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Monoclonal antibodies against AIV antigen and their significance in disease diagnosis

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Abstract
Avian influenza virus is a virus that has caused pandemics. It has global impact. Different groups of scientist has worked upon the virus to produce MABs against proteins present in virus to facilitate rapid diagnosis and confirmation of virus in early infection also to find the protein which can be utilized for can vaccine preparation in future. Ribonuleoprotein (RNP) can be used for the diagnosis though antigen is stable does not exhibit any significant antigenic variant but Anti-RNP antibody develops after infection but not following killed vaccines. While NS1 can differentiate well in infected and vaccinated animals. The surface proteins Haemagglutinase and Neuraminidase are present on different spike. They are responsible for antigenic variation. Haemagglutinase is a glycoprotein responsible for haemagglutination and haemadsorption. It enables virus to adsorb to mucoprotein receptors on RBCs as well as on respiratory epithelial cells. Anti-HA antibody is produced after infection and immunization. This antibody is protected by preventing adsorption of the virus to cells. Neuraminidase is a glycoprotein enzyme which destroys cell receptors by hydrolytic cleavage. The anti-neuraminidase antibody is formed after the infection and immunization. It is not as effective in protection as Anti-HA antibody. It is strain specific and exhibits variation.

Keywords: Monoclonal antibody, antigenic variation, haemagglutination

Introduction
This is a family of negative-sense, single-stranded RNA viruses. Influenza viruses are the only members of *Orthomyxoviridae* (8 segments). Viruses of this family have a predilection for the respiratory tract, but usually do not cause a serious disease in uncomplicated cases. Exceptions are human infections with viruses of avian origin. Principal viruses of veterinary importance are type A influenza viruses, which cause equine, swine, and avian influenza. Viruses have a segmented single-stranded RNA genome, helical nucleocapsids (each RNA segment + proteins form a nucleocapsid) and an outer lipoprotein envelope. The segmented genome facilitates genetic reassortment, which accounts for antigenic shifts. Point mutations in the RNA genome accounts for antigenic drifts that are often associated with epidemics. In either case, the changes are frequently associated with the HA (hemmaglutinin) and NA (neuraminidase) antigens. The envelope is covered with two different kinds of spikes, a hemagglutinin (HA antigen) and a neuraminidase (NA antigen). The viruses agglutinate red blood cells of a variety of species. Replication takes place in the nucleus.

The antigenic nature of influenza viruses is required to understand the epidemiology of influenza virus. The avian influenza virus (AIV) consists of following proteins:

1. The internal proteins consist mainly of nucleocapsid protein (NC), some matrix proteins (M1) and three polymerases (PA, PB1 and PB2). The proteins NC and M1 determine type specificity. Even being internal, these proteins (or peptides derived from them) may elicit cytotoxic T cells that are important in recovery from infection.
2. The nucleoprotein (NP) antigen (A, B, C) determines the virus type.
3. The surface proteins consists of two types haemagglutin and neuraminidase. Hem agglutinin (HA) is an envelope antigen (spike) that can attach to erythrocytes and cause agglutination.
4. Neuraminidase (N) is an envelope protein whose enzymatic activity results in the liquefaction of mucus thus contributing to viral spread. Specific antibody slows down the spread of virus. Neuraminidase also cleaves neuraminic acid to release progeny virus from the infected cell.
5. Influenza viruses are designated as follows: type/place/time of isolation/H and N content. In birds, there are approximately 15 H antigens (H1 - H15) and 9 N antigens (N1

- N9), which can be found in all possible combinations. An example would be H7 N3. Therefore, the type A virus: A/Bangkok/3/79 (H3N2) denotes, respectively, type A, isolated in Bangkok, local laboratory designate of number 3, first isolated in 1979, and envelope antigens of H3N2.

