Brief Report

Preparation of Anti-Idiotypic Antibody against Avian Influenza Virus Subtype H9

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To generate monoclonal anti-idiotypic antibodies (mAb2) against avian influenza virus subtype H9 (H9 AIV), BALB/c mice were immunized with purified chicken anti-H9-AIV IgG and the splenocytes of immunized mice were fused with myeloma cells NS-1. Hybridoma cells were screened by indirect enzyme-linked immunosorbent assays with both chicken and rabbit anti-H9-AIV IgG as coating antigens. One hybridoma cell clone secreting monoclonal antibody against idiotypes shared by both chicken and rabbit anti-H9-AIV IgG was established. Experiments demonstrated the mAb2 was able to inhibit the binding of hemagglutinin to anti-H9-AIV IgG and to induce chickens to generate hemagglutination inhibition antibodies, indicating this anti-species-sharing-idiotypic antibody bore the internal image of hemagglutinin on avian influenza virus. *Cellular & Molecular Immunology*. 2005;2(2): 155-157.

Key Words: avian influenza virus, anti-idiotypic antibody, sharing idiotype

Introduction

Avian influenza, as an acute and highly contagious disease caused by avian influenza virus (AIV), causes heavy economic losses in poultry. H9N2 was one of the main subtypes popular in china recently. Inactive vaccine was applied in fields but there were some shortcomings with it. Scientists are trying new ways to prevent avian influenza. Anti-idiotypic antibodies (Ab2, a-Id) bearing the structure complementary to the paratope of Ab1 and representing an "internal image" of the antigenic epitope recognized by Ab1 can induce specific antibodies and T-cell response to a given antigen (1). In this experiment, monoclonal Ab2 (mAb2) against H9N2 AIV was developed in order to be used in AIV vaccine.

Materials and Methods

Animals and cell line

Six-seven week-old female specific-pathogen-free (SPF) BALB/c mice and 4-week-old SPF white New Zealand rabbits were purchased from Laboratory Animal Center of Shandong University. SPF white leghorns were provided by Shandong Poultry Institute. All mice, chickens and rabbits were housed under pathogen free condition. NS-1 cell line (BALB/c mice myeloma cells) was obtained from Animal Disease Center of Shandong Agricultural University.

Antigen preparation

Five 15-day-old chickens were given an intra-muscular injection with an emulsion of inactive purified H9N2 AIV. Each chicken was given two booster injections at 14-day intervals with double dose. Titer of antibody against H9N2 virus was tested 10 days after the last injection. Blood of chickens with hemagglutination inhibition (HI) value over $1:2^{10}$ was collected and separated. Immunoglobulin G (IgG) was precipitated by saturated ammonium sulfate (SAS) precipitation, as described (2).

Mouse immunization and hybridoma generation

According to published protocol (3), the BALB/c mice were immunized with chicken IgG against H9N2 AIV (anti-H9-AIV) as antigen and the fusion between the splenocytes and myeloma cells NS-1 was performed. The hybridoma cells were cultured in HAT selection medium.

Hybridoma screening for anti-idiotypic antibody product

Three screening were done at 5-day intervals after growth was observed. Rabbit and chicken anti-H9-AIV IgG were used as the coating antigen respectively. Two set of indirect enzyme-linked immunosorbent assays (ELISA) were used to screen each of the clones. The protocol used for the

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Received Mar 18, 2005. Accepted Apr 18, 2005.

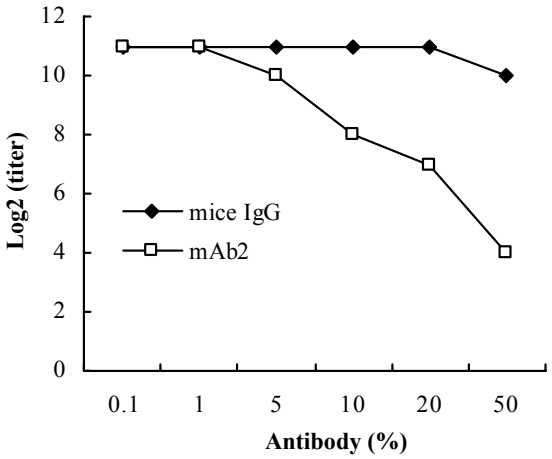

Figure 1. Interference of mAb2 to the HI titer of anti-H9-AIV IgG. HI titer of anti-H9-AIV IgG was detected in the presence of mAb2 or mice IgG. Mixture of mAb2 (1 mg/mL) or mice IgG (1 mg/mL) at a series dilution and H9 AIV (8 unit) was added into anti-H9-AIV IgG for the HI detection, as described under "Materials and Methods".

