Engineering a Better FLUture with Monoclonal Antibodies

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Abstract

Each year epidemiologists and public officials predict which influenza strain will be most prevalent and design a vaccine to target those specific strains; unfortunately, they are not always accurate. Traditional vaccines cause our bodies to produce antibodies that target two subtypes of influenza A, which both bind to the surface protein Hemagglutinin (HA). However, HA mutates quickly, leaving the body susceptible to new mutant strains of influenza. Fortunately, another surface protein, Neuraminidase (NA), mutates significantly slower than HA, which would make a vaccine that targets NA potentially more effective against a broader spectrum of influenza A and B subtypes. According to Stadlbauer et al., one H3N2 infected donor naturally created monoclonal antibodies (mAbs) against NA that are protective against various strains of influenza A and B. Our research explored the mAb 1G01 in relation to H3N2 NA using PDB 6Q23. We found that 1G01 targets the active site of NA resulting from attraction due to a difference in charges, thereby blocking binding to its substrate necessary for NA to support virus binding and replication. Therefore, implementing properties of anti-NA antibodies into current influenza vaccines could be used to improve their protectiveness against more strains of the virus, increasing the predictive reliability of current vaccines, and as a step towards a universal influenza vaccine. The Mahtomedi MSOE Center for BioMolecular Modeling MAPS Team used 3D modeling and printing technology to examine structure-function relationships of antibodies in relation to influenza. The visual model will be a valuable tool in developing our story.

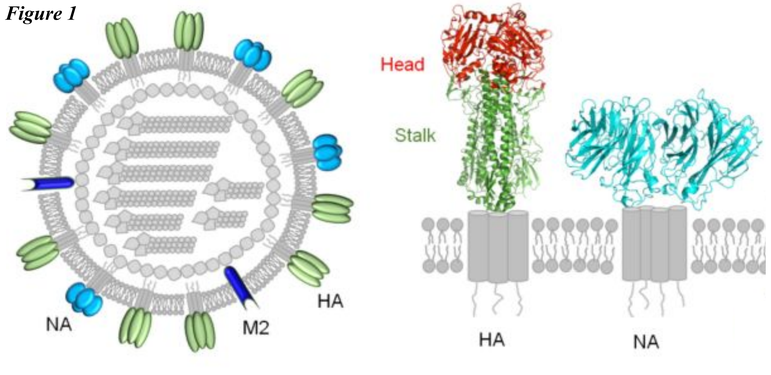
Differences Between HA and NA

Table 1: Data from Comparative Co-Evolution Analysis Between the HA and NA Genes of Influenza A Virus (Jang & Bae, 2018)

	Hemagglutinin	Neuraminidase
Human H3N2 Mean Substitution Rate of Nucleotides	**3.49 E-3**	**2.99 E-3**
Total (Human, Swine, and Avian) H3N2 Mean Substitution Rate of Nucleotides	**2.78 E-3**	**2.62 E-3**

Hemagglutinin (HA) is the influenza virus' major surface glycoprotein that most current vaccines target and is responsible for infecting host cells. Neuraminidase (NA) is the second major surface antigen which is critical for cleaving the terminal sialic acid from the host cell receptors and ensuring viron progeny does not bind to the same host cell (McAuley et al., 2019). An important difference is the size and relative number of Hemagglutinin as opposed to Neuraminidase. As seen below in Figure 1, the HA protrudes outward more than NA and is more prevalent on the virion surface; NA only accounts for about 10 to 20% of glycoproteins. Another key difference between HA and NA is the mutation rate. HA mutates at a higher rate than NA due to a number of factors including the polymerase error rate, immune pressure, and the globular head domain's high plasticity (Wong & Webby, 2013). As quantified in Table 1 above, the mean substitution rate for HA is higher across the three hosts studied for strain H3N2 influenza (Jang & Bae, 2018). NA has a slower drift, which allows antibodies to target NA across more strains and subtypes.