MABS against different proteins of AIV and their significance in disease diagnosis

The antigens of influenza virus can be classified as the internal antigens and the surface antigens. The internal antigens are RNP, Membrane and surface antigens consists of Neuramanidase and Haemagglutinin (HA) protein which are as follows:

Ribonucleoprotein (Rnp): It is found free in infected tissues and occurs in the supernatant when the virus containing fluid is centrifuged, it is called the soluble(S) antigen. It can be demonstrated by complement fixation test and immunoprecipitation tests. It is type specific and based on nature, influenza viruses are classified into types A, B, C. The RNP antigen of types A, B, C are distinct but all strains of any one type possess the same antigen. This antigen is stable does not exhibit any significant antigenic variant. Anti-RNP antibody develops after infection but not following killed vaccines. Following are MABs produced against Nucleoprotein:

1. 6 MABs against recombinant npof H9N2 AIV were obtained MABs obtained designated 4F4, 1C3, 1G11, 1C2, 1D10 and 2F7. The MABS 1G11 and 1D10 perform the best in titer and specificity. This finds its future for AIV study and development of method for rapid detection of AIV [1].
2. In order to develop rapid tests for AI antigen and antibody detection, two MABs against influenza NP were produced. These MABs are designated as F26-9 and F28-73 and able to recognize whole AI virus particles as well as the recombinant NP. Both of the MABs were tested in a slot blot for their reactivity against 15 subtypes of influenza virus.
3. Five monoclonal antibodies to nuclear protein of avian influenza virus (AIV) were developed against H9 subtype AIV. Specificity of these MABs was identified by immunofluorescent assay (IFA) and enzyme linked immunosorbent assay (ELISA). These five MABs which were named as 2C3, 6A5, 3H9, 7B4, 2H4 could react with all viruses of AIV-H9 strains in tests. The result of preliminary application showed that avian influenza viruses could be detected by MABs in IFA and ELISA. All these MABs will probably play important roles in preventing and monitoring avian influenza viruses [2].
4. The monoclonal antibody against H9 subtype of Avian influenza virus resulted in production two hybridoma cell lines secreting MABs against H9N2 AIV were obtained and named as 1E7 and 3D6. The results indicate that 3D6 is a monoclonal antibody against the NP protein of H9N2 AIV [3].
5. The RNP was used to immunize BALB/c mice to produce hybridoma secreting anti- NP MABs. Out of 11 anti-NP MABs produced 8D2-H5,8D2-H9, and 6D11-A7 were of IgM isotype and 5D10-C9 and 5D10-F11 were of igb2b type, while 3F3-D2,7D2-C9,7D2-G7, and 7D2-G8 were of IgG1 isotype. The MABs 3F3-D2 and 7D2-G8 showed high intensity positive reaction with RNP and a low intensity reaction with H5N1 virus in western blot analysis. The anti-NP MABs produced in the present work may be valuable in developing a competitive ELISA or immunochromatographic strip test – based assays for the rapid diagnosis of avian influenza [4].

Membrane Protein (M): This is membrane protein and is type-specific and distinct for A, B, C types of influenza viruses. The envelope lipid antigen is host specific and is determined by the species in which virus replication takes place. The following MABs against M antigen:

1. Four hybridoma cell lines secreting anti-AIV M2 MABs were obtained, designated 1E1, 2F8, 4E3 and 5D6. The Ag-Capture ELISA showed the MAB 2F8 could specifically reacted with H5 and H9 subtypes of AIV, but could not react with Newcastle disease virus (NDV) and Infectious bursal disease virus (IBDV).Four MABs against M2 were obtained in this study. The MAB 2F8 against AIV M2 could be used as the key reagent for establishment of AIV detection method [5].

Surface antigens: It consists of two antigens Neuraminidase (N) and Haemagglutinase. Haemagglutinase is a glycoprotein composed of two polypeptides-HA1 and HA2. It is responsible for haemagglutination and haemadsorption. It enables virus to adsorb to mucoprotein receptors on rbcs as well as on respiratory epithelial cells. Anti-HA antibody are produced after infection and immunization. This antibody is protected by preventing adsorption of the virus to cells. The HA is a strain specific and is capable of great variation. Fifteen distinct types HA subtype, named H1 to H15 have been detected. MABs against HA antigens are as follows:

1. Three hybridoma cell lines named 2H1, 2A3 and 1C8 against HA of AIV H9 were obtained. The immunoglobulin subclass of all 3 MABs was IgG1. Three MABs recognizing HA of H9 subtype of AIV were obtained, which may provide an useful tool for the antigenic analysis, the serological diagnosis, the epidemiological survey and the evaluation of AIV vaccine [6].
2. Six MABs generated against HA protein of H7N2 AIV outbreak of 1997-98 Pennsylvania [5] in this study 7 MABs against HA protein of AIV strain A/Newyork96/H [7].
3. 3. AIV H7 and H5 subtype specific-MABs have been successfully developed to enhance the Dot-ELISA and other MAB-based assays for AIV detection. Production and purification of the H7 and H5 MABs were made to provide essential reagents for Dot-ELISA and other immunoassays, and the current development of a novel Biosensor technique for rapid detection of AIV from clinical and field specimens. The AIV H5, H7 and N2 subtype-specific MABs can be effectively used for the detection of AIV by MAB based Dot-ELISA, other immunoassays, and Biosensor system, which offer great potential as useful methods for use in routine AIV diagnostics and surveillance programs, and are feasible for test-in-field or onsite for the rapid detection of AIV infections from field specimen [8].
4. Four monoclonal antibody against H7HA could react with the AIV. Among them, IF7 belongs to IgG2b, IA3, IB3, IC4 belongs to IgG1. And the four monoclonal antibodies can only react with the AIV Type H7HA, but not with the AIV Type H5HA or H9HA [9].

5. Three hybridoma cell lines against H9 subtype of AIV named 2H1, 2A3 and 1C8 against HA of AIV H9 were obtained. Three MABs recognizing HA of H9 subtype of AIV were obtained, which may provide an useful tool for the antigenic analysis, the serological diagnosis, the epidemiological survey and the evaluation of AIV vaccine [10].
6. To enhance the rapidity in diagnosing the spread of avian influenza virus (AIV) in chicken layer flocks, studies were initiated to develop more sensitive and specific immunological and molecular methods for the detection of AIV. In this study, the purification of the hemagglutinin protein (HA) from field isolates of H7N2, the production of MABs, and their evaluation as diagnostic reagents are reported. Hybridomas secreting antibodies specific for the HA protein were assayed by an ELISA and cloned using limiting dilution. The MABs produced were characterized by hemagglutination inhibition (HI), immunohistochemistry (IHC), indirect fluorescent antibody assay (IFA), Western blots, and IFA flow cytometry using various AIV subtypes (i.e., H4N2, H5N3, H7N2). Of the various MABs assayed, 6 had consistent and reproducible results in each of the assays used. The results obtained in this investigation enhanced the usage of the MABs to viral H protein in the surveillance of AIV in chickens [11].
7. Two hybridoma cell lines secreting the monoclonal antibodies to H5 avian influenza virus (AIV-H5), named 5A6, 5G7 were developed by two sub clones. The specificity of MAB was identified by means of indirect ELISA. The MABs prepared would be very useful in diagnosis and control of avian influenza [12].
8. To prepare monoclonal antibodies (MABs) against the hemagglutinin protein of H7 subtype of avian influenza virus (AIV). Four hybridoma cell lines secreting specific MABs named 2E2, 2A4, 5F5 and 7G5 were developed. The results of HI reactivity assay suggested that 2E2, 5F5 and 7G5 only reacted with H7 subtype of AIV but did not react with other subtypes of AIV, NDV and infectious bronchitis virus (IBV). However 2A4 reacted not only with H7 subtype of AIV but also with H15N8 reference strain of AIV at low HI level. These MABs can be used as a useful tool to analyze the HA structure of AIV. They also provide the effective reagents for the rapid detection of H7 subtype of AIV [13].

Neuraminidase Antigen (N)

