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Influenza and Non-Neutralizing Antibodies: A Defense in Disguise

Understanding the role of non-neutralizing antibodies in immunity against Influenza

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Table of Contents

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Abstract

Non-neutralizing antibodies (nNAbs) and Neutralizing antibodies (NAbs) are part of the adaptive immune system that play a protective role during infection. Neutralizing antibodies block the pathogens and prevent their spread by attaching to them, while non-neutralizing antibodies only attach to the pathogens but don't directly prevent infection. NAbs block viral infection by targeting surface proteins like hemagglutinin (HA), nNAbs take a different approach. They work by interacting with immune mechanisms such as Antibody-Mediated Complement dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis(ADCP), and antibody-dependent cellular cytotoxicity (ADCC), by targeting conserved viral areas, and hence help to eliminate infected cells and clear viral particles. The high rate of mutation in the influenza virus has proved to be a challenge in building a universal vaccine. nNAbs also provide cross-strain immunity by targeting conserved viral areas, such as internal proteins like nucleoproteins (NP) and Matrix-2 (M2) ion channels, which are less prone to mutate. Given this ability to target conserved regions, nNAbs may be essential to the development of future universal vaccination programs.By leveraging nNAbs' ability to activate immune responses against multiple viral strains, future vaccines could provide broader protection against evolving influenza viruses and emerging pandemics. This thesis investigates the role of these nNAbs particularly in influenza, their influence on immune response, potential in future vaccine development, and implications for broader disease protection strategies.

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Foreword

My interest in immunology began during my second year of studying biomedical sciences at the University of Groningen. A mandatory course which had to be followed by all students studying Biomedical sciences was my initiation in the world of immunology. The course focused on the intricate immune responses elicited by our immune system when our body is exposed to pathogens like influenza, this captivated my attention. The complexity of how our immune system combats pathogens like the constantly evolving viruses led me to dive deeper into who immunology plays an intricate role in defending against viruses.

When discussing my growing interest with my Academic Advisor Anna Henkel, I was encouraged to contact my thesis supervisor Dr. Anke Huckriede who does their research in the field of virology and immunology which was the perfect combination. After my meeting with Dr Anke Huckriede , I was encouraged to explore non neutralizing antibodies and their influence in influenza. Though this is a bit of a newer topic to me I was quite quickly intrigued by the readings and how these antibodies worked. Traditionally overshadowed by their neutralizing counterparts, nNAbs have shown promise in providing broader immune protection, especially in highly mutating viruses like influenza. Their role in immune responses such as antibody-dependent cellular cytotoxicity (ADCC), ADCP, and complement activation raised important questions about their potential in vaccine development and therapeutic interventions.

Through this thesis, I aimed to investigate the broader implications of nNAbs, not only in preclinical models but also in emerging human studies. This thesis has deepened my understanding of the complexities of the immune system and the potential future applications of nNAbs in public health. I hope this thesis contributes meaningfully to the ongoing dialogue in the field of immunology.

Introduction

Over the course of history, numerous influenza have occurred, which has resulted in the deaths of millions of lives across the globe, serving as one of the major causes of respiratory diseases in humans [The story of influenza, NCBI, 2005]. Influenza is responsible for significant morbidity and mortality worldwide, with complications ranging from mild respiratory symptoms to severe pneumonia, hospitalization, and death, especially among vulnerable populations such as the elderly, young children, and those with pre-existing conditions [Fleming et al.,2005].Seasonal influenza, commonly known as a flu, is an acute respiratory infection that is highly contagious, primarily affecting the throat, lungs, and nose. This infection has accounted for around 2,90,000 to 650,000 deaths and 3-5 million hospitalizations annually [WHO,2023].

