Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals

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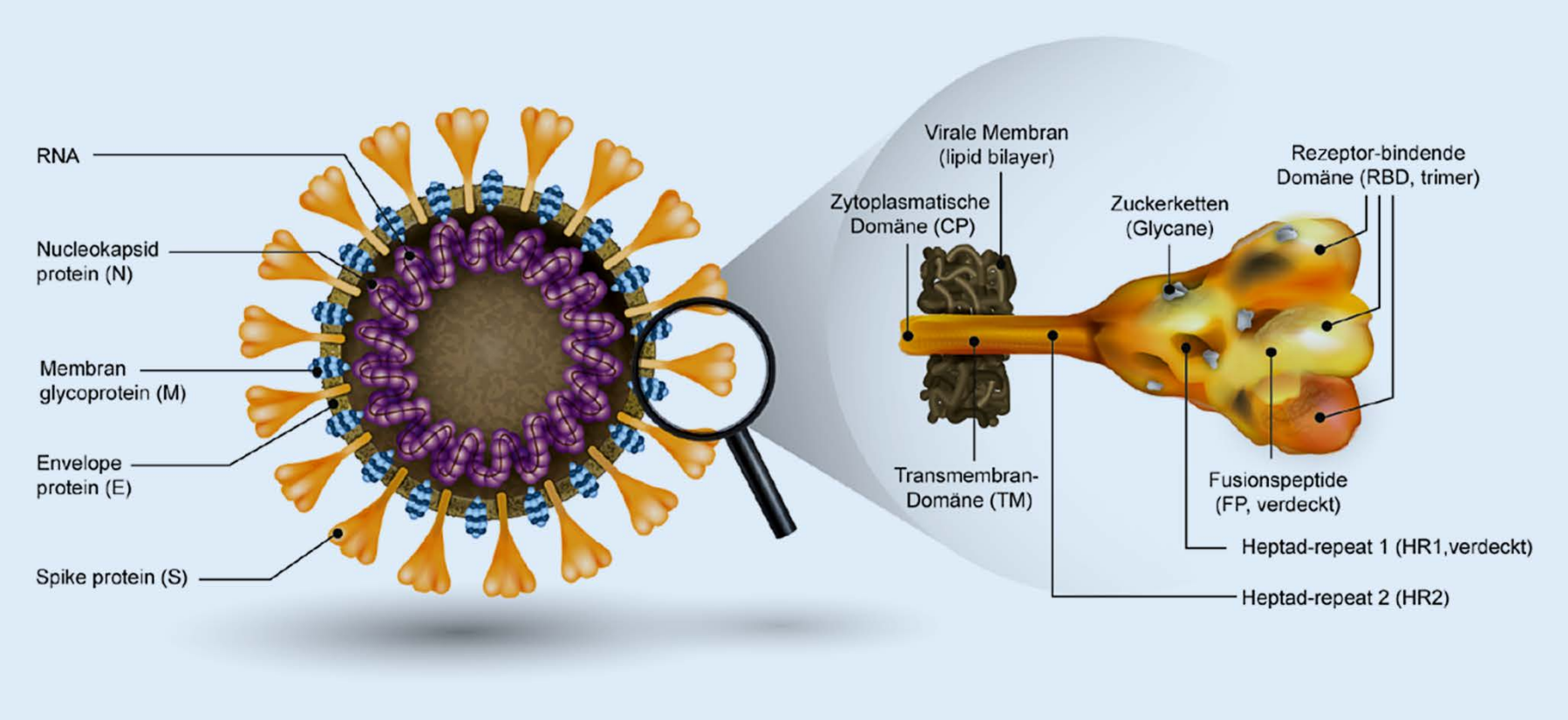
Introduction