It's a glycoprotein enzyme which destroys cell receptors by hydrolytic cleavage. The anti-neuraminidase antibody is formed after the infection and immunization. It is not as effective in protection as Anti-HA antibody. It is strain specific and exhibits variation. Nine different subtypes have been identified from N1-N9. The neuraminidase of influenza A viruses is a sialidase that acts as a receptor-destroying enzyme facilitating the release of progeny virus from infected cells. The monospecific anti-NA immune sera inhibited not only sialidase activity, but also influenza virus hemagglutination and infection of MDCK cells, suggesting that NA antibodies can interfere with virus attachment. Inhibition of both processes, virus release and virus binding, may explain why NA antibodies efficiently blocked virus dissemination *in vitro* and *in vivo*. Currently, 11 NA subtypes are known. Subtypes N1 to N9 have been detected in AIV, subtypes N1 and N2 are found in human influenza viruses, and subtypes N10 and N11 were recently discovered in bat influenza viruses [14]. The different nas of influenza A viruses can be phylogenetically classified into 3 groups, with group 1 comprising N1, N4, N5, and N8; group 2 comprising N2, N3, N6, N7, and N9; and group 3 containing N10 and N11 (Tong *et al*.,2013, Air 2012). Anti-NA immune sera showed broader reactivity than anti-HA sera in hemagglutination inhibition tests and demonstrated cross-subtype activity in sialidase inhibition tests. These remarkable features of NA antibodies highlight the importance of the NA antigen for the development of next-generation influenza virus vaccines. Neuraminidase (NA), the second major antigen of the viral envelope, is also subject to antigenic drift and shift, indicating that the antigen is under immune pressure, as well [15].

Non-structural protein (Ns)

The non-structural protein of influenza virus is expressed in large amounts in virus infected cells, but it was not detected in virions. The only protein found is non-structural, involve in multiple functions including regulation of slicing and nuclear export of cellular mRNA as well as stimulation of translation [16].Though its major functions seems to antagonize the interferon activity of host which depends on its ability to bind ds-RNA, a known potent inducer of interferon. It has been deduced from the studies that NS protein is also associated with virulence of virus. The following MABs have been produced against this:

1. Monoclonal antibody D9 against recombinant NS1 produced for the generation, identification, and epitope mapping of a NS1 protein-specific, was identified. Western blot assay results showed that MAB D9 reacted strongly with the recombinant NS1 [17].
2. Two MABs against NS1 of avian influenza virus (AIV), were belonging to IgG1 subclass and κ light chain. Western blot assay showed that the MABs were reacted with recombinant NS1. The result suggested that the peptide is a linear epitope of NS1, which facilitate further study on the function of NS1 and diagnostic method development for AIV detection [18].
3. Monoclonal Antibodies were raised against NS1 protein by immunizing BALB/C mice with recombinant NS1 protein and 12 clones were against recombinant NS1which are 1E5,3C10,4E8, 4E9,4C4, 5H8,6B9,8C6,8D10,9E8,9B7,10E2.10E2 was used for further study. It was characterized, purified and used for standardization of MAB based competitive ELISA for detection of antibodies against NS protein in field ser [19].

Conclusion

Above compilation reveals the facts that though all the protein play role in imparting virulence and pathogenicity to the influenza Virus directly or indirectly. But in diagnostics only few are as potent to be used as confirmatory for the presence of the virus. The Nucleoprotein is the best candidate to be a part of the diagnostic kits because of its consistency and non-variant antigenically. It can be utilized in competitive ELISA, Peptide Array technique, Immunofluorescent Assay, Immunochromatographic strip-test arrays. It helps in rapid diagnosis, prevention and monitoring of AIV. Membrane proteins can also be used for quick diagnosis but their effectivity is secondary to NP. Among surface antigens HA may provide an useful tool for antigenic analysis, serological diagnosis, epidemiological surveys and evaluation of AIV

vaccine. For Diagnostics tests it can be used in Haemagglutination Inhibition, Immuno Histo Chemistry, Indirect IFA, Western Blot, and Flow Cytometry.NA because of its action on virus attachment can be potent for future vaccine. But due to antigenic variation of HA, NA can work with type specific subtype of AIV which makes it difficult to work establish it as a vaccine candidate.

