

Adjuvant effects mediated by the carbohydrate recognition domain of *Agrocybe aegerita* lectin interacting with avian influenza H₉N₂ viral surface glycosylated proteins*

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Received Mar. 5, 2016; Revision accepted Sept. 30, 2016; Crosschecked July 18, 2017

Abstract: Objective: To evaluate the potential adjuvant effect of *Agrocybe aegerita* lectin (AAL), which was isolated from mushroom, against a virulent H₉N₂ strain in vivo and in vitro. Methods: In trial 1, 50 BALB/c male mice (8 weeks old) were divided into five groups ($n=10$ each group) which received a subcutaneous injection of inactivated H₉N₂ (control), inactivated H₉N₂+0.2% (w/w) alum, inactivated H₉N₂+0.5 mg recombinant AAL/kg body weight (BW), inactivated H₉N₂+1.0 mg AAL/kg BW, and inactivated H₉N₂+2.5 mg AAL/kg BW, respectively, four times at 7-d intervals. In trial 2, 30 BALB/c male mice (8 weeks old) were divided into three groups ($n=10$ each group) which received a subcutaneous injection of inactivated H₉N₂ (control), inactivated H₉N₂+2.5 mg recombinant wild-type AAL (AAL-wt)/kg BW, and inactivated H₉N₂+2.5 mg carbohydrate recognition domain (CRD) mutant AAL (AAL-mutR63H)/kg BW, respectively, four times at 7-d intervals. Seven days after the final immunization, serum samples were collected from each group for analysis. Hemagglutination assay, immunogold electron microscope, lectin blotting, and co-immunoprecipitation were used to study the interaction between AAL and H₉N₂ in vitro. Results: IgG, IgG1, and IgG2a antibody levels were significantly increased in the sera of mice co-immunized with inactivated H₉N₂ and AAL when compared to mice immunized with inactivated H₉N₂ alone. No significant increase of the IgG antibody level was detected in the sera of the mice co-immunized with inactivated H₉N₂ and AAL-mutR63H. Moreover, AAL-wt, but not mutant AAL-mutR63H, adhered to the surface of H₉N₂ virus. The interaction between AAL and the H₉N₂ virus was further demonstrated to be associated with the CRD of AAL binding to the surface glycosylated proteins, hemagglutinin and neuraminidase. Conclusions: Our findings indicated that AAL could be a safe and effective adjuvant capable of boosting humoral immunity against H₉N₂ viruses in mice through its interaction with the viral surface glycosylated proteins, hemagglutinin and neuraminidase.

Key words: Adjuvant; *Agrocybe aegerita* lectin; Carbohydrate recognition domain; Glycosylated protein; Avian influenza H₉N₂ virus

<http://dx.doi.org/10.1631/jzus.B1600106>

CLC number: S855.3

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* Project supported by the National Natural Science Foundation of China (Nos. 30771501 and 81102850), the National Basic Research Program (973) of China (No. 2011CB811302), the National Mega Project on Major Drug Development (No. 2009ZX09301-014-1), the Chinese 111 Project (No. B06018), and the Wuhan Municipal Project (No. 20110923296), China

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1 Introduction

In many countries, avian influenza H₉N₂ virus is still a major threat to the poultry industry. It causes a huge economic burden when it leads to mild respiratory abnormality, reduced egg production, immune suppressive disorders, and even mortality (Li *et al.*, 2011). Development of effective and safe vaccines is a priority in preparation for an influenza pandemic. Although vaccine has proved highly immunogenic in laboratory trials and prevents clinical diseases and reduces viral shedding in field conditions (Choi *et al.*, 2008), it cannot prevent vaccinated poultry from becoming infected and from shedding wild viruses in farm settings (Rahman *et al.*, 2012; Dabaghian *et al.*, 2014). Hence, it is important to improve vaccination strategies to control H₉N₂ outbreaks in poultry farms.

The immune-promoting activity of any given vaccination strategy is determined not only by the presence of the relevant antigenic components in the vaccine formulation, but also by the addition of suitable adjuvants which activate, promote, and extend the magnitude of a specific immune response (Liu, 1998; Li *et al.*, 2011). Emphasis is therefore being placed on the development of effective adjuvants to improve the efficiency of vaccines, especially inactivated viral and subunit vaccines.

