UNMASKING STEM-SPECIFIC BROADLY NEUTRALIZING EPITOPES BY ABOLISHING N-LINKED GLYCOSYLATION SITES FOR VACCINE DESIGN

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Targeting highly conserved HA stem regions has been proposed as a useful strategy for designing universal influenza vaccines. The influenza virus HA stem region, consisting of a HA1 N-terminal part and full HA2 part, contains several potential sites for the addition of N-glycans. We expressed a series of recombinant HA (rHA) mutant proteins with deleted N-linked glycosylation sites in the HA1-stem and HA2-stem regions of H5N1 and pH1N1 viruses. Unmasking N-glycans in the HA2-stem region (rH5HA N484A and rH1HA N503A) did not affect the trimeric structure of HA. Immunizations using rH5HA N484A and rH1HA N503A elicited more potent neutralizing antibody titers against homologous, heterologous and heterosubtypic viruses. Unmasking the HA2-stem N-glycans of rH5HA N484A induced higher levels of stem-specific CR6261-like and FI6v3-like antibodies, improved the ability of stem-specific anti-fusion antibodies, enhanced H5 stem helix A epitope-specific B and T cell responses in splenocytes, and provided better protection against both homologous and heterosubtypic virus challenges. These findings suggest that HA2-stem N-glycan unmasking holds potential as a useful design strategy for developing more broadly protective influenza vaccines.