Antibody response to DNA vaccine against H5N1 avian influenza virus in broilers immunized according to three schedules

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INTRODUCTION

Chickens are the main poultry species raised for meat, eggs and hobby. During the hundreds of years of breeding they have been subjected to intensive selection which could affect their immunological characteristics and susceptibility to avian influenza, a poultry disease of great importance (Alexander, 2007; Graham-Rowe, 2011). Although the threat of an H5N1 influenza outbreak is now less imminent than during the 2006 epidemic, it is still real. Construction of an effective vaccine against avian influenza is a challenging task due to the continuous threat of new outbreaks caused by new subtypes, like H7N9, which occurred recently in China (Gao et al., 2013b). Therefore, not only the control and surveillance of the influenza virus spread is important (Capua & Cattoli, 2013) but there is also an unquestionable need for modern veterinary vaccines that could be prepared without handling live viruses and would be consistent with DIVA European strategy (differentiating infection from exposure). DNA vaccines can potentially fulfill these criteria. In most experimental DNA vaccines for chickens two vaccine doses are administered one week (Mahmood et al., 2007; Song et al., 2010) or two weeks (Li et al., 2003; Li et al., 2013) apart, with the first dose applied at various age. The studies were often performed with SPF (specific pathogen-free) chickens, most frequently Leghorn White, which is a layer type breed (Yang et al., 2009; Chen et al., 2011; Gao et al., 2013a). Modern broiler lines, like Ross 508 (Mahmood et al., 2007; Song et al., 2013) or Chine’s Yellow Hair chickens, a traditional Asian meat breed (Xu et al., 2008; Song et al., 2010) have been used much less frequently in such experiments. Other breeds, like Rhode Island Red, were tested only sporadically (Haygreen et al., 2006). All of these breeds have different metabolism, life span or body weight. Differences in optimal vaccination protocols for these lines could be thus anticipated.

The present study is part of a broader research project focused on chicken immunity and vaccination against avian influenza. The main aim of this study was on analysis of antigen-specific humoral response to a DNA vaccine encoding hemagglutinin from the H5N1 influenza virus isolated in Poland from a wild swan, using the broiler line Ross 308 most popular in the poultry industry in Poland. The immunological response of these broilers to the DNA vaccine administered according to three different schedules was studied in order to establish an optimal vaccination program.

MATERIALS AND METHODS

DNA vaccine. Construction of the plasmid for DNA vaccination with inserted synthetic DNA encoding HA from the H5N1 A/swan/Poland/305-135V08/2006 (EpiFluDatabase [http://platform.gisaid.org]; Accession No. EPI156789), vaccine preparation and chicken immunization were described previously (Stachyra et al., 2014). Briefly, the HA gene was optimized to the domestic chicken codon bias and engineered to contain deletion of the proteolytic cleavage site (from Arg-341 to Arg-346). Next, it was cloned into the pCI mammalian expression vector (Promega GmbH, Germany) under control of CMV promoter. A dose of 125 µg of plasmid DNA mixed with 20 µl of Lipofectin transfection reagent (Life Technologies, USA) was used for each immunization.

Immunizations. Broiler (Ross 308) chickens were housed in cages in an experimental poultry house under standard commercial conditions. The vaccine was administered intramuscularly. The schedule of immuniza-
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Ezyme-linked immunosorbent assay (ELISA). In direct ELISA for detection of anti-HA antibodies in serum was performed in 96-well polystyrene plates (MediSorp, Nunc, Denmark) as described previously (Stachyra et al., 2014). Plates were coated overnight with 50 µl of affinity-purified, recombinant H5 HA (17-530, Δ341-346, 6xHis-tag at C-terminus) antigen (A/swan/Poland/305-135V08/2006 (H5N1)) produced in a baculovirus system by Oxford Expression Technologies, UK, diluted in PBS to 6 µg/ml. Goat anti-chicken IgY (Fc specific)–HRP IgGs (Pierce/Thermo Scientific, IL, USA) were used as secondary antibodies. All sera were diluted 200-folds.

Cells and influenza viruses. Madin–Darby canine kidney cells (MDCK) (Collection of the Department of Recombinant Vaccines, University of Gdansk, Poland) were cultured in Dulbecco’s Modified Eagle’s Medium (D-MEM) (Sigma–Aldrich, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 0.2% bovine serum albumin, 25 mM HEPES buffer, 100 U/ml penicillin, 100 µg/ml streptomycin, at 37°C under 5% CO$_2$.

The avian influenza virus A/ostrich/Denmark/725/96 (H5N2) (kindly provided by R. Manvell, AHVLA, Wey...
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bridge, UK) and human influenza virus (isolate 32U of the pandemic human influenza A/H1N1 virus) from the collection of the Department of Recombinant Vaccines, University of Gdansk, Poland were propagated in MDCK cells in the presence of 2 µg/ml TPCK (L-1-tosylamide-2-phenylethyl chloromethyl ketone) — trypsin (Sigma-Aldrich, USA). Viral stocks were stored at –70°C and titrated by plaque assay before use.