Many substances have been used as adjuvants in experimental vaccines, such as aluminum, Freund's complete adjuvant (FCA), immune stimulating complexes (ISCOMs), liposomes, polymeric microparticles, and saponins (Rajput *et al.*, 2007). Most of them have major disadvantages, including toxicity, allergy, poor activity, and potential neurotoxicity, which limit their practical application (Sivakumar *et al.*, 2011). Since numerous Chinese herbal medicines have been shown to have immune-enhancing effects (Yang *et al.*, 2008), and also have many other advantages such as extensive availability, low cost, reliable efficacy, and decreased risk of side-effects and toxicity (Kong *et al.*, 2004; 2006; Ung *et al.*, 2007), their potential use as new adjuvants for humans or animals has aroused much research interest in recent years.

Agrocybe aegerita lectin (AAL) is a 15.8-kD homodimeric protein, isolated from the medicinal mushroom *A. aegerita* by our laboratory, which shows anti-tumor activity by inducing apoptosis in cancer cells (Zhao *et al.*, 2003). We demonstrated that

AAL belongs to a galectin family with a unique carbohydrate recognition domain (CRD) that specifically recognizes β-galactose (Yang *et al.*, 2005; Feng *et al.*, 2010). AAL has antifungal, antibacterial, antiviral (Sun *et al.*, 2003), and antitumor functions and can improve immune function, and it is recognized that these functions are associated with the ability of its CRD to bind to glycan on glycosylated proteins (Rabinovich and Croci, 2012). We therefore hypothesized that AAL has adjuvant activity against the H₉N₂ virus by interacting with the glycosylated proteins, hemagglutinin (HA) and neuraminidase (NA) on the surface of the virus (Liu *et al.*, 2003).

2 Materials and methods

2.1 Vaccine preparation of AAL, AAL-wt, and AAL-mutR63H

Avian influenza H₉N₂ virus (Academy of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China) was cultured in 9-d-old embryonic eggs at 37 °C for 48 h. The allantoic fluid containing the viruses was harvested and purified by sucrose density gradient centrifugation. Purified vaccine viruses were inactivated by incubating at 75 °C for 15 min. The AAL from *A. aegerita* was extracted and purified by the chromatography method as previously described (Zhao *et al.*, 2003). Arginine in the 63th locus of the CRD region of AAL (Arg⁶³) is involved in the determination of the binding affinity of AAL to antigens (Feng *et al.*, 2010). Wild-type AAL (AAL-wt) and CRD mutant AAL (AAL-mutR63H in which histidine replaced arginine in the 63th locus of the CRD region) with a C-terminal 6× His-tag were expressed in *Escherichia coli* BL21 (DE3) and purified as previously described (Liang *et al.*, 2009). The recombinant AALs (with His-tag) were confirmed by anti-His antibody (Bioss, Beijing, China).

2.2 Mice, treatments, and sample collection

The Institutional Animal Care and Use Committee of Huazhong Agricultural University, China approved our animal protocol. In experiment 1, 50 BALB/c male mice (8 weeks old; Experimental Animal Center, Wuhan University, Wuhan, China) were divided into five groups (*n*=10 each group). All mice were allowed free access to commercial rodent

diets (Yamaguchi *et al.*, 2005) and water. After a week of acclimatization, the control group received a subcutaneous injection of 0.1 ml inactivated H₉N₂, and the alum group received a subcutaneous injection of 0.1 ml inactivated H₉N₂ with adjuvants of 0.2% (w/w) alum (Thermo, USA). The three other treated groups received a subcutaneous injection of 0.1 ml inactivated H₉N₂ with 0.5, 1.0, or 2.5 mg AAL/kg body weight (BW), respectively, four times at 7 d intervals (Huang *et al.*, 2008; Kim *et al.*, 2011). The inactivated viruses were mixed with the recombinant protein just prior to injection. Seven days after the final immunization the sera were harvested from the immunized mice (Kim *et al.*, 2011). The sera were prepared by centrifugation of the whole blood at 1000g for 15 min at 4 °C and stored at -80 °C (Sun *et al.*, 2014). In experiment 2, 30 BALB/c male mice (8 weeks old) were divided into three groups (*n*=10 each group) and received the same housing conditions as in experiment 1. The control group received a subcutaneous injection of 0.1 ml inactivated H₉N₂, while the two treated groups received a subcutaneous injection of 0.1 ml inactivated H₉N₂ with adjuvants of 2.5 mg AAL-wt/kg BW and 2.5 mg AAL-mutR63H/kg BW, respectively, four times at 7 d intervals (Huang *et al.*, 2008; Kim *et al.*, 2011). Seven days after the final immunization, serum samples were collected following the method described by Kim *et al.* (2011). No negative effects on the experimental animals were observed.

