Low-dose in vivo protection and neutralization across SARS-CoV-2 variants by monoclonal antibody combinations

Introduction:

Monoclonal antibodies (mAbs) are emerging powerful therapeutics that can provide rapid protection from either infection and/or disease from pathogenic agents. The isolation of mAbs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a significant approach to pandemic control. As SARS-CoV-2 variants continue to emerge, which evade vaccines and therapeutics, and identification of mAbs with broad activity that maintain efficacy are of vital importance as the pandemic continues to evolve.Here we identify several potent neutralizing antibodies directed against either the N-terminal domain (NTD) or the receptor-binding domain (RBD) of the viral spike protein. Administered in combinations, these mAbs provided low-dose protection against SARS-CoV-2 infection in the K18-human angiotensin-converting enzyme 2 mouse model, mediated by both neutralization and Fc effector functions. The RBD mAb WRAIR-2125, which targets residue F486 through a unique heavy-chain and light-chain pairing, demonstrated potent neutralizing activity against major SARS-CoV-2 variants of concern (VOCs), such as Beta and Delta. In combination with NTD and other RBD mAbs, WRAIR-2125 also prevented viral escape. Another mAb, WRAIR-2173, showed robust neutralization of the Omicron variant highlighting the breadth of the isolated mAbs. These data demonstrate that Mab combinations mAbs can confer potent protection and increased coverage against VOCs.

Materials and Methods:

Convalescent plasma samples from patients infected with SARS-CoV-2 were screened for. SARS-CoV-2 pseudovirus neutralization. SARS-CoV0-2 antigen-positive B cells were isolated from peripheral blood mononuclear cells (PBMC) via fluorescence-activated cell sorting (FACS). MAbs were synthesized, cloned, and purified from the antigen-positive B cells for further characterization. Binding specificity was tested by Luminex® based assay against a panel of 25 coronavirus antigens, and kinetics determined by biolayer interferometry. Antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), opsonization and complement deposition (ADCD) assays were performed to assess Fc effector function. Epitope mapping was assessed by binding competition and alanine scanning. Structural analysis was performed using X-ray crystallography and electron microscopy. In vivo protection was assessed in a K18-hACE2 transgenic mouse model of SARS-CoV-2 infection.

Results:

Using a B cell sorting strategy based on multiple SARS-CoV-2 probes, including a multivalent spike ferritin nanoparticle (SpFN), we isolated a panel of 117 antibodies from a SARS-CoV-2 convalescent donor with very high plasma neutralization activity. Neutralizing antibody epitopes mapped to the NTD supersite and several discrete areas on RBD within the viral Spike glycoprotein. A small subset (~10%) of antibodies displayed extremely potent 50% inhibitory concentration below 100 ng/ml. One mAb in particular, WRAIR-2125, targeted a conserved

area of RBD and potently neutralized the major SARS-CoV-2 VOCs, including Alpha, Beta, Gamma and Delta. Another RBD mAb, WRAIR-2173, also targeting RBD, displayed strong neutralizing activity against the Omicron variant. Aside from neutralization, SARS-CoV-2 mAbs also demonstrated Fc effector functions such as complement deposition (ADCD) and phagocytic activities (ADCP and ADNP) that may contribute to viral clearance and inactivation in vivo. When tested for viral escape in vitro, combinations of two mAbs were needed to prevent viral growth. Multiple antibodies were found to confer protection against SARS-CoV-2 in vivo in the stringent K18-hACE2 mouse challenge model. Combinations of NTD/RBD mAbs were particularly potent and conferred prophylactic protection at doses as low as 0.25 mg/kg. Therapeutic protection was observed at 2.5 mg/kg when the mAbs were administered 24 h after challenge.

Conclusions:
We isolated and characterized a panel of SARS-CoV-2 neutralizing antibodies targeting the NTD and RBD regions of the spike protein, adding to the current arsenal of potent neutralizing antibodies described. When tested in combination, mAbs prevented viral escape and provided stronger coverage across current circulating VOCs. These data indicate that mAb combinations offer advantages to combat contemporary and future SARS-CoV-2 variants, especially in immunocompromised populations or individuals who do not respond to vaccination. These combinations of mAbs bolster military medical readiness and protect the Warfighter from infectious disease in deployed settings.

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