- Genome sequence of SARS-CoV-2 bears **96% identity of bat coronavirus** and **79,5% SARS-CoV** (Zhou et al.)
- belongs to beta genus *Coronavirus* in the **Coronaviridae family** like SARS-CoV and MERS-CoV (Lu et al.)
- Clinically often **lymphopenia** and **pneumonia with higher pro-inflammatory cytokines in severe cases** - hypothesis that host immune system is involved in pathogenesis (Chan et al., Huang et al., Wu et al.)
- SARS-CoV or MERS-CoV have antibody responses but exhibited **defective expression of types I and II interferons (IFN-γ, IFN-α)**, indicative poor protective immune responses
- COVID-19 patients **showed nucleocapsid protein (NP)-specific antibody response** (Zhou et al.) and **several patients showed anti-S antibodies production** (Krammer et al.)
- Report of Thevarajan et al. showed **kinetics of T cell subpopulations (T_{FH}, CD4 and CD8) specific antibody responses** in one COVID-19 patient
- **Low level of neutralizing antibody** titer in Finland (Haveri et al.)
- Relationship of virus-specific T lymphocytes and neutralizing antibody titers remains uncharacterized

SARS-CoV-2



M. Ueffing, T. Bayyoud, M. Schindler, and F. Ziemssen, “Basic principles of replication and immunology of SARS-CoV-2,” *Ophthalmologe*, vol. 117, no. 7. Springer Medizin, pp. 609–614, Jul. 01, 2020, doi: 10.1007/s00347-020-01155-w.

Clinical and pathological Characteristics of study population

Table 1. Clinical and Pathological Characteristics of the COVID-19 Patients

Pt#	Sex	Age	Travel in Wuhan	Fever	Fatigue	Lymphocyte Count	Days in hospital	BT CT Scan	BT NA Test	Discharge CT Scan	Discharge NA Test
1	F	51	yes	yes	yes	1.1 × 10^9/L	33	patchy ground glass shadows on both lungs	P	improvement	N
2	F	42	no	no	no	2.5 × 10^9/L	27	multiple patchy ground glass and high-density shadows in both lungs	P	improvement	N
3	M	32	no	yes	no	1.7 × 10^9/L	36	exudative lesion of the right lower lung	P	improvement	N
4	M	49	no	yes	no	1.5 × 10^9/L	32	patchy ground glass shadows on both lungs	P	significant improvement	N
5	F	62	no	yes	yes	0.8 × 10^9/L	37	patchy ground glass shadows on both lungs	P	significant improvement	N
6	M	32	no	yes	yes	2.1 × 10^9/L	17	multiple ground glass shadows in both lungs	P	significant improvement	N
7	M	32	no	yes	yes	1.7 × 10^9/L	34	multiple ground glass lesions in the lower lobe of the right lung	P	significant improvement	N
8	F	57	yes	yes	yes	1.3 × 10^9/L	45	multiple flaky ground glass shadows in the subpleural areas of both lungs, some accompanied by consolidation	P	significant improvement	N
9	F	26	no	yes	no	2.9 × 10^9/L	12	right lung inflammation	P	normal	N
10	M	68	no	yes	no	0.7 × 10^9/L	14	multiple patchy ground glass shadows are seen in the left lung, and the upper lobe of the left lung is obvious	P	significant improvement	N
11	F	37	no	no	yes	1.9 × 10^9/L	12	double lung veins thickened	P	normal	N
12	F	29	no	yes	yes	1.9 × 10^9/L	13	ground glass in the pleura of the lower lobe of both lungs	P	normal	N
13	F	31	yes	yes	no	1.1 × 10^9/L	19	patchy ground glass shadows on both lungs	P	significant improvement	N
14	M	35	no	yes	yes	2.3 × 10^9/L	11	multiple ground glass shadows in both lungs	P	normal	N

Pt, patient; F, female; M, male; P, positive; N, negative; BT, before treatment; NA, nucleic acid.