Influenza viruses are classified into four types A, B, C, and D, with types A and B being the cause of most seasonal epidemics and hence being the most significant to study in terms of human health [Krammer et al., 2018]. The seasonal influenza epidemics are mainly caused by the two subtypes of influenza A virus H1N1 and H3N2 and the two lineages of influenza virus B B/Yamagata and B/Victoria [Lin X et al., 2023]. Influenza A viruses are further have subtypes based on their surface proteins, hemagglutinin (HA) and neuraminidase (NA), which play critical roles in the virus's ability to infect host cells [Taubenberger & Kash, 2010].Based on the combination of hemagglutinin (HA) and neuraminidase (NA) proteins produced on the virus surface, influenza A can be classified into many subtypes. There are eleven neuraminidase subtypes (H1-11) and eighteen hemagglutinin subtypes. Influenza A viruses, such H1N1 and H3N2, are classified into H and N kinds [Boktor & Hafner, 2023].

To evade the host's immune system often pathogens, especially viruses, go through antigenic drifts and shifts, which are changes or mutations to the viral surface antigens like HA and NA [How Flu Viruses Can Change: "Drift" and "Shift," 2024]. Due to these mutations it is difficult to develop a sustained immunity through natural infection or vaccination, hence posing a challenge to public health. This highlights the need to explore the intricacies of the immune system and how it responds to influenza [Brockwell et al.,2009]. Upon infection with influenza virus, the body responds by activating both the innate and adaptive immune response in order to fight the virus. The B cells produce antibodies which are part of the adaptive immune system that help fight the infection [Patel et al.,2021]. Neutralizing antibodies (NAbs) have been extensively studied for their role in immune protection and vaccine efficacy as they can directly prevent viral infection or replication by binding to specific sites on the virus [Krammer et al.,2019] . However, recent human studies have also highlighted the importance of non-neutralizing antibodies (nNAbs) in enhancing immune defense. Clinical trials for vaccines against influenza and HIV have found that nNAbs contribute significantly to protective outcomes [Yi-An Ko et al.,2021]. Damien et al., in their paper describe non-neutralizing antibodies (nNAbs) as a type of antibody which does not prevent infection but play other crucial roles in the immune mechanisms, such as mediating varios immune responses such as by activating the antibody-dependent cellular cytotoxicity (ADCC),antibody-dependent cellular phagocytosis and complement activation, while neutralizing antibodies are the type of antibody that directly block the entry of the virus by binding to the HA protein, preventing the virus from attaching to the host cells [Damien et al.,2008]. Non-neutralizing antibodies do not however prevent infection but rather help in fighting against the

infection by binding to the viral antigen and recruit other immune cells to enhance the defense of the overall immune response [Henry Dunand et al., 2016].In addition, nNAbs also have been found to broaden the response of the immune system by targeting the conserved regions of the viral internal proteins like HA and NA, because of which nNAbs can be a play a important role in the development of a universal influenza vaccine, hence providing a prolonged protection against a multiple strains of influenza virus [Mayr et al.,2017]

The significance of non-neutralizing antibodies in influenza still remains a relatively underexplored, but increasingly significant field of research. Although the primary goal of a standard influenza vaccine is to elicit neutralizing antibodies, there is mounting evidence that shows nNAbs also contribute significantly to providing a broader immune response and hence offering a wider protection against the virus. The aim of this thesis paper is to understand the role of non-neutralising antibodies in immunity against Influenza. By analyzing the existing literature, research studies, reviews and recent findings, this thesis seeks to understand the significance of nNAbs in the immunity against influenza by also highlighting the gaps in current research, and propose opportunities for further investigation.