2.3 Measurement of antigen-specific antibodies

The H₉N₂-specific IgG, IgG1, and IgG2a antibodies in the sera were analyzed by enzyme-linked immunosorbent assay (ELISA) as described previously with minor modifications (Sun and Liu, 2008). Briefly, the 96-well microtiter plates were coated with 100 μl of H₉N₂ solution (50 μg/ml in 50 mmol/L carbonate buffer, pH 9.6) for 24 h at 4 °C. The wells were washed three times with 200 μl of phosphate buffer saline (PBS) containing 0.05% (v/v) Tween 20 (PBST), and then blocked with 100 μl of PBST within 0.5% (v/v) bovine serum albumin (BSA) at 37 °C for 1 h. One hundred microliters of diluted serum samples were then added to triplicate wells, and then incubated at 37 °C for 1 h, followed by washing three times. Aliquots of 100 μl of horseradish peroxidase-conjugated goat anti-mouse IgG, IgG1, or IgG2a (SouthernBiotech, China) were added and incubated

for 1 h at 37 °C. An assay was developed using 3,3',5,5'-tetramethylbenzidine (TMB) (Beytime Biotechnology, China), and the reaction was stopped by adding 0.5 mol/L H₂SO₄. The absorbance at 450 nm was measured by an ELISA plate reader.

2.4 Hemagglutinating assays

The hemagglutinating assays were performed as described previously with minor modifications (Yang *et al.*, 2005). Briefly, a serial two-fold dilution of AAL-wt or AAL-mutR63H solution in microtiter U-plates (50 μl) was mixed with 50 μl of 1.0% suspension of mouse erythrocytes in PBS (pH 7.2) at 20 °C. The initial concentration was 1 mg/ml. The results were read after about 1 h, when the control (no AAL added) had fully precipitated. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was defined as one hemagglutination unit. Specific activity was the number of hemagglutination units per mg of protein.

2.5 Immunogold electron microscope assays

Immunogold electron microscope assays were performed as described previously (Pena *et al.*, 2013). Briefly, 10 μl purified H₉N₂ viruses were adsorbed onto formvar-coated and carbon-vaporized 300-mesh copper grids (Electron Microscopy Sciences, Hatfield, PA, USA). The grids were blocked in Tris-buffered saline (TBS) containing 0.2% (v/v) BSA at room temperature (RT) for 1 h, and washed three times with TBS with 0.3% Tween 20 (TBST). They were then incubated with TBS (control), 10 μl AAL-wt (500 ng/ml), or AAL-mutR63H (500 ng/ml) at RT for 1 h. Subsequently, the grids were washed in TBS and incubated with rabbit anti-His (anti-AAL) at RT for 1 h, and then incubated with goat anti-rabbit IgG antibody conjugated to 15-nm gold beads (Bioss, Beijing, China) at RT for 1 h. Finally, the grids were negatively stained with 2% (v/v) phosphotungstic acid (PTA) for 30 min, dried, and examined under a transmission electron microscope (Hitachi H-7000FA).

