect targets of antibodies that were significantly associated with the presence of histopathology features and anti-HLA DSA. The gene names corresponding to the targets of antibodies that were significantly increased in AMR/mixed patients of the external cohort (Montreal) are highlighted in turquoise. The nodes with a triangle shape represent targets of non-HLA antibodies increased in AMR/mixed rejection in both discovery and validation cohorts. The gene names of the non-HLA as well as HLA antibody targets that we previously found to be differentially expressed at the protein level in the AMR glomeruli or tubulointerstitium[48] are colored in red. Purple edges connect proteins that are correlated with histopathological features, orange and turquoise edges connect protein targets of antibodies increased in AMR and mixed samples compared to ATN and ACR, respectively. Red edges connect proteins identified as deregulated in Clotet-Freixas et al, *JASN*, 2020.

associated with intimal arteritis, linking the action of these antibodies in autoimmune diseases to lesions characteristic of AMR. Interestingly, in a subgroup of patients with AMR/mixed rejection but no DSA, we observed a persistent increase in the levels of IgG anti-CENP-B antibodies, suggesting that these antibodies may represent markers or participants in DSA negative AMR.

The targets of non-HLA and HLA antibodies are highly interconnected in a protein–protein interaction network (Figure 5). Furthermore, integration with our recent kidney tissue proteomics data set[48] highlighted that proteins perturbed in the AMR tissue are directly connected with the targets of the immune response. The key hubs in this network are chaperones HSP90, which participate in renal immunity.[84] HSP90 was previously elevated in the serum of patients with kidney AMR.[85] We demonstrated that anti-HSP90 antibodies were strongly associated with the presence of chronic glomerulopathy. PCNA is another hub in the network, connecting centromere proteins, PDH and HSP90. Upon injury, increased PCNA expression indicates increased cell cycle entry, which may lead to adverse events, such as hypertrophy or mitotic catastrophe.[86,87] In turn, antigen presentation and HLA-ligation can trigger proliferation in endothelial cells.[82,88] Proliferative stress in kidney AMR may result in abnormal centromere function and affect the turnover of centromere-related proteins, such as CENP-B. In conclusion, proteins disrupted in kidney tissue during AMR interact directly with the targets of anti-HLA and non-HLA antibodies. Further studies aimed at deciphering the biology of these antibodies and their target proteins are warranted.

Our study has several strengths. We carefully selected a group of well-characterized kidney transplant recipients with longitudinally collected serum samples. Using an innovative protein microarray, we studied non-HLA antibody changes in different subgroups and over time. We integrated the findings from this study with clinical/histopathologic data and with our prior proteomics-based study.[48] The key findings were reproduced in a second, external cohort. Our study also has limitations. After excluding sera affected by plasmapheresis and IVIG, the study of antibody dynamics was limited to a smaller subset of patients. This is an explorative and hypothesis-generating study of retrospectively collected sera from 2 independent patient cohorts. Despite the novelty and concordance of our key findings across the 2 cohorts, we acknowledge that they warrant validation in bigger, prospective studies. These studies should also examine the potential value of monitoring anti-CENP-B antibodies in AMR patients that are DSA negative. Finally, the current study pinpoints novel and interesting associations, but basic research studies in vitro and in vivo are required to establish their causal relationship with AMR.

In conclusion, our approach revealed a novel link between increased pretransplant levels of IgG anti-Ro/SS-A (52 kDa) and anti-CENP-B and the development of AMR after kidney

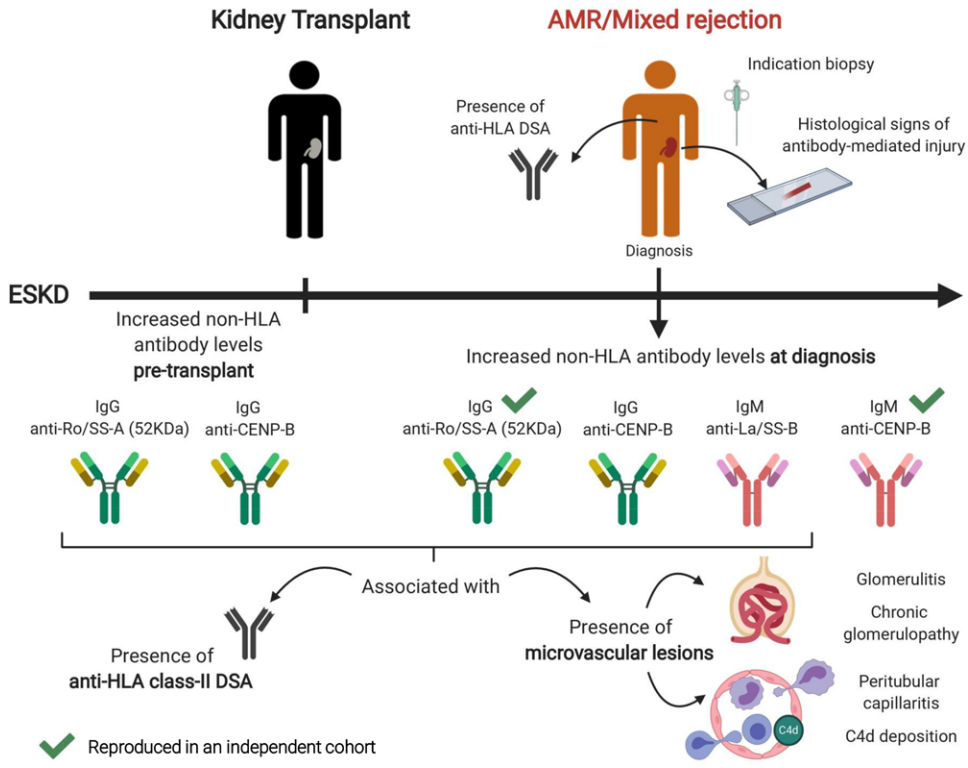

FIGURE 6. Summary of the key non-human leukocyte antigen (HLA) antibodies associated with kidney antibody-mediated rejection (AMR). Summary of relevant non-HLA antibodies increased in AMR/mixed rejection before transplant and at the time of diagnosis. IgG antibodies are depicted in green, and IgM antibodies are illustrated in red. The green ticks indicate that increased levels of IgG anti-Ro/SS-A (52 kDa), IgG and IgM anti-major centromere autoantigen (CENP)-B, and IgM anti-La/SS-B in AMR/Mixed rejection patients were reproduced in a second, independent cohort. DSA, donor-specific antibodies; ESKD, end-stage kidney disease; Ig, immunoglobulin.

transplantation. Together with IgM anti-La/SS-B, IgG anti-Ro/SS-A (52 kDa) and anti-CENP-B were also increased at the time of AMR diagnosis, both early and late after transplant. These antibodies were associated with the presence of microvascular lesions and anti-HLA class-II DSA, suggesting that they may synergize with class-II DSA and induce endothelial injury in AMR.

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