4.1 Mechanisms of Non-Neutralizing Antibodies (nNABs)

In spite of the rising acknowledgement of their role in viral infections like influenza, nNAbs, unlike NAbs, have still been relatively understudied. Even though nNAbs do not prevent the entry of the virus, they engage the immune system in ways that promote the clearance of infected cells and viral particles. Their role in viral immunity has gained interest, especially for viruses like influenza, which go through constant mutation [Patel et al., 2021].nNAbs engage with viral components in such a way that enhances the response of the immune system. By attaching to these viral regions, nNabs trigger various mechanisms. Some mechanisms are mediated by intracellular activity, while others depend on external effector cells or proteins. nNAbs interact with the immune system through the constant engagement of the Fc region of the antibody with the Fc(fragment crystallizable region) receptors(FcR) which are found on various immune cells like macrophages, natural killer (NK) cells and dendritic cells. These interactions can lead to various mechanism like antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and Antibody-Mediated Complement dependent cytotoxicity, which help in clearing the virus from the body [Chandler et al.,2023].

Furthermore, nNAbs are capable of connecting the innate and adaptive immune responses.Their engagement with FcRs result in antigen presentation and triggering of adaptive immune responses, which are needed for forming immunological memory[Tay et al.,2019]. Moreover, nNAbs are known for their ability to target different viral epitopes, which enables them to adapt to viral mutations. This characteristic is especially important for viruses such as influenza, as immune flexibility is needed to effectively combat new viral strains due to continuous mutation [Krammer et al.,2015]. Through these functions, nNAbs contribute to a more comprehensive immune defense.

4.1.1 Antibody Dependent Cellular Cytotoxicity (ADCC)

Antibody Dependent Cellular Cytotoxicity (ADCC) is a type of immune response through which immune cells are activated to kill target cells which are infected with viral particles. This process involves the Fc gamma receptor IIIa ,FcγRIII (CD16) expressed on granulocytes such as natural killer cells and neutrophils [Chandler et al.,2023]. Once the NK cell recognizes the Fc region of the antibody bound to the target cell, it becomes activated, leading to the release of cytotoxic granules that contain perforin and granzymes. Granzymes enter through the pores made by perforin, inducing apoptosis leading to cell death [Katz et al., 1980].It was shown that a virus like influenza virus was able to elicit such antibody responses in humans [Jegaskanda et al., 2013].

4.1.2 Antibody-Dependent Phagocytosis (ADCP)

Antibody-Dependent Phagocytosis (ADCP) enhances the clearance of infected cells by activating phagocytic cells such as macrophages, monocytes, and dendritic cells. It involves the interaction of nNAbs with viral antigens, engaging Fc receptors like FcγRI (CD64) and FcγRIIA (CD32A) on these phagocytes, leading to more efficient engulfment of antibody-coated pathogens. Once the pathogen is engulfed, phagolysosomes form, where enzymes and reactive oxygen species destroy the pathogen [Chandler et al., 2023]. This process also promotes antigen presentation, boosting the overall immune response [Gao et al., 2020]. Notably, a study conducted by Dunand et al. found that nNAbs when a person is infected with influenza primarily triggered ADCP rather than ADCC in vitro. This suggests that, for influenza, ADCP might play a more significant role in protection when it comes to nNAbs [Dunand et al., 2019].

4.1.3 Antibody-Mediated Complement dependent cytotoxicity (CDC)

Complement activation represents another critical component of the immune response, enhancing the ability of antibodies and phagocytic cells to clear pathogens [Chandler et al., 2023]. Complement activation results in the production of a number of effector proteins like C1,C2, and C4 that interact with each other to eliminate the pathogen [Sedova et al.,2019]. These complement proteins C1,C2, and C4 ultimately form C3 convertase. The cleaved component C3b of C3 helps in the opsonization facilitating uptake by phagocytes. These proteins also form a Membrane Attack Complex (MAC) which by inserting into the pathogens membrane forms pores and disrupts the membrane which ultimately leads to the lysis of the cell [Merle et al.,2015].

