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Assessing cross-neutralization activity for SARS-CoV-2 spike mutants

SSI 02. November 2020, 10.2.e , senior researcher

SSI has so far identifed seven unique mink mutations/changes in the spike protein the sars-cov-2 variants in mink and infected peiople with contact to the farms. We have tested the first variant from farm-1 and here we have tested a variant with four simultaneous changes in the spike. This variant is dominating farm-6, 9, 10, 14, 18 (CHCNR 99768, 10967, 92288, 91966, 97712) and in the present experiments we isolated this same mink variant virus from a human patient and used it to evaluate if this virusvariant was sensitive or not to neutralizing antibodies (antisera) from a collection of convalescent individuals.

Virus culture

A mink and human clinical sample containing the SARS-CoV-2 virus with the spike mutations of interest is identified. The virus is grown on mammalian cells, a monkey kidney cell line named VeroE6, from the clinical sample in two subsequent rounds. Prior to use, nucleotide sequencing of the isolated virus is used to confirm that the cell culture process did not introduce additional mutations that were not present in the original clinical sample. To date, all viruses evaluated for cross-neutralization of antisera were identical in the spike protein compared to that found in the clinical sample.

Neutralization assay in Biosafety-3 laboratory

To determine if a mutant virus has reduced sensitivity to existing neutralizing antibodies, a predetermined amount of the mutant virus is mixed with serially diluted sera from patients that were infected with SARS-CoV-2 early in the Danish epidemic. The same sera is mixed with the same amount of virus from a strain that does not have the spike mutations. The virus and sera are incubated for 1 hour to allow the antibodies in the sera to bind to the virus. The virus/antibody mix is added to VeroE6 cells and incubated for 24 hours. Those antibodies that neutralize the virus will block its ability to infect cells and thus lead to a lower amount of virus present in the cells. After 24 hours, the amount of virus in the cells are measured by fixing the cells to the cell culture plate. This fixation process exposes the virus proteins produced during the infection of the cells. One of these proteins is the SARS-CoV-2 nucleocapsid protein. In a standard ELISA targeting the nucleocapsid protein, the amount of virus is determined. Using appropriate controls for each virus evaluated, a 50% cut-off value is calculated. The serum dilution at the 50% cut-off value is reported as the neutralization titer.

Results

Convalescent sera from 9 people (10 serum samples were tested, but one was inconclusive, so only 9 are reported) previously infected with SARS-CoV-2 early in the Danish epidemic were used to evaluate cross-neutralization activity against a mutant virus. Each serum sample was tested in duplicate with the mutant virus and the reference virus (without spike mutations).

	Serum sample – Neutralisation Titer								
	1	2	3	4	5	6	7	8	9
Reference virus	39	68	54	67	186	>1280	846	186	291
Mutant virus	28	5	9	30	67	>1280	606	54	177
% Difference	-28%	-93%	-83%	-55%	-64%	0%	-28%	-71%	-39%

The mutant virus resulted in reduced neutralization activity for 8 out of 9 sera tested when compared to a virus that lacks the spike mutations. The effect varied between the sera and ranged from a reduction of 25% to as high as 93% neutralization activity.

In conclusion, this indicate that mink virus variants with four of the seven identified mink-mutations/changes in the spike protein show less sensitivity (resistance) for neutralizing antibodies from persons with previous covid-19 infection. It is important to note that these results are preliminary and are in the process of being confirmed in an independent repeat experiment, but suggest that mink-specific mutation potentially can influence virus' sensitivity to protective antibodies.