2.6 Lectin blot assays

Lectin blot assays were performed as described previously with minor modifications (Matsumura *et al.*, 2007). Briefly, the protein extracts of the H₉N₂ virus were isolated by methanol and chloroform, and 60 μg of the extracted protein was then subjected to 15% (0.15 g/ml) sodium dodecyl sulfate-polyacrylamide

gel electrophoresis (SDS-PAGE). One of the samples was treated with PNGase F (New England BioLabs, MA, USA) to remove the glycan on glycosylated protein following the manufacturer's instructions. After electrophoresis, the gels were blotted onto polyvinylidene difluoride (PVDF) membranes which were incubated overnight with 5% (v/v) BSA in TBST (50 mmol/L Tis-HCl, 150 nmol/L NaCl, 0.1% Tween 20, pH 7.4) at 4 °C, and then for 100 min with 500 ng/ml of AAL-wt or AAL-mutR63H in TBST. After being washed with TBST, the membranes were incubated with anti-His (anti-AAL) antibody (Bioss, Beijing, China) at RT for 100 min. Staining was then performed with ECL Western blot detection reagents as previously described (Sun *et al.*, 2013).

2.7 Co-immunoprecipitation assays

Co-immunoprecipitation (Co-IP) assays were performed as described previously (Rajik *et al.*, 2009). Briefly, the extracted protein of H₉N₂ virus was diluted to a concentration of 1–2 µg/µl in 50 µl Co-IP buffer, and was then incubated overnight with 50 µl of 100 µmol/L AAL-wt at 4 °C. Anti-His (anti-AAL) antibody, anti-HA antibody (Sino Biological Inc., Beijing, China), and IgG antibody were added to the mixture, respectively, and rotated gently for 3.5 h at 4 °C. Subsequently, protein G plus-agarose beads were added, and the mixture was rotated gently for 3 h at 4 °C. The beads were collected by centrifugation at 100g for 3 min at 4 °C, and then washed three times with ice-cold NP40 lysis buffer. Finally, the proteins were eluted from the beads by boiling in SDS loading buffer for 5 min, and analyzed by Western blot assay using the anti-His (anti-AAL) antibody and anti-HA antibody with appropriate secondary antibodies.

2.8 Statistical analysis

Data generated from animal studies were analyzed using Student's *t*-test by using SAS 8.2 (SAS Institute). Data are presented as mean±standard error (SE) with the significance level set at *P*<0.05.

3 Results

3.1 Adjuvant activity of AAL on antibody titers against H₉N₂ in mice

AAL was successfully extracted and purified by chromatography and confirmed by anti-AAL for

Western blot assay (Figs. 1a and 1d). In the first animal experiment, after 4 weeks of treatment, compared to mice immunized with H₉N₂ alone (control), IgG antibody levels in the sera were increased (*P*<0.05) by 97%, 147%, and 93% when mice were co-immunized with inactivated H₉N₂ and alum, AAL (1.0 mg/kg BW), and AAL (2.5 mg/kg BW), respectively (Fig. 2a). IgG1 antibody levels in the sera were increased (*P*<0.05) by 237%, 340%, and 372% when mice were co-immunized with inactivated H₉N₂ and AAL at the doses of 0.5, 1.0, and 2.5 mg/kg BW, respectively (Fig. 2b). In addition, IgG2a antibody levels in the sera were increased (*P*<0.05) by 60%, 133%, and 145% when mice were co-immunized with inactivated H₉N₂ and AAL at the doses of 0.5, 1.0, and 2.5 mg/kg BW, respectively (Fig. 2c).

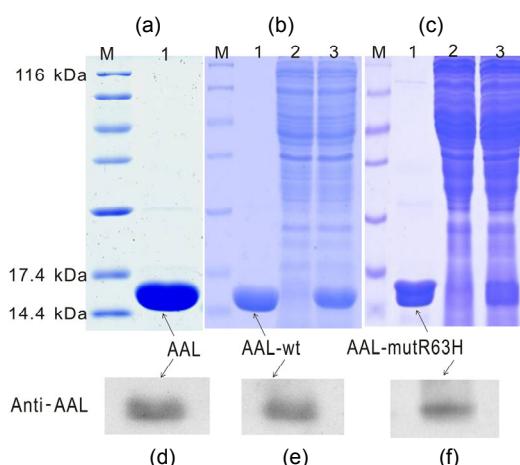


Fig. 1 Purified AAL (a), AAL-wt (b), and AAL-mutR63H (c) applied to 15% SDS-PAGE and stained with Coomassie brilliant blue, and Western blot analysis of the purified AAL (d), AAL-wt (e), and AAL-mutR63H (f) using rabbit anti-AAL antibody