4.2 Fc Receptor Interactions and Fc Receptor Polymorphism

The binding affinity between nNAbs and Fc receptors can vary, influencing the overall immune response and effectiveness in clearing infections. Fc receptors, particularly the Fc gamma receptors (FcγRs) have the most important role in mediating immunological responses in nNAbs [Amiah et al.,2020]. It was found that for the implementation of the effector functions of nNAbs, the most significant isotopes were IgG and IgM [Sedova et al.,2019]. A study by Shibuya et al., showed that FcγRs such as FcγRI, FcγRIII, and FcγRIV initiated ADCC or ADCP-mediated cross-protection when mice were infected with influenza [Shibuya et al.,2020]. Monocytes and macrophages express the FcγRIIa receptor (CD32A),which enhances phagocytosis, and FcγRIIIa present on NK cells participates in ADCC [Sedovo et al.,2019].

Polymorphism in Fc receptor (FcR) genes is important in how antibodies and immune cells interact, ultimately affecting the effectiveness of antibody treatments and immune reactions.These polymorphisms, include single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), which can influence both the structure and function of Fcγ receptors [Li et al.,2014]. Variability in immune responses to influenza can arise due to variations in FcγR polymorphisms across individuals. This diversity may impact the effectiveness of protection provided by antibodies and must be taken into account when developing vaccines [Thulin et al.,2018].

4.3 The role of nNABs, Cross-Reactivity and Broader Protection

Non-neutralizing antibodies are crucial as they play a major role in protection against influenza by evoking multiple immune responses.They enhance immune defense through effector mechanisms like ADCC and ADCP. Unlike neutralizing antibodies (NAbs), which block viral entry, nNAbs help clear infected cells by targeting conserved regions, offering potential cross-protection across multiple influenza strains.
One of the most compelling features is nNAbs' ability to recognize highly conserved epitopes, which allows them to provide cross protection across different influenza strains. Nucleoprotein (NP) is an important internal protein that is highly conserved across various influenza strains, nNABs can bind to these NPs when it is displayed on the surface of the infected host cell. Another critical component is an internal protein called the M2 Ion channel Protein, they help in uncoating the virus. These non

neutralizing antibodies can bind to these channels on the surface of the infected cells. nNABs can also bind to the Hemagglutinin (HA) and Neuraminidase (NA) in infected host cells [Boudreau et al.,2019] as well as to viral antigens through their Fragment antigen binding region(Fab) and facilitate immune responses through their Fc region [Ko et al.,2021]. Following antigen binding, the Fc region of the nNAb interacts with Fc receptors on immune cells initiating effector functions [Boudreau et al., 2019]. Banguru et al., in their study, highlighted that the non-neutralizing antibodies recognise the HA globular head and by binding to it limit the spread of the influenza virus and protect against infection. And DiLillo et al. showed that nNAbs targeting these more conserved regions of the influenza virus were more effective in activating immune cells such as natural killer (NK) cells and neutrophils . Similarly a higher cross protection was also observed in humans across multiple influenza strains when nNABs target regions like M2 ion channels and nucleoprotein (NP) instead of surface proteins like HA [Dunand et al.,2014][Bangaru et al., 2019]. Additionally, nNAbs that target the conserved M2 protein have been shown to provide protection against multiple influenza subtypes in mice [Gao et al.,2020]. Even though non-neutralizing antibodies have been found to target both the HA head and stem regions of the influenza virus, however, they tend to focus more on the stem region. This is important because antibodies targeting the stem can recognize a wide range of influenza A viruses, offering broad protection [Boudreau et al.,2019].