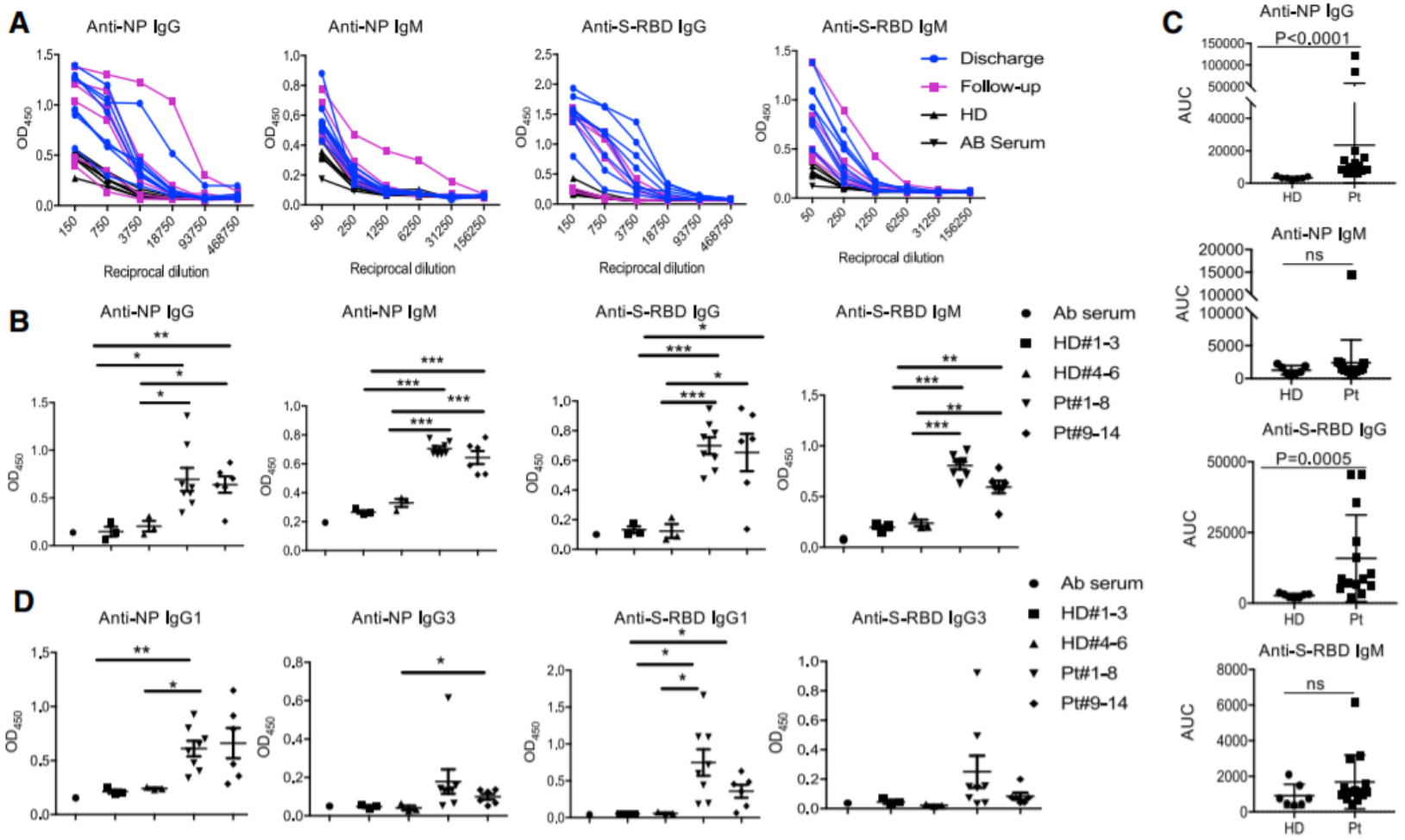
- All showed **initially mild symptoms** via CT scan
- Patients 1-8 were **newly discharged**
- Patients 9-14 were two weeks post discharge (**follow up patients**)
- Patient 5 and 10 showed lymphopenia
- **Three Healthy donors** were obtained before the SARS-CoV-2 outbreak
- **Three** additional **healthy donors** were **close contacts** of the patients
- Human AB serum from healthy male ABD donors were used as negative control