M, sizes (kD) of molecular weight markers; Lane 1, purified AAL, AAL-wt, or AAL-mutR63H; Lane 2, flow-through; Lane 3, crude extract AAL

3.2 Hemagglutinating and adjuvant activity assays of AAL-wt and AAL-mutR63H

To analyze the role of the CRD of AAL by site-directed mutagenesis, Arg⁶³ at the domain was replaced with His⁶³ based on the three-dimensional structures of AAL to produce the mutated AAL (AAL-mutR63H) which was then purified and confirmed by Western blot. This showed that Arg⁶³ is a key function site of AAL (Figs. 1c and 1f) (Feng *et al.*, 2010). We also produced and purified the recombinant

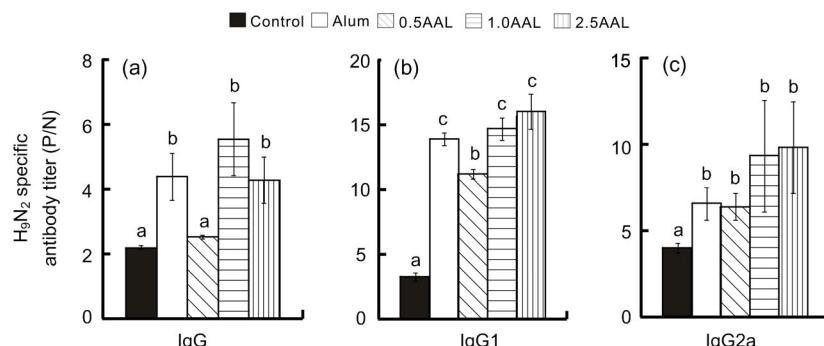


Fig. 2 Effects of co-immunization with inactivated H₉N₂ and AAL or alum on IgG (a), IgG1 (b), and IgG2a (c) antibody levels in sera of mice

Values are expressed as mean±SE, n=10 each group. Values without sharing a common letter differ significantly ($P<0.05$). 0.5AAL: 0.5 mg AAL/kg BW; 1.0AAL: 1.0 mg AAL/kg BW; 2.5AAL: 2.5 mg AAL/kg BW

AAL-wt and confirmed it by Western blot (Figs. 1b and 1e). The hemagglutinating assay showed that AAL-wt has hemagglutinating activity (1:2–1:128), while no activity was found in AAL-mutR63H (Fig. 3a). The second animal experiment showed that AAL-wt significantly enhanced ($P<0.05$) H₉N₂-specific IgG levels in the sera of mice, but that AAL-mutR63H had no significant effect ($P>0.05$) (Fig. 3b).

3.3 Immunogold electron microscope, lectin blot, and co-immunoprecipitation assays

The immunogold electron microscope results showed that, compared with the control group, AAL-wt accumulated intensively on the surface of the H₉N₂ virus, but only a few of the mutant AAL-mutR63H did (Fig. 4). To test whether the two glycosylated proteins, HA and NA, on the surface of the H₉N₂ virus could be the binding targets of AAL's CRD, we performed lectin blot analysis (Fig. 5a). The results showed that both glycoproteins NA (55 kD) and HA (26 kD) were displayed on the AAL-wt-incubated PVDF membrane (Lane 1); however, these two bands disappeared after the N-linked glycan of virus was removed by PNGase F (Lane 2) or in the AAL-mutR63H-incubated PVDF membrane (Lane 3). These data indicated that AAL interacted with the glycosylated proteins HA and NA, while the mutant AAL-mutR63H lost its ability to interact with these two proteins. The interaction of AAL with HA was further confirmed by Co-IP assay (Fig. 5b). Protein extracts of H₉N₂ virus were incubated with AAL-wt/