Another key characteristic of nNAbs is its ability to provide cross protection against multiple strains of influenza. Unlike NAbs, which are often specific to a single strain, nNAbs work by recognising highly conserved epitopes like the HA stem [Krammer et al.,2015]. For instance a research study done by Tan et al., upon analysis found that transferring non neutralizing human serum with strong HA reactivity into H7N9 influenza infected mice reduced the virus levels in the lungs. It was also seen that non-neutralizing antibodies like 1H5 provided protection in mice when infected due to interactions with Fc receptors or complement proteins [Tan et al.,2016]. Damien et al. also showed that nNAbs against influenza have shown to elicit CDC, and increase the T-cell responses associated with dendritic cell function enhancing the immunity across subtypes [Damian et al.,2008]. This cross reactivity makes nNAbs particularly valuable in the context of seasonal influenza, where the circulating strains change from year to year [Jegaskanga,2018]. In fact, some nNAbs induced by vaccines like FluMist combined the effect between pre-existing and vaccine responses in influenza strain A and B, suggesting nNAbs may build upon pre-existing cross-reactive immunity.Furthermore, Ko et al. conducted an experiment in mice to understand the influence and protection of the non-neutralizing antibodies when infected by influenza virus. The antibody studied 651, was non-neutralizing and showed a broad recognition of viral antigens and could activate immune cells like natural killer (NK) cells and alveolar macrophages. The infected mice that were treated with this antibody displayed a significant reduction in inflammation and also portrayed lower mortality rates. These immune cells elicited responses like ADCC and ADCP, which help clear infected cells and reduce the viral load. The study also demonstrated that alveolar macrophages play a critical role in modulating immune responses without causing harmful inflammation, which is important in diseases like influenza where inflammation can worsen symptoms [Ko et al.,2008].
By targeting conserved elements, nNAbs offer the potential for more universal protection, this especially important for mitigating the effects of antigenic drift and offer a potential solution by providing cross protection against pandemic strains as well as seasonal influenza.

4.4 nNABs in Influenza in Humans

In humans, nNAbs have been shown to provide broad protection across different influenza strains, particularly those targeting conserved viral proteins. A study by Rijnink et al. illustrates the potential of nNAbs targeting conserved internal influenza A virus (IAV) proteins as a strategy for broad protection against multiple influenza strains. The researchers isolated human monoclonal antibodies (mAbs) against M1 and NP from two individuals that were affected with the H3N2 virus to demonstrate the cross reactivity in Influenza A virus. In vitro assays revealed that while these antibodies were non-neutralizing; anti-NP mAbs displayed higher levels of ADCC and ADCP as compared to the levels of anti-M1 mAbs, suggesting Fc-mediated effector functions of these antibodies [Rijnink et al.,2023].

Antibodies specific to the nucleoprotein (NP) of influenza A virus, which are involved in ADCC, were also detected in children vaccinated with seasonal inactivated influenza vaccines. These NP specific antibodies, which interact with FcγRIIIa and activate natural killer (NK) cells, were identified in both healthy individuals and those infected with influenza. Studies have shown that serum from healthy donors containing NP specific antibodies can activate NK cells against virus-infected cells expressing NP, demonstrating their role in immune defense [Demminger et al., 2020].Another study conducted by Tong et al. highlighted the importance of Fc-mediated effector functions in influenza by demonstrating that live attenuated influenza vaccine (LAIV) induced more non-neutralizing antibodies effector responses compared to that in inactivated influenza vaccine (IIV). Live attenuated influenza vaccine (LAIV) contains a live, weakened influenza virus and is made to mimic a natural infection. These LAIV-induced effector functions include ADCC, ADCP, and antibody-dependent complement activation (ADCD). On the other hand, inactivated influenza vaccine (IIV) contains dead viruses or viral proteins, inducing immunity primarily through the production of antibodies. This study suggests that LAIV promotes higher levels of antibody dependent cell functions across various influenza strains, particularly against neuraminidase, and induces greater NK cell activation and monocyte phagocytosis [Tong et al., 2023]. It was also seen that the conserved antigenic site A on the H7 influenza virus could be important for human immunity. This antigenic site remains unchanged between the two different strains of the H7 virus allowing for cross reactive protection in humans. Hence suggesting that the vaccine targeting this site provides broad protection against the H7 virus strains[Tan et al.,2016].
In addition, Kristensen et al. found that immunization with IIV enhances ADCC activity against the hemagglutinin (HA) proteins of influenza virus in adults [Kristensen et al., 2016]. Similarly, Zhong et al. revealed that when humans were vaccinated with the 2014/2015 seasonal vaccine, significant ADCC activity specific to hemagglutinin (HA) was observed against antigenically drifted circulating H3N2 viruses [Zhong et al., 2016]. These findings underscore the importance of nNAbs in humans when infected with influenza and highlight the potential for future vaccines to induce these functional antibody responses.