Results

Detection of SARS-CoV-2-Specific Antibodies in COVID-19 Convalescent Subjects - Methods

- Production of **recombinant pET28-N-6XHis** (linking 6 copies of His tag to the C terminus of NP in the pET28-N-vector) in **E. Coli**
- E. Coli transformed with pET28-N-6xHis was lysed and tested by Coomassie blue staining to confirm NP expression at 45.51kDa
- NP was purified using Ni-NTA affinity chromatography and gel filtration (90% purity), expression of NP confirmed by anti-FLAG antibody
- Production of **S-RBD (receptor binding domain)** and **main protease** in **Baculovirus insect expression system** and purified to a purity of 90%

SARS-CoV-2 NP- and S-RBD-specific antibodies in COVID-19 convalescent individuals



Results

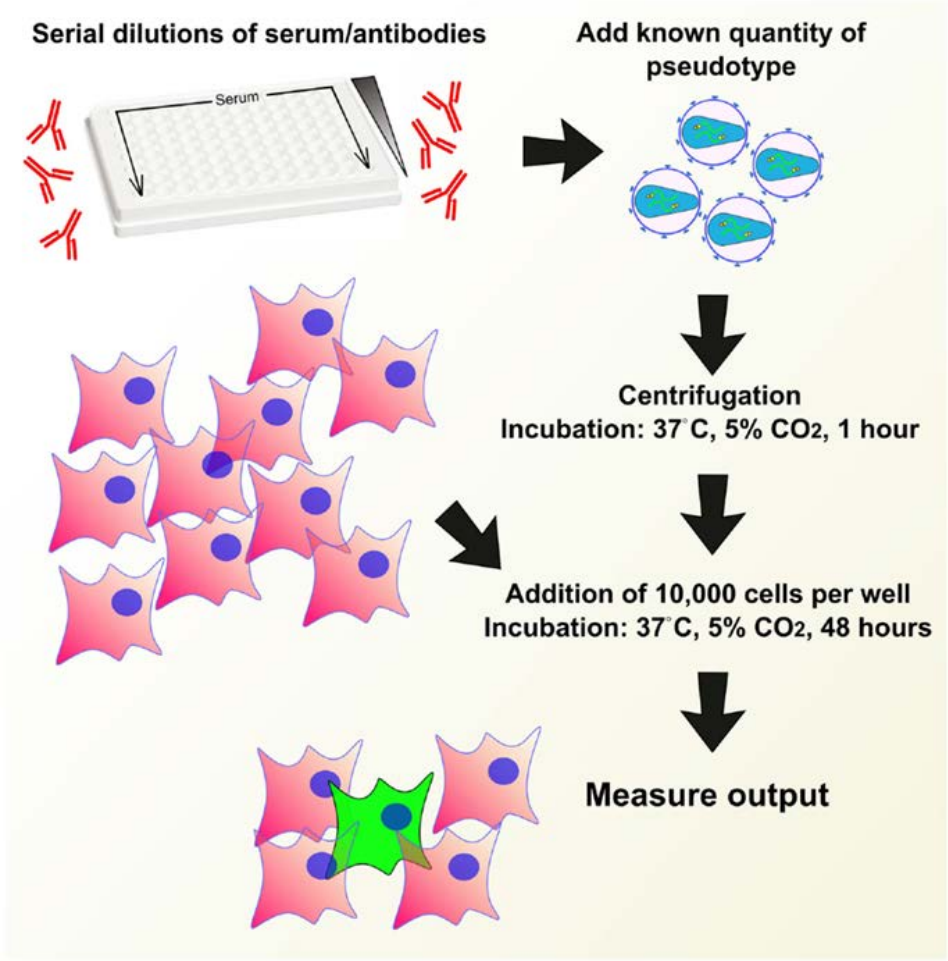
Detection of SARS-CoV-2-Specific Antibodies in COVID-19 Convalescent Subjects

