Disparity between levels of anti-RBD IgG and anti-nucleocapsid protein IgG antibodies in Covid-19 recovered kidney transplant patients

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Disparity between levels of anti-RBD IgG and anti-nucleocapsid protein IgG antibodies in Covid-19 recovered kidney transplant patients

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To the editor: We read with great interest Chavarot's[1] study, demonstrating anti-SARS-CoV-2 anti-nucleocapsid (N) protein IgG decline rapidly following SARS-CoV2 infection in kidney transplant patients (KTx-pts), independent of illness severity, but it did not address the dynamic interplay with IgG antibodies against the spike protein Receptor Binding Domain (Spike-RBD).

We studied 25 KTx-pts and 23 normal controls all of whom had nasopharyngeal RT-PCR-confirmed Covid-19 (1 control confirmed by blood Elisa) and subsequently tested negative for SARS-CoV-2 and recovered. All patients had stable engraftments for an average of 18.6 months (1-52) at time of viral infection.

We used multiplexed microsphere-based assays for the detection of IgG antibodies against viral N protein and spike-RBD. Most KTx-pts (22/25, 88%) were positive for anti-spike-RBD IgG antibodies and only 28% were positive for anti-N IgG antibodies (Figure 1A and Table S1A). All 23 controls developed both anti-N and anti-RBD IgG antibodies (Figure S1A). In a subgroup of KTx-pts (n=12), in which both age (54.5 years old) and infection time (35.8 days) were comparable to a subgroup of controls (n=16), we found that while the levels of anti-RBD IgG antibodies in patients were very heterogenous, they were not statistically different from those of normal controls (p=0.60). Levels of anti-N IgG antibodies in transplant patients, on the other hand, were significantly reduced, when compared to that of the controls (p=0.0022) or compared to that of the anti-RBD IgG in the same group of transplant patients (p=0.0449). This result (Figure S1B) suggests that anti-N IgG antibodies, but not anti-RBD IgG antibodies, were predominately affected in KTx-pts.

Longitudinal analyses of anti-N and anti-spike-RBD antibodies were studied in 6 KTx-pts with multiple sera samples available. Figure 1B shows heterogenous yet rapid induction of anti-RBD IgG antibodies with persistence for at least 100 days (MFI>700) and still present at 200 days in 4 patients. However, significantly lower levels of anti-N antibodies were produced, and by day 100 only one patient had anti-N IgG antibodies. This patient was noted to have pre-existing anti-N IgG antibodies detected 138 days prior to SARS-CoV-2 infection, consistent with previous exposure to another type of epitope sharing- corona viruses[2].

Even with different methodologies, our results are consistent with Chavarot et al.[1] finding that anti-nucleocapsid (N) protein IgG was induced in KTs-pts and that these antibodies rapidly decline over time. SARS-CoV-2 N protein shares high degree of amino acid identity with the SARS-CoV (90%) and MERS-CoV (45%). The role of immunodominant anti-N IgG antibodies in providing protective anti-viral immunity is currently unknown. By comparing levels of anti-N antibodies with those of concurrent anti-RBD antibodies over a prolonged period in transplant patients, our findings provide a rare opportunity to look into the immunological dynamics of these individuals, further extending current understanding of anti-SARS-CoV-2 immune response in immunocompromised patients. In summary, our results clearly demonstrate a disparity between the levels anti-N and anti-RBD IgG antibodies in CoVid-19 recovered post-transplant patients. Our findings of persistence of anti-RBD IgG antibodies suggest that recovered transplant patients may have developed long lasting anti-RBD IgG antibodies, with potential neutralizing effect against common strains[3] or some of the new SARS-CoV-2 variants[4]. Larger studies are needed to estimate the degree of acquired protection against reinfection.

Figure 1 A: Detection of anti-N IgG and anti-RBD IgG antibodies in Covid-19 recovered KTx-pts. 25 SARS-CoV-2 positive patients were tested by multiplexed microsphere-based SARS-CoV-2 IgG assays (Luminex Corps, Austin, TX). Results are shown as median fluorescence intensity (MFI). The positive threshold (700 MFI) is represented by a horizontal line for both IgG antibodies; each patient is represented by a vertical line. Scatter plot analysis elucidates the level of both types of IgG antibodies at time (days) post-Covid confirmation for each patient. **B: Longitudinal analysis of anti-viral IgG antibodies in 6 KTx-pts.** Samples were analyzed for the presence of anti-N IgG and anti-RBD IgG antibodies after SARS-CoV-2 infection at different time points. For each patient, a sample taken prior to their exposure to SARS-CoV-2 (before Feb. 2020) was used as an internal control, with value typically <100 MFI. Day "0" is designated as the day the SARS-CoV-2 infection was confirmed. Antibody positivity was set as previously described. Except for one patient (case 14) who had under detectable (656 MFI) anti-N IgG antibodies on a pre-pandemic date, no other patients had pre-existing anti-N or anti-RBD IgG antibodies.