Figure 1

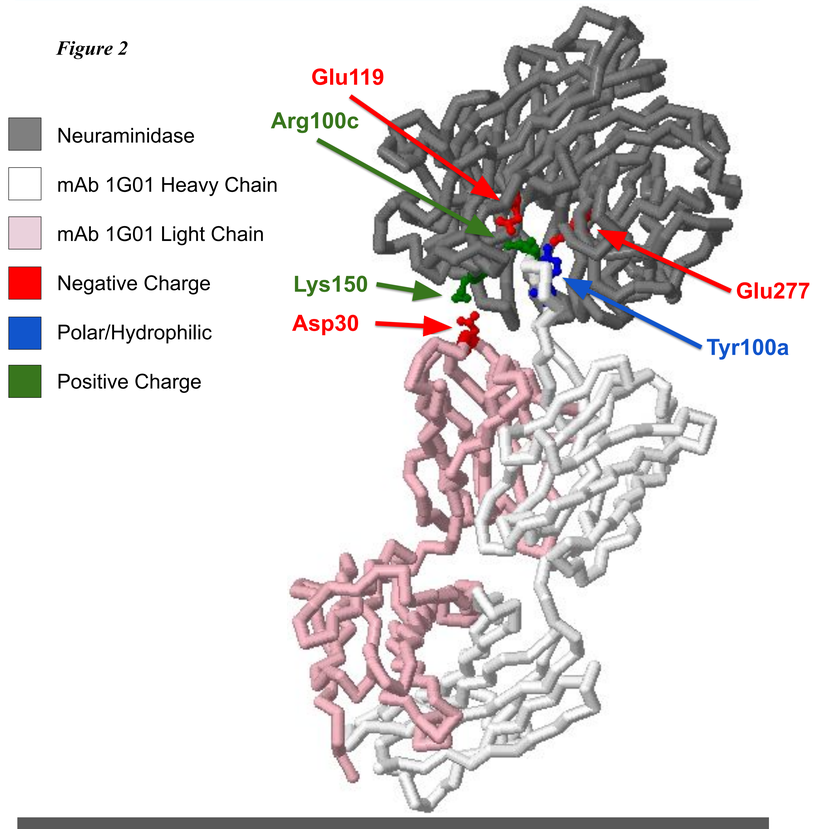


Antibodies

Antibodies are Y-shaped proteins which identify and latch onto antigens in the body. They can neutralize the antigens they bind to or, if unable to, will induce a chemical reaction with other proteins in the bloodstream that will cause the antigen to be incapacitated through other means. Since antibodies are structured by four protein chains, two long and two short, known as heavy and light chains respectively, they are able to create more than 2.6 billion different binding sites. Some antibodies, known as polyclonal antibodies, interact with many different epitopes on its target antigen. On the other hand, monoclonal antibodies only interact with one type of epitope on its target antigen (*Antibody*). This study focuses on monoclonal antibodies, specifically the human mAb 1G01 (Stadlbauer et al., 2019).

Influenza and 1GO1 mAB Model

Figure 2



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mAb 1G01 Broadly Protective

A human donor infected with H3N2 influenza provided three monoclonal antibodies for the study (Stadlbauer et al., 2019). Of the three mAbs analyzed, we focused on the most broadly protective: 1G01. This mAb binds to all influenza A group 1 NAs (N1, N4, N5, and N8) as well as all group 2 NAs (N2, N3, N6, N7, and N9) and both influenza B virus lineages (Stadlbauer et al., 2019). This is a considerable improvement over current intramuscular vaccines which target HA and only cover 4 strains (*Influenza Vaccine for the 2021-2022 Season,* 2021). The activity of all influenza A group 1 and 2 NAs and one influenza B NA were inhibited as a result of the antigen binding fragment (Fab) of 1G01 binding within the active site of NA, fully blocking it. We hypothesized that a difference in charge is the largest contributor to binding. Specifically, we postulated that negative amino acids on the 1G01 mAb are attracted to the positive amino acids on the NA. However, of the fourteen interactions we identified after modeling the binding at the active site, only one was a result of a salt bridge between a negative amino acid on the mAb and a positive amino acid on NA. We discovered it is a variety of interactions that contribute to binding. Other interactions included three salt bridges between a positive amino acid on the mAb and a negative amino acid on NA, and eight potential short hydrogen bonds (SHBs). The latter is likely a significant contributor to binding. The donor amino acids that are most common in short hydrogen bonds are Serine, Threonine, and Tyrosine whereas the most common acceptors are Aspartic acid and Glutamic acid (Zhou et al., 2022). All eight of the potential short hydrogen bonded pairs we identified contained at least one of these common residues, four of which contained two common residues. Furthermore, we identified one of each of the most likely pairs to form SHBs: Tyr-Asp and Tyr-Glu (Zhou et al., 2022). With a variety of strong electrostatic and short hydrogen bonding interactions leading to increased binding, the mAB becomes much more effective in smaller quantities. In fact, the 1G01 mAb demonstrates an IC_{50} of 1.35 nM allowing for the mAB to maintain potency in smaller doses, which reduces systemic toxicity for vaccine recipients. Most importantly, the mAb 1G01 was found to provide full protection from morality and almost full protection from weight loss in a mouse model (Stadlbauer et al., 2019).

Applications and Limitations

Neuraminidase antibodies are currently being used for therapeutic treatment. However, we propose implementing NA antibodies within vaccines as well. Since the 1G01 antibody IC_{50} is comparatively small, it could be possible to add NA binding monoclonal antibodies to existing influenza vaccines without compromising current universality. If the 1G01 antibody could be implemented to create a universal influenza vaccine, it could remain effective for a longer period of time, possibly even protecting against viruses across multiple influenza seasons. The NA targeted vaccine would protect against more influenza strains, and reduce infections rates for the public.

While the implementation of NA in influenza vaccines may improve general infection rates, it is important to consider the unknown effects that it could have long-term. Similar to how HA mutates faster due to selective pressure against it, targeting NA surface proteins may also cause NA-targeting vaccines to become obsolete. However, if scientists were able to construct an antibody using 1G01 that could cover all eleven subtypes, NA antigenic drift would effectively be neutralized (with a small margin of error). Further, due to their co-evolution, antigenic drift in either HA or NA could impact the other's mutation rates. This could cause an overall decrease in current vaccine effectiveness and viral strain relativity (Jang & Bae, 2018). Current HA immune pressure has demonstrated minimal effect on current NA mutation rates, so we hypothesize that NA immune pressure would only have a marginal effect on HA mutation rates, although more research needs to be conducted to find concrete evidence.

Conclusion

Influenza NA mutates significantly slower compared to the other major surface glycoprotein HA. The NA-targeting mAb 1G01, derived from a human donor, is more protective against many strains of influenza than most current HA-targeting vaccines. Pathologists and public health officials would be able to apply NA-targeting mAbs in vaccines to increase their universality and longevity. Despite possible limitations of an NA targeting antibody, we encourage further research regarding implementation of 1G01 or similar mAbs into influenza vaccine development. We believe that researching NA's immune pressure impact on HA would be the next step in creating a universal vaccine.