4.5 Therapeutic and Vaccine Implications

Non-neutralizing antibodies (nNAbs) are now a major focus in the field of influenza treatment and vaccine research. Contrary to neutralizing antibodies that block viral entry, non-neutralizing antibodies can provide protection through various Fc-effector functions. NAbs function by binding to viral surface proteins, such as HA and NA, and preventing the virus from entering host cells. This direct mechanism of action makes NAbs highly effective at neutralizing specific viral strains. However, viruses like influenza mutate quite rapidly, which means that these NAbs are no longer able to identify the virus and become effective against that specific strain [Dong et al., 2020]. While NAbs are highly strain-specific, nNAbs offer cross-protection by targeting conserved viral elements. [Jegaskanda et al., 2018].

A study conducted in 2016 by Dunand et al. showed that vaccines designed to trigger both NAbs and nNAbs offered more robust protection against influenza virus strains. By integrating nNAbs into vaccines, it may be possible to achieve broader and more efficient immunity. This protective mechanism was further illustrated in experiments by Dunand et al. where both neutralizing and non-neutralizing H7-reactive antibodies protected mice in passive transfer experiments.Certain cross reactive non-neutralizing antibodies that were able to offer protection by recruiting effector cells through Fc-mediated effector activation. [Dunand et al.,2016]

Experimental vaccines that elicit nNAbs have shown promise in preclinical and early-stage clinical trials. For example, the M2 virus-like particles (VLPs), which mimic the structure of the virus but are non-infectious, are used in vaccines to stimulate an immune response specifically targeting the conserved M2 protein on the viral surface. While the M2-VLP vaccination did not induce significant neutralizing activity it was seen that the non-neutralizing humoral immunity was able to show protection. These antibodies were able to recognise viral components across different subtypes and contribute to cross protective immunity by clearing the virus through effector mechanisms like opsonophagocytosis, where immune cells engulf and destroy antibody-coated viruses [Zhang et al.,2024]. Studies have shown that NA specific antibodies reduce illness, including mortality in mice models, by inhibiting viral release from cells and reducing efficiency of replication. The research by Chen et al. underlined that NA can provide broad protection against influenza by inducing antibodies that target conserved NA epitopes, which remain relatively stable across various strains. Antibodies that react to NA can prevent viral release and also impact viral replication, providing protection against both human and avian influenza strains[Chen at al.,2018]. Another study suggested that mice and ferrets vaccinated with NA antigens showed significant protection against homologous and heterologous influenza virus challenges, with reduced viral lung titers and morbidity [Skarlupka et al.,2022]. This suggests that optimizing NA content and structure in vaccines could enhance protection . These results indicate that nNAbs are crucial in providing protection, particularly when neutralizing antibodies are less efficient because of virus mutations. The capacity of nNAbs to boost the immune reaction beyond simply neutralizing is a crucial benefit of vaccine creation.

Discussion and Conclusion

Traditional methods for assessing influenza vaccine efficacy, such as hemagglutination inhibition (HI) and neutralization assays, primarily detect neutralizing antibody responses and often overlook the protective potential of non-neutralizing antibodies (nNAbs) [Ko et al., 2021]. Although nNAbs do not directly neutralize the virus, they provide essential defense through Fc-mediated mechanisms. The limitations of traditional efficacy measurements underscore the need for broader evaluation methods that encompass nNAbs' protective effects, which contribute valuable immune defense components. Developing assays to measure both neutralizing and non-neutralizing responses could lead to vaccines offering broader protection against influenza strains [Dilillo et al., 2016].