Supplementary materials

Table S1: Cohort of Covid-19 recovered kidney transplant patients (KTx-pts). Forty-one KT-pts positive for SARS-CoV-2 between March 2020 to Jan 2021 were retroactively identified through Columbia University Irving Medical Center (CUIMC) medical records. The study was approved by Columbia University Institutional Review Board. Availability of patients' (n=25) post-infection samples were identified through HistoTrac Software. LUD, living unrelated donor; LRD, living related donor; CAD, cadaver donor; na, no available; ^, 2nd or more graft- recipient. Positivity for both anti-RBD and anti-N IgG antibodies was preset to be 700 MFI by manufacturer.

Figure S1 A: Detection of anti-N IgG and anti-RBD IgG antibodies in Covid-19 recovered controls. A group of 23 were similarly tested by multiplexed microsphere-based SARS-CoV-2 IgG assays. The positive threshold (a vertical line) is as previously described; each individual is represented by a vertical line. **B: Decrease in the development of anti-N IgG antibodies in Covid-19 recovered KTx-pts, but not in recovered controls.** Levels of anti-N IgG and anti-spike-RBD IgG in 13 recovered KTx-pts, were compared to a group of recovered controls (n=16) who had similar age and infection time. Average (range) age for the patient group was 54.5(37-72); for the control group, 55.8(30-76). Average (range) infection days for the patient group was 35.8(15-38); for the control group, 39.1(9-36). Statistics were performed with Mann-Whitney test using Graphpad Prism Software. *, significant; **, very significant.; NS, not significant; Tx, KTx-pts; NR, normal recovered controls.

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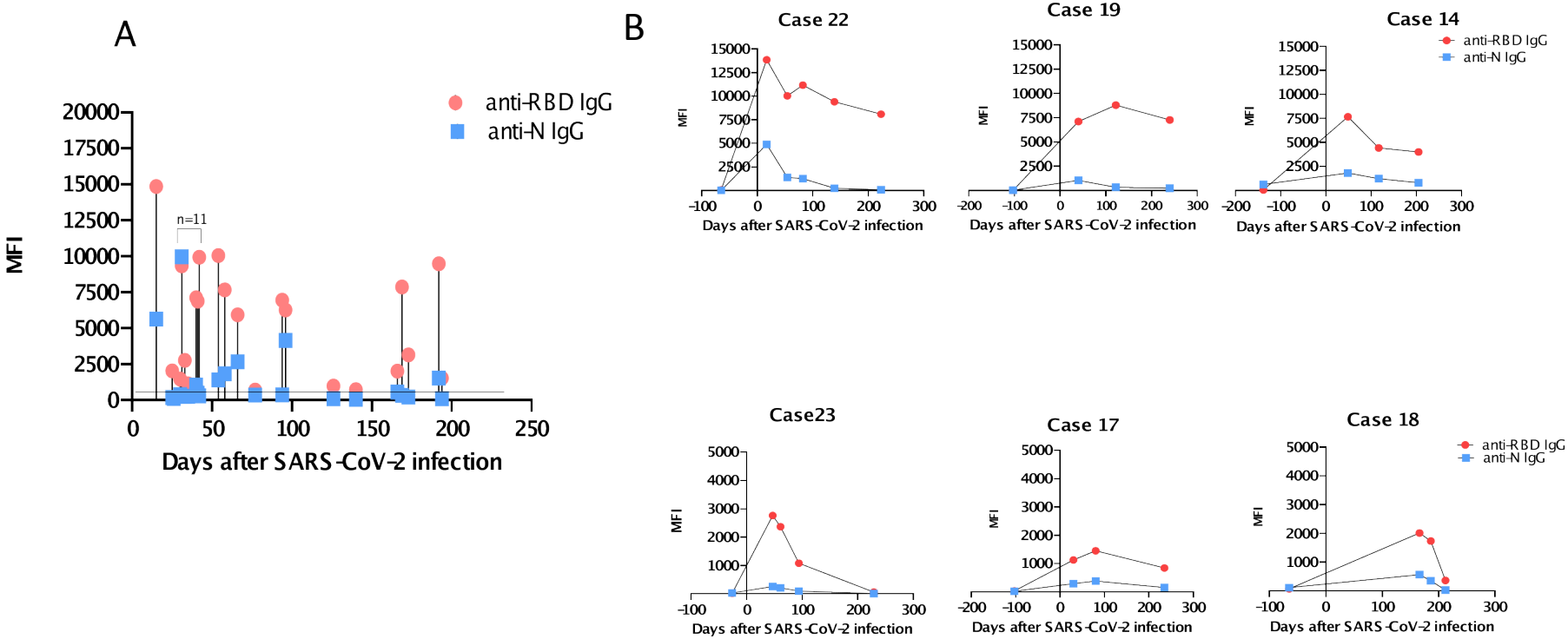
A
anti-RBD IgG
anti-N IgG
MFI
n=11
Days after SARS-CoV-2 infection
B
Case 22
Case 19
Case 14
Case23
Case 17
Case 18
anti-RBD IgG
anti-N IgG
MFI
Days after SARS-CoV-2 infection