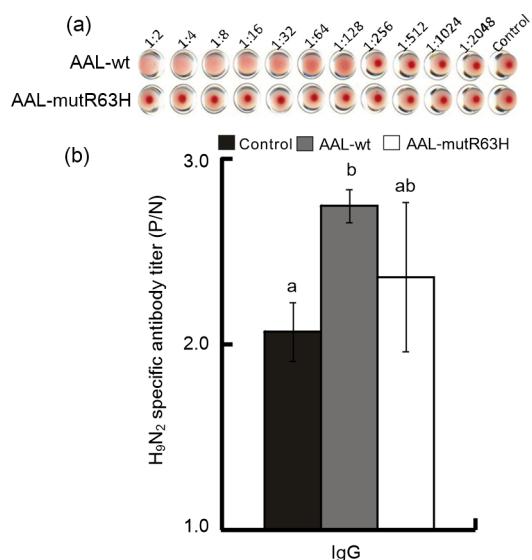


Fig. 3 Effects of AAL-wt and AAL-mutR63H on hemagglutinating activity (a) and IgG antibody level (b) in the sera of mice

Values are expressed as mean±SE, n=10 each group. Values without sharing a common letter differ significantly ($P<0.05$)

AAL-mutR63H, and then with anti-HA, anti-AAL, and anti-IgG antibodies. Finally, the total extracts and the precipitates were Western-blotted against anti-HA and anti-AAL (Fig. 5b). HA/AAL-wt was detected in AAL-wt/HA immunoprecipitates with H₉N₂ virus protein extracts, indicating that the HA/AAL-wt was captured by the anti-AAL-wt/HA antibody. The Co-IP results further indicated that AAL interacted with the glycosylated proteins HA. In contrast, no interaction was found between HA and AAL-mutR63H.

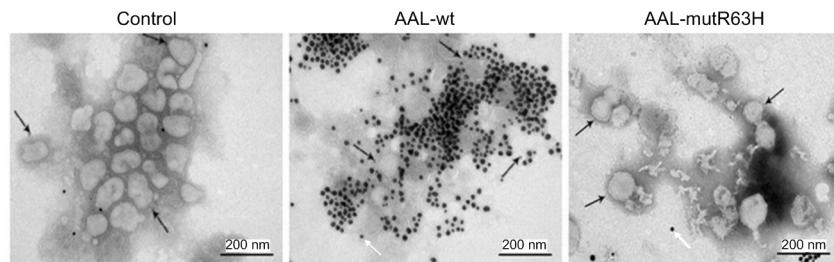


Fig. 4 Immunogold electron microscope analysis of the interaction between avian influenza H₉N₂ virus and the CRDs of AAL-wt and AAL-mutR63H

Black arrow: H₉N₂ virus; White arrow: gold particle

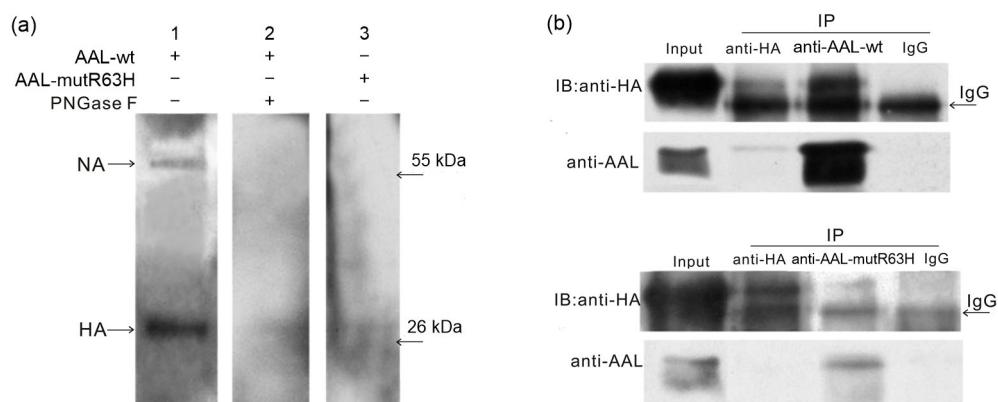


Fig. 5 Lectin blot (a) and co-immunoprecipitation (Co-IP) (b) analysis of the interaction between avian influenza H₉N₂ viral surface glycosylated proteins, HA and NA, and the CRDs of AAL-wt and AAL-mutR63H