References

1. Liu FF, Jin ML, Zhang AD, Dan HB *et al*. Preparation and partial characterization of monoclonal antibodies specific for nuclear protein of avian influenza virus Chinese Journal of Cellular and Molecular Immunology. 2006; 22(5):648-9, 653.
2. Xi Bao, Yu Fen Zi, Mian Yi, Xue Za Zhi. Preparation and characterization of monoclonal antibodies against M2 protein of avian influenza virus. Chinese journal of cell and molecular biology. 2008; 24(5):475-78.
3. Xi Bao, Yu Fen Zi, Mian Yi, Xue Za Zhi. Preparation and characterization of monoclonal antibodies against the hemagglutinin of H9 subtype of avian influenza virus. 2004; 20(6):702-4.
4. Yu Fen Zi, Mian Yi, Xue Za Zhi. Preparation and partial characterization of monoclonal antibodies specific for nuclear protein of avian influenza virus. Xi bao Chinese Journal of Cellular and Molecular Immunology. 2006; 22(5):648-9, 653.
5. Wang X, Castro AE, Castro MD, Lu H, Weinstock D, Soyster N *et al*. Production and evaluation criteria specific of monoclonal antibodies to heamagglutinin of the H7N2 sub type of avian influenza virus Journal Veterinary Diagnosis Investigation. 2000; 12:50(3):509.
6. Huaguang Lu, Lin Lin, Ronghui Wang, Yanbin Li, Bill Scheuchenzuber, Jiabo Liu *et al*. Development of H5 subtype-specific monoclonal antibodies and MAB-based assays for rapid detection of H5 avian influenza Article, 2005.
7. Sheng- xi WU, Meng YAO, Ren-jian HU. Generation of Monoclonal Antibody to Avian Influenza Virus: Journal of Chongqing Institute of Technology (Natural Science), 2011.
8. Xue-xia1 WEN, Hong-mei BAO, Jia-shan SUN, Yu-hui ZHAO, Yun-he WANG, Yong JIANG *et al*. Preparation of monoclonal antibodies against NS1 of avian influenza virus and the antigenic epitope identification Chinese journal of Preventive Veterinary Medicine, 2010.
9. Wang Kai, Jin Ningyi, Lu Huijun, Han Song, Xu Yiming, Hu Zhongming. Preparation of monoclonal antibody against Chinese Journal of Preventive Veterinary Medicine. 2005; 27(6):454-456.
10. Li YQ, Yang J, Zhang L, Wei L. Preparation and characterization of monoclonal antibodies against M2 protein of avian influenza virus Chinese Journal of Cellular and Molecular Immunology. 2008; 24(5):475-478
11. Xi Bao, Yu, Fen Zi, Mian Yi, Xue Za, Zhi. Zhang PH, Zhang SH, Sun QY, Liu XF. Preparation and characterization of monoclonal antibodies against the hemagglutinin of H9 subtype of avian influenza virus. Chinese journal of Cellular and Molecular Immunology. 2004; 20(6):702-4.
12. Wang AE, Castro MD, Castro H, Lu D. Production and characterization of Monoclonal Antibodies against Nucleoprotein of Avian Influenza Virus: Bhat S., Bhatia S., Sood R., Bhatnagar H., Pateriya A., Venkatesh G.,; Monoclonal antibodies in immunodiagnosis and immunotherapy. 2013; 32:6.
13. Li YJ, Jiao XA, Pan ZM, Sun L, Wang Cb, Zhang SH *et al*. Chinese Journal of Cellular and Molecular Immunology [01 Oct 2007] - Development and characterization of monoclonal antibodies against H7 hemagglutinin of avian influenza virus. 2007; 23(10):953-955.
14. Xu X, Cox NJ, Bender CA, Regnery HL, Shaw MW 199 6. Virology, 1996.
15. Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M *et al*. New world bats harbor diverse influenza A viruses. Plos Pathogy, 2013, 9.
16. Sandbulte MR, Westgeest KB, Gao J, Xu X, Klimov AI, Russell CA *et al*. Discordant antigenic drift of neuraminidase and hemagglutinin in H1N1 and H3N2 influenza viruses Proc Natl Acad Sci USA. 2011; 108:20748-20753.
17. Air GM. Influenza neuraminidase. Influenza Other Respiriratory Viruses. 2012; 5:245-256.
18. Rai M, Bhatia S, Malik YPS. Development and characterization of monoclonal antibodies against Ns1 protein of avian influenza; hybridoma. 2009; 29(2):183-186.
19. Jiashan Sun, Xiurong Wang, Xuexia Wen, Hongmei Bao, Lin Shi, Qimeng Tao *et al*. Identification of a Highly Conserved Epitope on Avian Influenza Virus Non-Structural Protein 1 Using a Peptide Microarray, 2016.