Case	Age	Sex	Race	Graft type	Engraftment days at time of infection	Serum days after infection	Anti-SARS-CoV-2 N IgG Ab	Anti-SARS-CoV2-RBD IgG Ab	Serum Donor Specific Ab
1^	51	M	W	LUD	1593	94	-	+	-
2	45	F	na	LUD	1362	77	-	+/-	-
3	57	M	W	LRD	1307	192	+	+	-
4	29	M	B	na	1278	96	+	+	na
5^	37	M	B	CAD	1152	173	-	+	-
6	70	F	B	CAD	1130	126	-	+	-
7^	25	F	W	CAD	1068	140	-	+	+
8	72	M	na	CAD	1053	35	-	+	-
9	62	M	B	LUD	894	169	-	+	-
10	69	M	na	LUD	691	66	+	+	-
11	69	F	B	LUD	471	41	-	+	-
12	47	M	na	LUD	392	15	+	+	-
13	49	M	W	LUD	333	42	-	+	-
14	52	F	W	CAD	320	58	+	+	-
15	56	M	W	LRD	278	31	+	+	-
16	54	F	W	LUD	169	164	-	+	-
17	51	M	na	CAD	130	30	-	+	-
18	33	F	B	LUD	126	166	-	+	+
19	37	F	A	CAD	116	40	+	+	-
20	60	F	W	CAD	116	275	-	-	-
21^	50	F	B	CAD	59	315	-	-	-
22	45	M	na	CAD	48	54	-	+	-
23	66	F	na	CAD	48	33	-	+	-
24	55	F	na	CAD	29	25	-	+	-
25^	55	M	W	LRD	23	26	-	-	-

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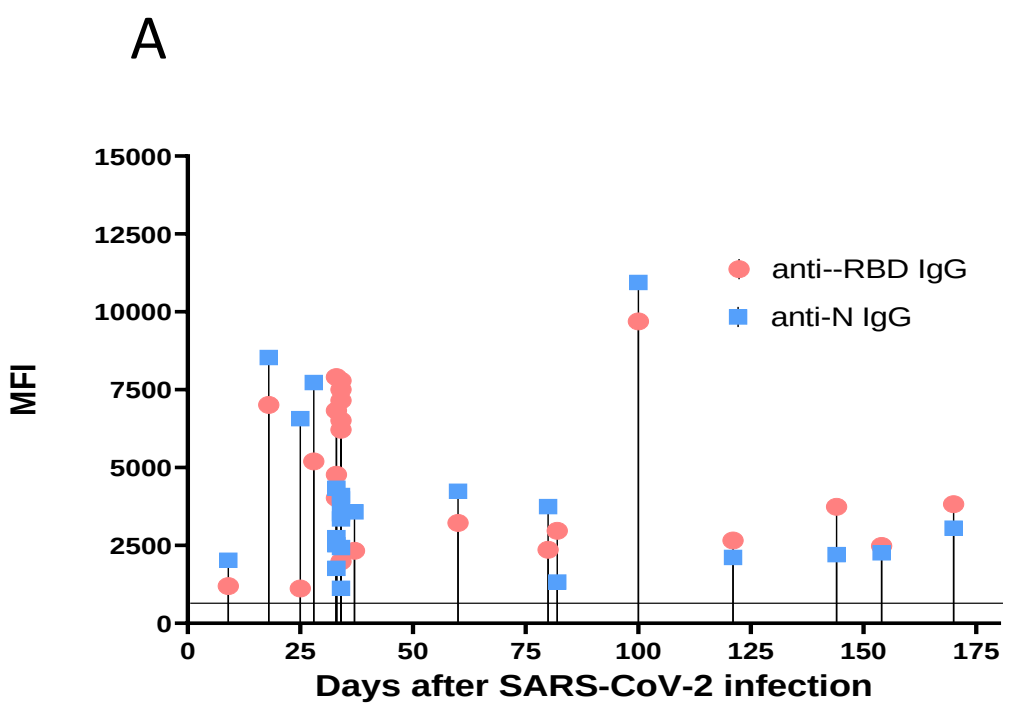
A
MFI
15000
12500
10000
7500
5000
2500
0
0
25
50
75
100
125
150
175
Days after SARS-CoV-2 infection
anti--RBD IgG
anti-N IgG