Input is mixed HA with AAL-wt or AAL-mutR63H

4 Discussion

The two novel findings from the present study are: (1) AAL has an adjuvant activity against avian influenza H₉N₂ virus in mice; (2) the mechanism of AAL's adjuvant activity is associated with the interaction between the CRD of AAL with the H₉N₂ virus surface glycosylated proteins HA and NA. Levels of total IgG, IgG1, and IgG2a in serum have been described as valuable parameters of humoral immunity (Li *et al.*, 2011). The first animal study showed that co-injecting inactivated H₉N₂ virus with ≥ 1.0 mg AAL/kg BW induced higher levels of antigen-specific IgG, IgG1, and IgG2a antibody titers in mice than injecting inactivated H₉N₂ avian influenza virus (AIV) vaccine alone. Furthermore, the levels of IgG, IgG1, and IgG2a were similar in the mice co-injected with inactivated H₉N₂ virus with AAL or alum. These outcomes indicated that AAL, like alum, has an effective adjuvant activity that enhances antigen-specific humoral immune responses. Similar results

were obtained from recent studies in which Korean mistletoe lectin was found to have the ability to serve as a mucosal adjuvant (Jung *et al.*, 2011) and mushroom lectin as an adjuvant of hepatitis B virus (HBV) DNA vaccine (Gao *et al.*, 2013). AAL is attractive as a new adjuvant for animal H₉N₂ vaccines, since it can be isolated from mushroom *A. aegerita*, which is extensively available and has reliable efficacy but lower risks of side-effects and toxicity than alum salts or other adjuvants (Aguilar and Rodríguez, 2007; Sivakumar *et al.*, 2011).

To validate our hypothesis that the CRD plays a vital role in AAL's adjuvant activity, we obtained AAL-mutR63H, by replacing Arg⁶³ at CRD with His⁶³ (Feng *et al.*, 2010), and studied its biological function. The in vitro study showed that AAL-wt had haemagglutination activity, but that AAL-mutR63H lost this activity. The second animal study showed that AAL-wt enhanced the levels of IgG in the sera of mice, while AAL-mutR63H had no significant effect. Taken together, these outcomes indicated that the

CRD of AAL plays a key role in the adjuvant effect of AAL against the H₉N₂ virus in mice. Similar results were obtained by Dodson *et al.* (1999), who reported that the CRD of the *Entamoeba histolytica* Gal/GalNAc lectin played key roles in infection and immunity.

Since lectin's antiviral function and ability to improve immune function are associated with the ability of its CRD to bind to glycan on glycosylated protein (Rabinovich and Croci, 2012), we carried out immunogold electron microscope, lectin blot, and Co-IP assays to determine whether AAL's adjuvant activity against the H₉N₂ virus was associated with the ability of its CRD to bind to the surface glycosylated proteins HA and NA. The immunogold electron microscope results showed that AAL-wt bound strongly to the surface of the H₉N₂ virus, but the mutant AAL-mutR63H had lost this ability. Since HA and NA are the two major glycosylated proteins on the surface (Liu *et al.*, 2003), lectin blot and Co-IP were done to determine AAL's ability to bind to the surface through its CRD interacting with these two glycosylated proteins. The lectin blot results showed that AAL-wt interacted with both HA and NA, but AAL-mutR63H lost its ability to interact with these two proteins. The interaction between AAL and the HA protein was further confirmed by Co-IP assay. Overall, the immunogold electron microscope, lectin blot, and Co-IP assays demonstrated that the CRD of AAL plays a vital role in interacting with the glycosylated proteins HA and NA on the surface of the H₉N₂ virus.

The glycosylation of viral envelope proteins plays an important role in viral immune evasion by masking antigenic sites from immune recognition (Kreisman and Cobb, 2012). Previous reports have shown that N-linked glycosylation can limit the neutralizing antibody response to simian immunodeficiency virus (SIV) and shield the virus from immune recognition (Reitter *et al.*, 1998), and that the glycosylations of HA and NA are significant for influenza virus to evade the host immune system (Klenk *et al.*, 2001; Tsuchiya *et al.*, 2002a; 2002b). Therefore, we speculated that the enhancement of antigenicity of the H₉N₂ virus by AAL may be related to the interaction of AAL with glycosylated proteins on the virus surface by exposing antigenic sites to immune recognition (Fig. 6a). Since AAL is a homodimeric protein

with two CRDs (Zhao *et al.*, 2003), which may link two viral particles, an antigen depot is formed (Fig. 6b). The antigen depot can provide a slow release of antigen, which can continue the stimulation of the immune system (Lindblad, 2004).