Immunoperoxidase monolayer assay (IPMA). MDCK cell monolayers in 12-well plates were infected for 1 h at 37°C with either avian influenza virus A/osstrich/Denmark/725/96 (H5N2) or isolate 32U of the pandemic human influenza A/H1N1 virus. Unbound viral particles were removed by washing with serum-free medium, and the cell monolayers were overlaid with fresh serum-free medium containing 1.2% Avicel (FMC BioPolymer, USA) and 2 µg/ml TPCK-trypsin. After 3 days, cells were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde and virus-infected cells were immunostained by incubating for 1 h with collected sera diluted 1:8 in PBS containing 1% Tween 20 and 5% fetal bovine serum (FBS). Anti-chicken horseradish peroxidase (HRP)-conjugated antibody (Santa Cruz Biotechnology, USA) was used as secondary antibody (diluted 1:1000 in PBS containing 1% Tween 20 and 5% FBS). Infected cells were detected using the Vector Nova-Red kit (Vector Laboratories Ltd., UK). The infected cells appeared as red plaques on unstained background of uninfected cells.

Statistical analysis. For comparison of ELISA results in different groups the ANOVA Tukey HSD (Honestly Significant Difference) test for unequal n was applied, using STATISTICA 6.0 (Statsoft, Poland). The results were considered significant for p < 0.05.

RESULTS AND DISCUSSION

Three groups of broiler chickens were immunized intramuscularly either once or twice with 125 µg of DNA vaccine per dose and the level of anti-HA antibodies in sera of the immunized chickens was determined by the ELISA test at different time points. The experiments were performed in poultry house cages, using standard commercial conditions, which allowed us to observe natural reactions to the immunization. The study lasted 6 weeks, which corresponds to the broiler life span in commodity production. The broiler chickens grow very fast and have intensive metabolism. Therefore, the immunization was started as early as possible; the first dose was administered on the seventh day after hatching. The second dose (if applied) was administered, depending on the group, on day 21 or 35. The ELISA test demonstrated clear differences between the groups. The mean values of the levels of anti-HA antibodies in the experimental groups are shown in Fig. 1B. They were higher in the group immunized once than in the control group, but the difference was statistically nonsignificant. The second vaccination (booster) resulted in an increased humoral response regardless of the booster time. A statistically significant increase of the anti-HA antibody titer was observed one week after the second dose in both groups that were immunized twice. The anti-HA level remained high in those groups until the end of the experiment (42 or 49 days). These results indicate that two doses of the experimental DNA vaccine were required to elicit a high level of anti-HA antibodies. Interestingly, large biological variation was observed among responders within the experimental groups (Fig. 1B). Similar substantial inter-individual variation has been described in other DNA vaccine studies with chickens (Shan et al., 2011), which could be related to differences in DNA uptake by muscle fibers.

A considerable number of publications report chicken immunization with DNA vaccines, especially with avian influenza antigens. Such studies sometimes included measurement of IgY humoral response in serum samples and analysis of its dynamics (Jalilian et al., 2010; Liniger et al., 2012), but most of them concentrated on hemag-
glutinin inhibition tests and virus neutralization assays (jiang et al., 2007; shan et al., 2011; lim et al., 2012). in this study, the specificity of the anti-ha antibodies induced by our experimental dna vaccine was detected preliminarily using the ipma test which enables detection of cells exposing ha on their surface, such as cells infected by the virus. two serum samples collected at day 42, one from a non-immunized chicken (negative in elisa, no. 228) and one from a positive individual with a high titer of antibodies, immunized twice on days 7 and 21 (no. 141) were analyzed. the results clearly indicated that the serum negative in elisa remained negative in the ipma test, while the positive serum with a high anti-ha titer was able to detect cells infected by the h5n2 (a/ostrich/denmark/725/96) virus (fig. 2). interestingly, the positive serum also detected cells infected by a serologically distant pandemic h1n1 strain of human influenza a virus, which apparently indicates the presence of broad-specificity antibodies in the serum (fig. 2). the discovered cross-reactivity and presumed ability of our experimental dna vaccine to induce antibodies with broad specificity is a very interesting observation justifying further detailed studies.

acknowledgements

we thank anna doraczyńska-filipczak for excellent veterinary support and katarzyna florys (iba), józef kapusta (ibá), katarzyna szatrój (ibb) for their help and cooperation during immunization experiments and immunological analysis.

acknowledgments of financial support

this work was supported by innovative economy program, grant no. wnd-poig.01.01.02-00-007/08.

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