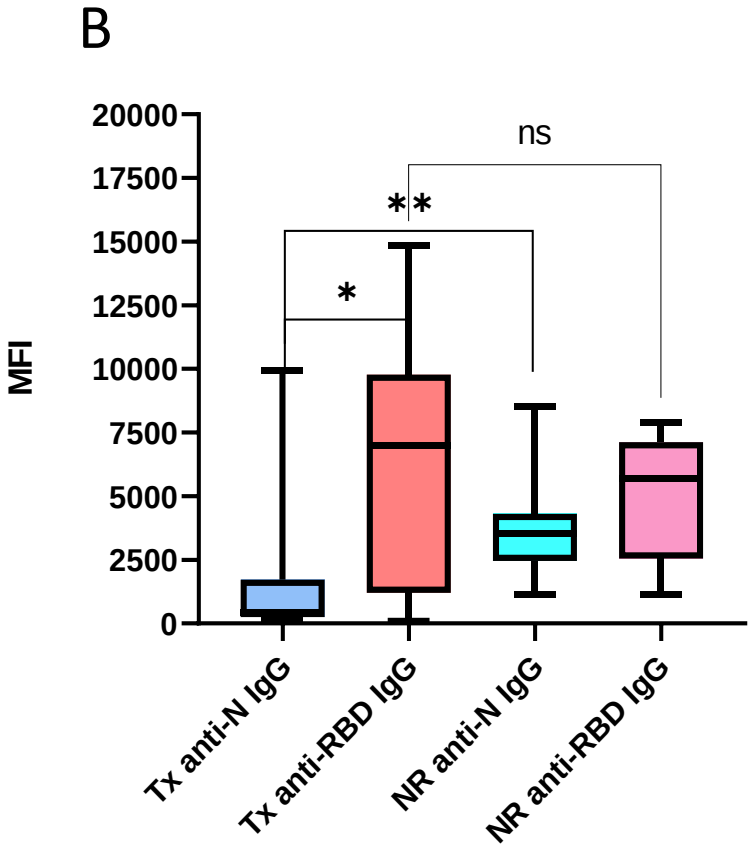
B
MFI
20000
17500
15000
12500
10000
7500
5000
2500
0
ns
**
*
Tx anti-N IgG
Tx anti-RBD IgG
NR anti-N IgG
NR anti-RBD IgG

Figure S1 A: Detection of anti-N IgG and anti-RBD IgG antibodies in Covid-19 recovered controls. A group of 23 were similarly tested by multiplexed microsphere-based SARS-CoV-2 IgG assays. The positive threshold (a vertical line) is as previously described; each individual is represented by a vertical line. **B: Decrease in the development of anti-N IgG antibodies in Covid-19 recovered KTx-pts, but not in recovered controls.** Levels of anti-N IgG and anti-spike-RBD IgG in 13 recovered KTx-pts, were compared to a group of recovered controls (n=16) who had similar age and infection time. Average (range) age for the patient group was 54.5(37-72); for the control group, 55.8(30-76). Average (range) infection days for the patient group was 35.8(15-38); for the control group, 39.1(9-36). Statistics were performed with Mann-Whitney test using Graphpad Prism Software. *, significant; **, very significant.; NS, not significant; Tx, KTx-pts; NR, normal recovered controls.