Although typical vaccine efficacy assays do not measure these nNAb activities, they are essential for comprehensive immune protection. New vaccines aimed at conserved NA regions, like those created with the COBRA method, have great promise in producing broadly reactive nNAbs. Anti-NA antibodies work by blocking the NA cleavage activity in which NA cuts off the terminal sialic acid from glycans on both the host cell and emerging virus [Zhu et al.,2019].This results in the new virus to be released from the host cell. AN example of this type of vaccine is the COBRA-derived N1-I antigen that has produced antibodies that lower viral levels and improve symptoms in animal tests [Allen et al.,2018][Sautto et al.,2019]. These results suggest that anti-NA antibodies may play a role in providing prolonged protection against various flu strains, including those that could cause a pandemic. Although anti-NA antibodies by themselves may not block the virus from entering cells, they can still help decrease the replication and transmission of the virus during an infection, which works alongside the protective actions of neutralizing antibodies. This suggests that adding NA antigens to future vaccines with HA antigens may boost overall immune protection [Krammer et al., 2018].This integration could also assist in reducing antigenic drift, which happens when viral surface proteins change over time, making some neutralizing antibodies less useful. As the HA and NA proteins evolve separately, including antigens that trigger both neutralizing and non-neutralizing antibodies may offer enhanced cross-protection (Dong et al., 2020). In addition, focusing on preserved NA regions could broaden immunity against various strains, supporting the progress of developing a universal influenza vaccine.

Unlike neutralizing antibodies, which focus on viral surface proteins, nNAbs target conserved regions that exhibit lower mutation rates. This characteristic enables nNAbs to offer cross reactive protection, which is particularly significant for rapidly evolving viruses like influenza, where cross-protection is vital for the development of effective vaccines. By engaging immune cells through ADCC and ADCP, nNAbs facilitate viral clearance, thereby limiting infection spread and mitigating disease severity [Tong et al., 2023]. The ability of nNAbs to provide protection across influenza strains is especially valuable for vulnerable populations, such as older adults and immunocompromised individuals. These groups may have weaker neutralizing antibody responses and could benefit from the additional layer of protection nNAbs offer [Laursen et al., 2018]. Incorporating nNAbs into vaccine design could help address this limitation by enhancing immune response through mechanisms other than direct viral neutralization. As vaccine development continues to progress, designing formulations that elicit both neutralizing and non-neutralizing antibodies will be essential for achieving broad protection across diverse influenza strains. Additionally, investigating the role of Fc receptor polymorphisms in non-neutralizing antibody (nNAb) efficacy could lead to personalized vaccine strategies. Moreover, further studying the long-term effects of nNAb-mediated protection and their impact on viral evolution may inform future vaccine design [Lin et al., 2021]. Research in both animal models and humans has demonstrated that nNAbs can decrease viral load, inflammation, and mortality rates. nNAbs have

protective effects due to their capacity to trigger processes like antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement activation [Jegaskanda et al., 2018]. Future studies need to prioritize optimizing vaccine strategies to elicit both neutralizing and non-neutralizing antibody responses for complete protection. Furthermore, exploring the interaction between nNAbs and T-cell responses may result in improved immunization strategies [Ko et al., 2021]. Moreover, further studying the long-term effects of nNAb mediated protection and their impact on viral evolution may inform future vaccine design [Lin et al.,2021].The research highlights the important role of nNAbs in both preventing and treating influenza. These antibodies provide wider protection against different influenza strains by focusing on conserved epitopes and activating different immune response mechanisms. Research in both animal models and humans has demonstrated that nNAbs can decrease viral load, inflammation, and mortality rates. nNAbs have protective effects due to their capacity to trigger processes like ADCC, ADCP, and complement activation [Jegaskanda et al.,2018].Future studies need to prioritize optimizing vaccine strategies in order to elicit both neutralizing and non-neutralizing antibody responses for complete protection. Furthermore, exploring the interaction between nNAbs and T-cell responses may result in improved immunization strategies [Ko et al.,2021]. As we learn more about nNAbs, we should also investigate their potential in fighting other viral infections besides influenza.