Measurement of IgG and IgM of patients and healthy donors were analyzed:

- No significant antibody response to main protease in sera from several patients (suggestion: no antigen for humoral immunity)
- NP- and S-RBD-specific IgM and IgG antibodies were detected in the sera of newly discharged patients, compared with healthy donor groups
- Anti-SARS-CoV-2 IgG antibodies were more obviously observed than IgM in the follow-up patients (9-14), when compared with healthy donors
- AUC for NP- and S-RBD-specific IgG antibodies were significantly higher compared to AUC of control sera
- IgG isotypes were tested in all patients and controls: anti-NP and S-RBD IgG was mainly IgG1 isotype, newly discharged and follow up patients showed similarly amounts of anti-NP IgG1
- One patient (5) showed higher amounts of anti-NP IgG3, two patients (4 and 5) showed higher amounts of anti-RBD IgG3
- No IgG2 types were detected (either NP- or S-RBDS protein)

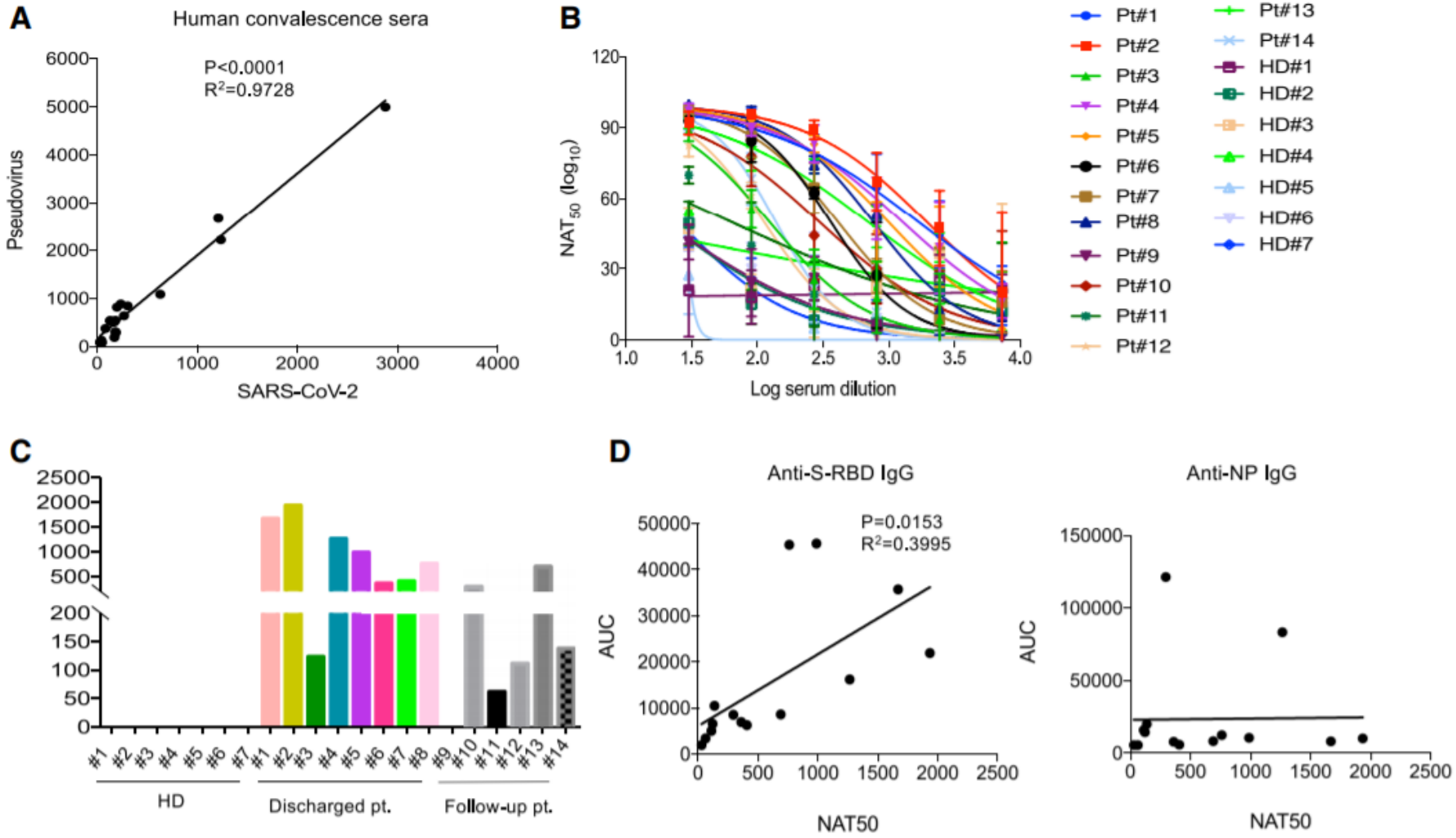
Results

Measurement of Neutralizing Antibody Titers from COVID-19 Convalescent Subjects

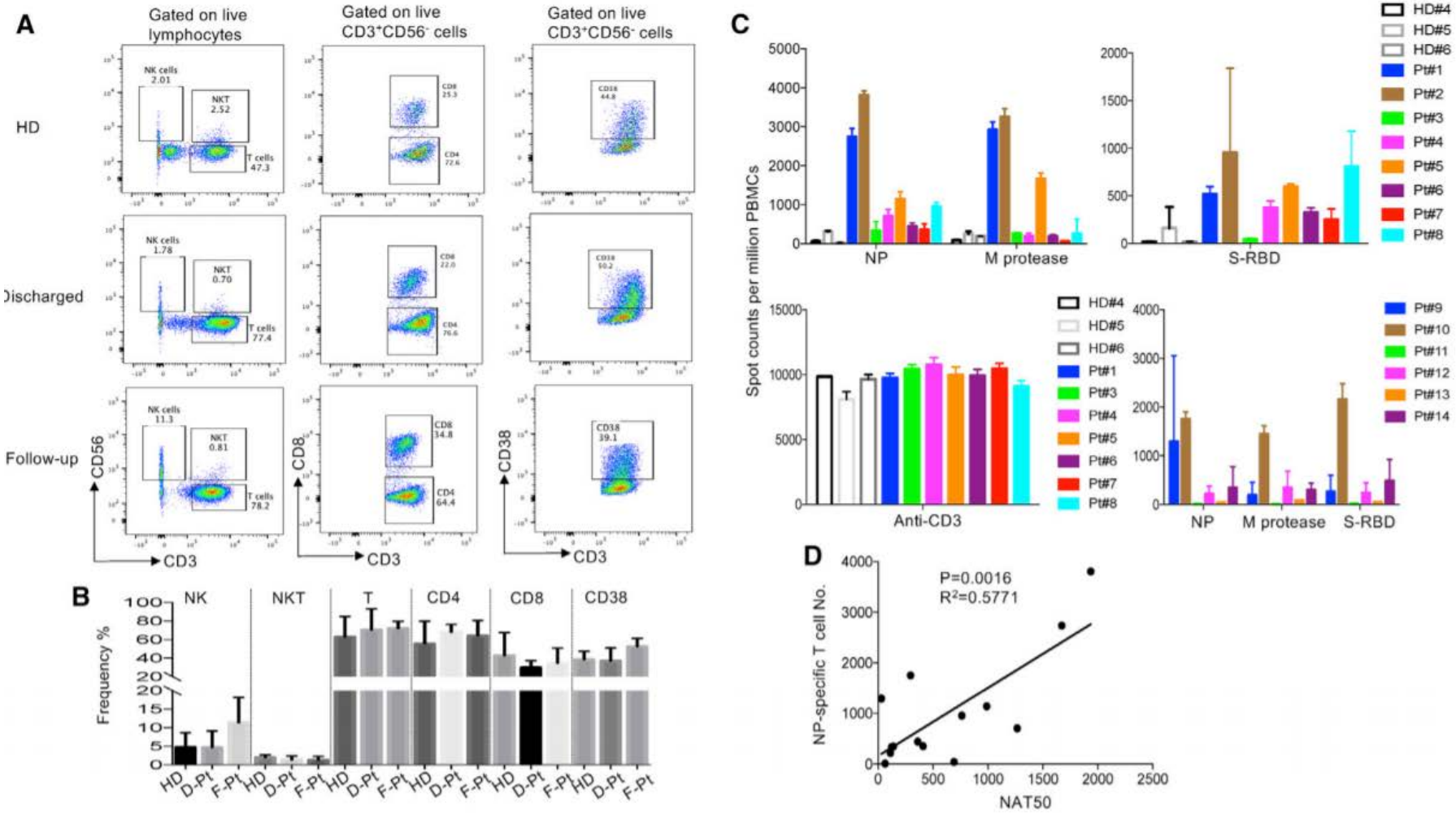


- A pseudovirus particle-based neutralization assay was performed:
  - Patients 1,2,4,5 and 8 (all within the newly discharged group) had high neutralizing antibody titers
  - All follow-up patients had neutralizing antibody titers, with exception of patient 9