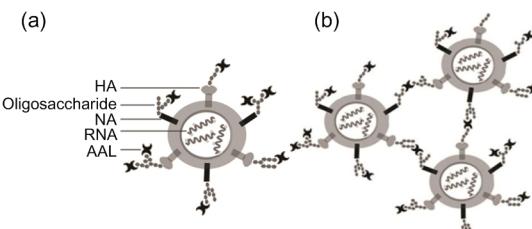


Fig. 6 Schematic illustration of potential mechanisms of AAL serving as an adjuvant of avian influenza H₉N₂ vaccine
 (a) The enhancement of antigenicity of the H₉N₂ virus by AAL may be related to the interaction of AAL with the glycosylated proteins on the virus surface, resulting in the exposure of the antigenic sites to immune recognition. (b) AAL as a homodimeric protein with two CRDs may link two viral particles and form an antigen depot. The antigen depot can provide a slow release of antigen, continuing the stimulation of the immune system

5 Conclusions

In summary, AAL purified from mushroom *A. aegerita* and AAL-wt both exhibited adjuvant effects against avian influenza H₉N₂ virus. The site-directed mutant AAL-mutR63H (Arg⁶³ at the CRD of AAL replaced with His⁶³) lost haemagglutination activity in vitro and adjuvant activity in vivo, indicating that the CRD of AAL plays a vital role in the adjuvant effect against the H₉N₂ virus in mice. Furthermore, the immunogold electron microscope, lectin blot, and Co-IP assays demonstrated that the CRD of AAL interacted with the glycosylated proteins HA and NA on the surface of the H₉N₂ virus, which resulted in the exposure of the antigenic sites to immune recognition and the formation of an antigen depot. This may explain why the antigenicity of the H₉N₂ virus was enhanced. These findings indicate that the use of AAL as a novel adjuvant offers a new prophylaxis strategy against enveloped H₉N₂ viruses.

Compliance with ethics guidelines

Li-bao MA, Bao-ying XU, Min HUANG, Lv-hui SUN, Qing YANG, Yi-jie CHEN, Ya-lin YIN, Qi-gai HE, and Hui SUN declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文摘要

题 目: 杨树菇血凝素糖识别域与禽流感病毒 H₉N₂ 表面糖蛋白结合介导的佐剂效果

目 的: 探讨杨树菇血凝素 (AAL) 作为疫苗佐剂的作用机理, 为有囊膜病毒疫苗佐剂的研究提供新思路和数据。

创新点: 首次研究 AAL 作为禽流感疫苗佐剂的作用机理。

方 法: 大肠杆菌重组表达、纯化野生型的 AAL(AAL-wt) 和糖识别结构域 (CRD) 突变型的 AAL (AAL-mutR63H)(图 1); 动物试验证实 AAL-wt 具有免疫佐剂活性, AAL-mutR63H 失去免疫佐剂活性 (图 2); 免疫胶体金电镜 (immunogold electron microscopy, IEM) 试验发现 AAL-wt 能吸附 H₉N₂ 病毒粒子, 而 AAL-mutR63H 不能吸附病毒粒子 (图 4); 凝集素印迹 (lectin blot)、免疫共沉淀 (co-immunoprecipitation, Co-IP) 试验证明了 CRD 区与 H₉N₂ 病毒中血球凝集素 (HA) 和神经氨酸酶 (NA) 这两种糖蛋白的相互作用 (图 5)。

结 论: AAL 的 CRD 区是发挥佐剂作用的关键区域, CRD 区与禽流感病毒表面的 HA 糖蛋白结合, 可能暴露病毒的免疫识别位点, 或者将病毒粒子连接在一起, 形成大的病毒复合体, 增加病毒的抗原性 (图 6)。

关键词: 杨树菇凝集素; 禽流感病毒 H₉N₂; 糖识别结构域 (CRD); 血球凝集素 (HA); 神经氨酸酶 (NA)