論文の内容の要旨

論文題目： Characterization of human monoclonal antibodies against influenza A virus and its application to antibody therapy
（A 型インフルエンザウイルスに対するヒトモノクローナル抗体の性状解析と抗体治療への応用）

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Influenza virus is an RNA virus that circulates among humans, causing acute respiratory infections. It is estimated that three to five million people are infected with influenza virus every year, causing a serious health burden and economic impact due to reduced productivity. Therefore, influenza virus poses a constant and serious public health threat.

To counter the threat of influenza virus, antiviral therapy is one of the main protective strategies. In particular, neuraminidase (NA) inhibitors are currently used to treat influenza virus infection. NA is the virus surface protein consisting of a stalk and a head portion, and the latter of which possesses enzymatic NA activity that cleaves the interaction between viruses and the host cell surface, allowing release of progeny viruses. NA inhibitors bind to the active site on NA molecules and inhibit virus release. These inhibitors have been employed successfully for more than a decade. However, resistant strains appeared and circulated between 2007 and 2009. Although the current dominant strains are susceptible to NA inhibitors, the emergence of resistant viruses is always a concern. Therefore, several therapies that target highly conserved features of influenza virus, such as antibodies to the hemagglutinin (HA) conserved region, are being developed. The virus surface protein HA consists of a globular head and a stem region, and mediates binding of the virus to host cells via interactions between its receptor binding site (RBS) and the terminal sialic acids on host cell glycans. Antibodies targeting the functionally conserved residues within the RBS of the HA head possess high protective efficacy by directly blocking sialic acid receptor binding; such antibodies would be expected to be powerful anti-influenza agents. However, it is not clear how readily mutant viruses can escape from anti-RBS antibodies or whether such mutants are biologically fit to compete with wild-type viruses.

Another powerful tool to control influenza virus infection is vaccination, which can induce protective antibody responses. However, influenza viruses can evade virus-specific host immunity through antigenic drift. Therefore, influenza vaccines must be reformulated frequently to antigenically match circulating strains. For the development

of a long-term effective vaccine, it is important to know the exact antigenicity of the circulating strains and predict their future antigenic drift. However, the antigenicity of NA is not as well understood as that of HA. And, although some studies have shown the importance of anti-NA antibodies for protection, the current vaccine does not efficiently elicit antibodies against NA. In this study, I aimed to improve protection strategies through isolation and characterization of human monoclonal antibodies (mAbs) induced by vaccination or infection.

In chapter I, I focused on the antigenic drift of A(H1N1)pdm09 virus. Since the 2017 Southern Hemisphere influenza season, the A(H1N1)pdm09-like virus recommended for use in the vaccine was changed because human, but not ferret, sera distinguish A(H1N1)pdm09 viruses isolated after 2013 from the previously circulating strains. An amino acid substitution, lysine to glutamine, at position 166 (H3 numbering) in the major antigenic site of HA was reported to be responsible for the antigenic drift. Here, I established two human anti-A(H1N1)pdm09 HA monoclonal antibodies, 1429B72/2-7 and 1429C45/1-5, which failed to neutralize A(H1N1)pdm09 viruses isolated after 2013. By passaging the virus in the presence of these monoclonal antibodies, I obtained escape mutant viruses that possessed an amino acid substitution at position 129, 165, or 166. Some escape mutants showed better growth kinetics and became the dominant population in the competitive replication assay. These findings indicate that antigenic variant viruses with a mutation at position 129 or 165 could be selected by the immunological pressure of human antibodies that recognize the epitope around position 166 that are abundant in some individuals, leading to the emergence of epidemic strains with such mutations. In combination with computational analyses and other methods, in vitro selection of potential antigenic drift mutants may improve the selection of vaccine seed viruses.

In chapter II, I isolated and characterized three human monoclonal antibodies that neutralize A(H1N1)pdm09 viruses: 1428A33/1 and 1428B5/1 were specific to H1pdm09-HA, whereas F3A19 recognized both H1pdm09- and H5-HA. I passaged viruses 9–25 times to obtain escape mutants, which possessed mutations within the RBS of HA, and in some instances failed to obtain such mutants even after 30 passages. Our results suggest that it is more difficult for viruses resistant to our three mAbs to emerge. Furthermore, the escape mutant viruses showed significantly less efficient replication in vitro, indicating that mutant viruses that are able to escape from these three anti-RBS mAbs would be unlikely to dominate the parental viruses. Given that the potential emergence of escape mutant viruses is one of the main disadvantages of mAbs as antiviral treatments, mAbs such as ours that rarely produce escape mutant viruses should be useful

as protective antibodies.

Finally, in chapter III, I evaluated the antigenic properties of NA. Antibodies possessing neuraminidase inhibition (NI) activity are produced in infected individuals and are important for protection against seasonal influenza virus infection. To evade such NI antibodies, amino acid mutations are accumulated around the enzymatic center of NA. Although many amino acid changes accumulate on the lateral surface of the NA head over time, which is far away from the enzymatic center, the role of these mutations remained unknown. Here, I isolated human mAbs that recognize the lateral surface of the NA head of A(H1N1)pdm09 virus isolated during the 2015–2016 influenza season. All seven mAbs activated Fcγ receptor (FcγR)-mediated signaling pathways in effector cells, and five of the mAbs possessed NI activity, but the other two did not; yet all seven protected mice from lethal challenge infection via their NI activity or FcγR-mediated antiviral activity or both. Serological analysis of individuals who were infected with A(H1N1)pdm09 virus revealed that some possessed or acquired the anti-NA lateral surface antibodies upon infection. I also found antigenic drift on the lateral surface of the NA head between 2009 isolates and 2015 isolates. Our results demonstrate that anti-lateral surface mAbs without NI activity can provide protection by activating FcγR-mediated antiviral activity and can drive antigenic drift at the lateral surface of the NA head. These findings suggest that the assessment of antigenic drift by using the NI assay alone is insufficient and that methods that allow us to consider antigenic changes that cannot be detected using NI assays need to be developed for better selection of vaccine candidate strains.