  - Significant correlation between neutralizing antibody titers and AUC of anti-S-RBD IgG, but not of anti-NP IgG

Measurement of Neutralizing Antibody Titers in COVID-19 Convalescent Individuals



T cell Responses to Recombinant SARS-CoV-2 Proteins in COVID-19 Convalescent Individuals



Results

Cellular Immune Responses to SARS-CoV-2 in COVID19 Convalescent Subjects

- For exploration of cellular immune responses to SARS-CoV-2, PBMCs from the whole blood was isolated and phenotypically analyzed by flow cytometry
  - Found a trend toward an increased frequency of NK cells in follow-up patients
  - No significant difference in percentages of T cells among these two groups and the healthy donors
- PBMCs were treated with recombinant NP, main protease, and S-RBD followed by IFN-γ ELISpot analysis:
  - Number of IFN-γ-secreting NP-specific T cells in patients 1,2,4,5 and 8 were much higher than other patients (had also strong humoral and cellular immune responses)
  - Main protease-specific T cells were detected in patient 1,2 and 5
  - Patients 1,2,4,5,6,7 and 8 showed S-RBD-specific T cells (lower numbers of IFN-γ-secreting S-RBD specific T cells)
  - In follow-up group only patient 10 (lymphopenia before treatment) had high numbers of IFN-γ-secreting S-RBD specific, main protease specific and NP-specific T-cells (suggestion: recovered patients have only low numbers of viral T cells in PBMCs