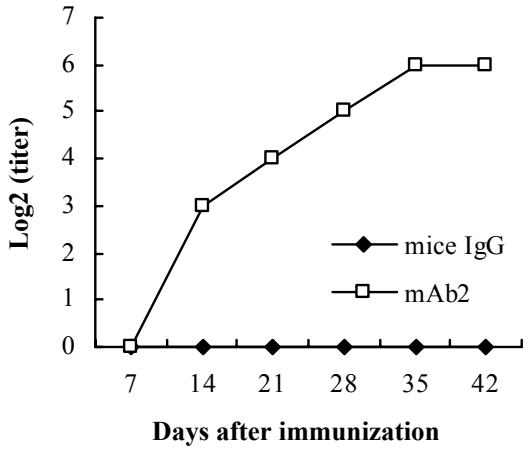

Figure 2. HI response against AIV in the sera of immunized chickens. The antibody response was measured using HI test after vaccination with mAb2 or mice IgG. No HI response was detected in control chickens vaccinated with mice IgG.

generation of rabbit antibodies was same as that of chicken.

Dilution cloning and production of mAb2

The positive hybridoma cells were sequentially cloned for two times by the limited dilution method and the ascites were produced by the mice injected with hybridoma cells (3). The mAb2 was precipitated from the cell free supernatant or ascites by SAS precipitation.

Identification for subclass and specificity

The mAb2 was characterized for subclass in agar gel precipitation (AGP) with the goat anti-mouse subclass antisera. To test the specificity of the mAb2, an indirect ELISA was done to determine the reaction between the mAb2 and chicken IgG against Newcastle disease virus (anti-NDV), chicken IgG against infectious bursal disease virus (anti-IBDV), chicken IgG against Marek's disease virus (anti-MDV), chicken IgG against H5 AIV, chicken IgG or rabbit IgG.

HI interference

To determine if the mAb2 can mimic the epitope for hemagglutinin on AIV to recognize cellular receptor, HI interference experiment was performed. Mixture of mAb2 or mice IgG at a series of dilution and 8 unit of H9N2 virus was added into wells of HI plate into which anti-H9-AIV IgG was coated previously. After hatching for 15 minutes, HI plate was added 1% chicken red blood cells to detect the HI value of anti-H9-AIV IgG.

Ability to stimulate chicken producing HI antibodies

Four chickens were inoculated with an emulsion of 200 μg mAb2 and complete Freund adjuvant at age of 15 days. Two booster injections were given at 14-day intervals with an emulsion of 400 μg mAb2 and incomplete Freund adjuvant. Two chickens were inoculated with mice IgG as control. HI titers were test against H9N2 AIV once a week, after first injection.

Results and Discussion

Establishment of hybridoma cell clone secreting mAb2

On day 15 and 20, five wells was detected for positive reaction, among 106 wells with growing cells. The strongest positive well was cloned for 2 times until each clone in the plate was ascertained to coming from one cell. The acquired hybridoma cell clone was named E6. The indirect ELISA titer of supernatant fluid was 1:32 (with chicken anti-H9-AIV IgG as the coating antigen) or 1:16 (with rabbit anti-H9-AIV IgG as the coating antigen). In previous preparation for monoclonal anti-idiotypic antibody, mouse mAb1 and conjugative were used as immunogen (4-6). In this research, purified chicken anti-H9-AIV IgG was used, thus sparing a step to establish hybridoma cell clone secreting mAb1 when it was unavailable. Our research indicated that this method for mAb2 production was feasible. As heterologous antibody, chicken anti-AIV IgG stimulated mice to generate antibodies easily, as demonstrated by the indirect ELISA (titer reaching 1:10,000) detecting antibodies in immunized mice, in that both the isotypic epitopes and idiotypic epitopes on the antibodies were recognized by mice immunity system and could be used as carrier determinants by each other, not requiring such conjugative as keyhole limpet hemocyanin.

Identification for subclass and specificity

The AGP test showed the mAb2 reacted positively to goat anti-mouse-IgG2b antibodies, rather than anti-mouse-IgG1,

anti-mouse-IgG2a, anti-mouse-IgG3 and anti-mouse-IgM. The indirect ELISA demonstrated the mAb2 reacted positively to chicken anti-H9 IgG but negatively to chicken IgG, rabbit IgG, chicken anti-NDV IgG, chicken anti-H5-AIV IgG, chicken anti-IBDV IgG and chicken anti-MDV IgG.

HI interference and ability to stimulate chicken producing HI antibodies

The results shown in Figure 1 and Figure 2 indicated the mAb2 was able to inhibit hemagglutinin on AIV in binding chicken anti-H9-AIV IgG competitively and induce chicken generating HI antibodies. Thus it should be one of internal images of hemagglutinin. Previous research has proved anti-hemagglutinin antibodies were the most among the polyclonal antibodies (7). So when mice were vaccinated with chicken anti-H9-AIV IgG, the idiotype information in anti-hemagglutinin antibodies was tendency to be passed on. In order to sift out the mounts of mice anti-chicken antibodies, rabbit anti-H9-AIV IgG was used as coating antigen in indirect ELISA, for detecting antibodies against the idiotypes shared by both rabbit and chicken anti-H9-AIV IgG. Although internal image and anti-species-sharing-idiotypic antibodies were different (8), anti-species-sharing-idiotypic antibodies stood more chance to be internal image than anti-private-idiotypic antibodies, in the assumption that species-sharing-idiotype was in the center of paratopes on the antibodies rather than the edge. The antibodies complementary to these idiotypes mimicked antigen epitopes more accurate and took more chance to be internal image of antigen epitope.

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