Nevertheless despite their potential, nNAbs come with limitations and potential dangers. A downside of nNAbs is antibody-dependent enhancement (ADE), which is a phenomenon seen in viral infections like. Antibodies in ADE help the virus enter host cells instead of stopping it, which could make the infection worse. ADE happens when antibodies attach to the virus without deactivating it, enabling the virus to infect cells through Fc receptors and boost its reproduction [Sawant et al.,2023]. While ADE has not been conclusively seen in influenza cases, it is important to thoroughly assess the effectiveness of nNAbs in vaccine development to prevent unintended immune reactions[Xu et al.,2021]. Comprehensive knowledge of the role of nNAbs in influenza immunity and the conditions that may lead to ADE is crucial to prevent vaccines designed to target nNAbs from unintentionally worsening infections.Furthermore, though animal studies have shown the effectiveness of nNAbs in decreasing viral levels and enhancing survival rates, the evidence in humans is somewhat scarce. Animal research offers important insights into immune processes, although human immune reactions may vary greatly [Sedovo et al.,2019]. Additional research is required to apply these results in clinical environments, confirming the effectiveness and safety of nNAbs in humans. Moreover, the function of nNAbs could depend on the situation, as some research shows strong protection, especially when paired with neutralizing antibodies, while other studies propose that their impact may change based on viral strain, immunity level, or experimental settings[Yegorov et al.,2022]. This variability underscores the importance of conducting more detailed studies on the particular situations in which nNAbs provide the best protection. Future research on nNAbs in influenza could further look at the vaccine efficacy against different strains, by targeting the conserved regions. Through long term clinical trials, scientists can also experiment how nNAbs might play a role in immune memory in high risk populations like the elderly and people with weak immune systems.These strategies could be useful in creating therapy in emergency situations like an outbreak. Regardless, it is still important to take into consideration the potential challenges that ADE and other complexities could cause.

In conclusion, even though neutralizing antibodies are still the main priority for creating flu vaccines because they can prevent the virus from entering cells, non-neutralizing antibodies play a crucial role in immune defense with their diverse Fc-mediated activities. Their capacity to focus on preserved viral areas and offer broad protection across different strains resonates with the objectives of creating a

universal vaccine, especially for influenza, a virus known for its constant changes in antigens. As vaccine plans progress, adding non-neutralizing antibodies (nNAbs) with neutralizing antibodies could enhance immune responses for groups with weakened neutralizing responses, like older adults and those with compromised immune systems. Future vaccines should aim to stimulate both types of antibodies for a more robust and long-lasting immune defense. Additionally, gaining knowledge on how nNAbs interact with T-cell responses may offer guidance for improving vaccination techniques. The significance of incorporating nNAbs in vaccine creation becomes more apparent with the rise of new influenza strains, as they provide extra protection avenues in cases where neutralizing antibodies are scarce or when viral changes evade standard immune barriers. More research is needed to completely understand the potential of nNAbs, but they show potential for improving immunity to influenza and working towards vaccines that can protect against quickly changing viral dangers.

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Afterword

This thesis has been a meaningful and insightful journey into exploring the role of non-neutralizing antibodies in influenza and their potential in shaping future protective strategies. The research process has deepened my understanding of immunological responses and the complexities of disease mechanisms. While this exploration is just one step, I believe it is a significant move towards enhancing our ability to protect against influenza in the future.

I would like to express my sincere gratitude to my thesis supervisor Dr Anke Huckriede, whose guidance and support were invaluable in helping me identify this crucial research topic. Their expertise and encouragement have been essential throughout this process. Writing this thesis has not only strengthened my scientific knowledge but also contributed to my personal and academic growth and I am proud of the outcome and the insights I have gained along the way.

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