  - Taken all patients together there was a correlation between titer of neutralizing antibodies and NP-specific T-cells -> development of neutralizing antibodies may be correlated with activation of anti-viral T-cells (**collaborative humoral and cellular immune response is needed for effective virus clearance**)

Discussion

- Humoral and cellular immunity were detected in newly discharged patients
- Neutralizing antibody titers significantly correlated with numbers of NP-specific T cells
- Findings suggest B and T cells participate in immune-mediated protection to viral infection
- Implications in designing an effective vaccine (BioNTech/Pfizer and Moderna mRNA vaccinations already use S-Proteins as targets)
- Production of S-RBD-specific antibodies were detected in recovered in patients and discover virus neutralization in these patients
- Correlation between S-RBD production and neutralizing antibody titers but no correlation between NP production and neutralizing antibody was found
- S-RBD-specific T cell production of IFN-γ was also noted, suggesting that S-RBD induced broader T cell immune responses
- Detection of variable antibody titers in recovered patients (<30) to 1,936.
- Patient 9 had no significant serum virus-neutralizing activity (had anti-NP and S-RBD IgM but no significant IgG or IgG1 production, but had detectable virus-specific cell function)
- No correlation between age and neutralizing antibody titers (small sample size?)
- No differentiation of CD4+ and CD8+ T Cell responses