



Evidence of the fast change of a dominant epitope in the hemagglutinin of Asian highly pathogenic H5N1 viruses

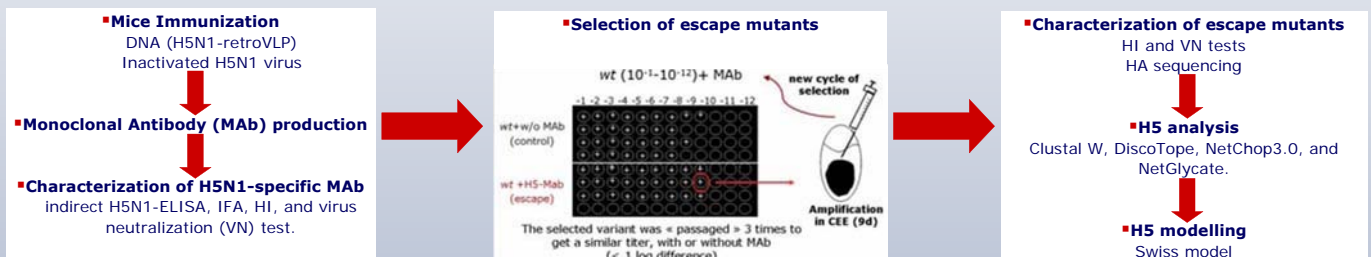
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Introduction and Objectives

- The highly pathogenic avian influenza (HPAI) H5N1 viruses have the RNA error-prone polymerases without proofreading capacity with high mutation rates, thus, they exist as heterogeneous populations of closely related variants characterized by quasispecies. Monoclonal antibodies (MAbs) targeting the hemagglutinin (HA) can be used for selecting antigenic variants (also known as escape mutants) of the influenza virus.
- The present study aimed at identifying amino acid changes in the hemagglutinin of the highly pathogenic A/crested eagle/Belgium/01/2004 (H5N1) H5N1 isolate, a clade 1 virus, that resulted in resistance to H5 protein-specific MAbs. In addition, the HA analysis from obtained H5N1 escape mutants and the impact of amino acid change by molecular homology was done.

Materials and Methods



Results and Discussion

- In the present study, H5N1 HPAI specific MAbs were obtained and then used for selection and characterization of H5N1 escape mutants. Two MAbs reacted with H5N1 HPAI viruses: 8C5 and 5A1 showed 62.84% and 46.47% of binding with clade 1 virus, but only 2.38 and 2.01% of binding with clade 2 virus. Similar results were obtained by IFA (Fig.1), VN and HI test (Tab.1).

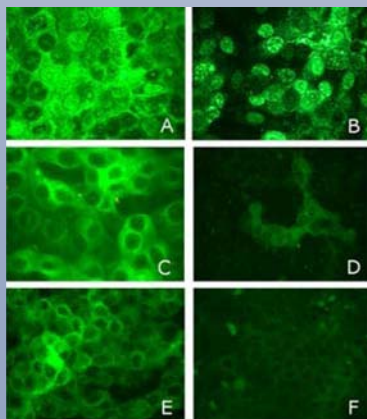


Fig. 1: MDCK cells were infected with H5N1 virus (clade 1 and clade 2) at MOI of 0.01 and detected using different MAbs by immunofluorescence assay. HB65 from ATCC, MAb against NP protein, recognize infected MDCK cells with H5N1 clade 1 (A) and H5N1 clade 2 (B). H5N1 clade 1 infected cells were recognized by MAbs 8C5 (C), 5A1 (E), whereas those infected MDCK cells with H5N1 clade 2 didn't react with MAb 8C5 (D) neither 5A1 (F). (1000x).

- The obtained MAbs were used for the selection of escape mutants (8C5v, 5A1v). HI titers obtained with both escape-mutants using the respective MAbs were 32-fold less than HI titers with wild type virus using the same MAbs (Tab. 1).

- The sequencing results show that 8C5v and 5A1v had an amino acid change (Lys→Glu) at position 189 in the H5 HA1 subunit. Interestingly, when looking at publicly available HA sequences, H5N1 clade 1 viruses have Lys (K) at this position; whereas 2.1 and 2.2 viruses have Arg (R), and clade 2.3 viruses have Lys (K) at the same position (Fig.2).

Tab. 1: After the escape mutant selection, the expression of biologically active epitopes on the HA protein of 8C5v and 5A1v were assessed by HI and virus neutralization test.

MAB	HI titer			VN titer		
	wt	8C5v	5A1v	wt	8C5v	5A1v
8C5	5	<2	<2	11	<2	<2
5A1	5	<2	<2	8	<2	<2
Positive serum	8	7	6	13	12	11
Negative serum	<2	<2	<2	<2	<2	<2

References

- Kaverin, N.V. et al., 2007. *J Virol* 81: 12911-7.
- Philpott, M. et al., 1990. *J Virol* 64: 2941-7.
- Wu, W.L. et al., 2008. *J Virol* 82: 1798-807.



Fig. 2: Schematic representation of Clustal W from HA1 protein showing differences of site 2 (residues 155-160 and 186-197) when compared 8C5v, 5A1v, wt, the most representative sequences of clade 1, clade 2.1, clade 2.2, clade 2.3, and 1jsm (PDB code). The blue box represents the amino acid residue 189

- The H5 HA protein has 3 antigenic sites (1-3). Our results show that the amino acid change at position 189 corresponds to antigenic site 2 of H5 HA1 (Fig.3). A very recent study suggest that 189-Lys alone has little effect on antigenic variation; conversely, our results are in good agreement with another very recent study, which suggests that the residue 189 is antigenically significant.

- An additional mutation (Lys→Glu) was observed at position 29 in the HA2 subunit. The K29E mutation resulted in the lack of a proteasomal cleavage site. The Glu-29 in the HA2 subunit could be an advantage for H5N1 viruses since the virus can induce a lower CTL response.

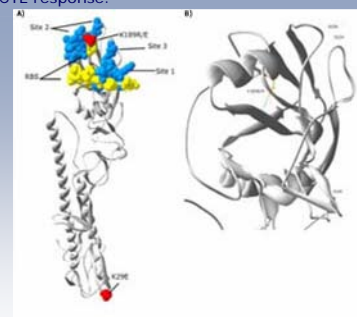


Fig. 3: (A) Antigenic sites, receptor binding site (RBS), and the amino acid changes of HA protein from H5N1 escape mutant viruses were based on the homology modelling using prototype 1jsm. Three antigenic sites of H5 HA escape mutants are shown in blue. RBS of H5 HPAI viruses is shown in yellow. Locations of 2 amino acid changes from H5N1 escape mutants (K189E in the HA1, K29 in the HA2) are represented in red. (B) Structural comparison of residues at position 189 in the HA1 from H5N1 viruses. Lys presented on wt is shown in orange, Glu of 8C5v and 5A1v is shown in red, and Arg of clade 2 is shown in green.

- Therefore, the residue at position 189 can be considered as one of the dominant epitopes in H5N1 viruses and clade 2 viruses evolved from clade 1 viruses under the selection pressure on this epitope. Conversely, the amino acid change in the HA2 subunit can represent a random selection of viral quasispecies.

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