Appendix

The immune response to H7N1 whole virus vaccine in mice
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Abstract. Influenza vaccination is the main method of prophylaxis, but in a pandemic scenario vaccination will occur in an unprimed population. In this study we provide preliminary data on the kinetics of the immune response to a highly pathogenic avian H7N1 virus vaccine in mice. BALB/c mice were immunised intramuscularly with 1 or 2 doses (15 µg HA) of whole egg grown inactivated A/Chick/Italy/13474/99 H7N1 vaccine. Mice were sacrificed at 0, 3–7 and 21 days after each dose of vaccine. The Enzyme-Linked ImmunoSpot Assay (ELISPOT assay) was used to examine the class and IgG subclass of antibody secreting cell (ASC) response in the spleen and bone marrow. Sera were collected at the time of sacrifice and analysed by haemagglutination inhibition. In the spleen only IgM ASC were detected after first dose of vaccine whereas IgG, IgA and IgM were found after the second dose of vaccine. In our experience, this avian strain appears to be less immunogenic than whole H3N2 virus vaccine. Our data for this murine model confirm studies by others suggesting that a pandemic vaccine virus of avian origin may be considerably less immunogenic in man than the H1, H2 and H3 human subtypes. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

An outbreak of a low pathogenic avian H7N1 influenza in domestic poultry started in 1999 in Italy. By late December 1999 a highly pathogenic avian influenza virus had emerged, causing widespread disease with a mortality close to 100% [1]. The influenza viruses that cause this kind of disease in domestic poultry have always been H5 and H7 subtypes [1], highlighting the need for a better understanding and increased knowledge of these influenza A subtypes as vaccine strains. Therefore, a highly pathogenic H7N1 avian virus from the Italian outbreak, which proved lethal in mice (personal communication John Wood, NIBSC), was chosen as a candidate vaccine strain. In this study we provide...
preliminary data on the kinetics of the immune response to this highly pathogenic avian H7N1 virus vaccine in mice.

2. Materials and methods

BALB/c mice (6 weeks old) were purchased from Bomholt Gaard (Denmark) and housed according to the Norwegian law on the use of experimental animals. The experimental design is presented in Fig. 1.

Mice were immunised intramuscularly into the quadriceps muscles with 1 or 2 doses (15 μg HA) of egg grown inactivated whole virus A/Chick/Italy/13474/99 (H7N1) vaccine, kindly provided by NIBSC (both hind legs 50 μl per leg). Mice were sacrificed at 0, 3–7 and 21 days after each dose of vaccine. Mice were anaesthetised using viva/hypnorm, exsanguinated by cardiac puncture and the blood, spleen, femur and tibia bones of the hind leg were collected.

HI assays were carried out using eight haemagglutinating units of the low pathogenic A/Turkey/Italy/3889/99 (H7N1) virus which is antigenically similar to the highly pathogenic A/Chick/Italy/13474/99 (H7N1) and 0.7% turkey red blood cells. The sera were incubated overnight at 37 °C with receptor destroying enzyme (RDE) (Denka Seiken, Tokyo, Japan) to remove non-specific inhibitors, and then inactivated at 56 °C.

ELISPOT was used to investigate the influenza specific antibody secreting cell (ASC) response in the spleen and bone marrow. Lymphocytes were separated from the spleen for use in the ELISPOT assay using lymphoprep density gradient centrifugation [2]. Briefly, the ELISPOT plates were coated with 10μg/ml of split virus vaccine (A/Chick/Italy/13474/99 (H7N1)) overnight at 4 °C. The lymphocytes (400,000/well) were then incubated in a humidified CO₂ incubator at 37 °C overnight. After incubation, the influenza specific ASC were detected using biotinylated class (IgG, IgA and IgM) and subclass (IgG1, IgG2a, IgG2b and IgG3) specific antibodies from Southern Biotechnology, USA. The spots were then counted using a dissection microscope and the mean number of class and IgG subclass specific ASC per 500,000 lymphocytes was calculated for each individual mouse.

3. Results and discussion

No HI antibody titre was detected after the first dose of vaccine, but after the second dose there was an increase in HI antibody titre even though the titre remained low (GMT at
21 days post second dose was 32) (Fig. 2). Avian viruses often produce low HI titres in an HI assay using turkey red blood cells (rbc). Stephenson and coworkers [3] have found greater sensitivity using horse erthrocytes and these could be used to reassess the low antibody response.

IgM influenza-specific ASC were detected after first dose of vaccine whereas IgG, IgA and IgM ASC were found after the second dose of vaccine (Fig. 3). The number of influenza-specific ASC after vaccination remained at a low level compared with the level
of ASC routinely achieved with H3N2 vaccine, confirming the low HI antibody response observed. The IgG subclass response had the following distribution; IgG2a>IgG2b>IgG1>IgG3. Low numbers of ASC (day 3 and day 5 post second vaccination) were detected in the bone marrow (data not shown).

4. Conclusions

In our experience, this avian strain appears to be less immunogenic than whole H3N2 virus vaccine. Our data for this murine model confirm studies by others suggesting that a pandemic vaccine virus of avian origin may be considerably less immunogenic in man than the H1, H2 and H3 human subtypes.

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References
