A Comparison of the Antibody Responses between Specific Pathogen-Free and Commercial Layers Immunized with an Influenza Vaccine Prepared from Inactivated Non-Pathogenic H5N1 Virus by Single Shot

Takashi SASAKI1), Norihide KOKUMAI1)*, Toshiaki OGITANI1), Takashi IMAMURA2), Akira SAWATA3), Zhifeng LIN3), Yoshihiro SAKODA3) and Hiroshi KIDA3,6)

1)Avian Biologics Department, Kyoto Biken Laboratories, Inc., 24–16 Makishima-cho, Uji, Kyoto 611–0041, 2)Division 2, Second Research Department, The Chemo-Sero-Therapeutic Research Institute, Kikuchi 869–1298, 3)Research Center for Biologicals, The Kitasato Institute, Kitamoto 364–0026, 4)Research Department, Nippon Institute for Biological Science, Ome 198–0024, 5)Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818 and 6)Research Center for Zoonosis Control, Hokkaido University, Sapporo 061–0020, Japan

(Rceived 1 December 2009/Accepted 28 January 2010/Published online in J-STAGE 9 February 2010)

ABSTRACT. It is known that antibody responses in chickens against invading organisms or antigens are considerably different among different lines. Thus, an avian influenza vaccine was prepared from inactivated whole particles of the virus of non-pathogenic strain A/duck/Hokkaido/Vac-1/04 (H5N1) using an oil adjuvant containing anhydromannitol-octadecenoate-ether and injected intramuscularly into each ten 10-week-old specific pathogen-free (SPF) white leghorn chickens and commercial layers of Julia and Boris-Brown to obtain comparative data for antibody responses until 6 weeks after vaccination. Despite significant partial differences of antibody titer between the chicken lines, this study clearly showed that the vaccine induced good and sufficient antibody response in both SPF chickens and commercial layers.

KEY WORDS: avian influenza, chicken lines, H5 virus, vaccine.

The basic measure undertaken for control of outbreaks of highly pathogenic avian influenza (HPAI) in poultry is stamping out in Japan and many advanced nations. Vaccination is allowed as an optional tool to decrease the amount of virus shedding from infected chickens when stamping out is not effective enough to control the disease [2]. Although commercial vaccines prepared from viruses of the North American lineage are available, they may be less effective for control of HPAI outbreaks caused by infection with viruses of the Eurasian lineage [9]. It is desirable that the antigenic properties of the virus used to manufacture a vaccine is as similar as possible to the viruses of outbreaks in the field [1, 4]. Thus, our test vaccine was prepared from an Eurasian lineage virus.

We have developed an H5N1 gene reassortant virus of the Eurasian lineage that is non-pathogenic in chickens and chicken embryos and exhibits good multiplication in embryonated chicken eggs [9]. Subsequently, we prepared test vaccines using this gene reassortant virus and determined the optimal antigen concentration in relation to protective potency against a currently prevalent Eurasian lineage HPAI virus [7]. We also demonstrated that the test vaccine induced protective immunity against HPAI virus from 8 days after vaccination [4].

Several academic reports have described chicken line differences in immunological responses against foreign antigens or vaccination [3, 5, 11]. Dunnington et al. described the existence of a considerable difference in cecal lesions after challenge of Eimeria tenella between 2 lines of chicken with high and low antibody responses [3]. A significant difference in antibody response against sheep red blood cells was observed using high and low responder chicken lines [5, 11]. Comparative studies on the differences of antibody responses in chicken lines are of prime importance in evaluating vaccine efficacy precisely for control of highly contagious diseases such as avian influenza. Then, all our previous studies were conducted using specific pathogen-free (SPF) white leghorn chickens reared in a highly sanitary environment [4, 6–8]. Our concerns about whether the test results for vaccine efficacy with SPF chickens are analogous with commercial layers cannot be neglected [4, 6–8]. So, it is important to obtain comparative data for antibody responses between SPF chickens and typical commercial layers reared in common poultry farms. As the test vaccine used in this study is oil adjuvanted, it is difficult to use for meat chickens, especially broilers whose feeding period is too short to recover from the local reaction at the injection site. Thus, we compared the antibody responses between SPF chickens and commercial layers of Julia and Boris-Brown to confirm the potency of test vaccine in this study.

A Eurasian lineage reassortant non-pathogenic avian influenza (A1) virus, A/duck/Hokkaido/Vac-1/04 (H5N1; Dk/Vac-1/04), was used for vaccine preparation. The virus was generated by reassortment of A/duck/Mongolia/54/01 (H5N2) and A/duck/Mongolia/47/01 (H7N1) [9]. To prepare virus suspensions, the Dk/Vac-1/04 virus was inocu-
lated into the allantoic cavity of embryonated chicken eggs and incubated at 34°C for 48 hr. Allantoic fluid of the eggs was harvested as Dk/Vac-1/04 virus suspension and inactivated by incubation with formalin at a final concentration of 0.2% for 3 days at 4°C. Inactivation was confirmed by inoculation of the formalin-treated samples into embryonated chicken eggs. The inactivated Dk/Vac-1/04 virus suspension was diluted to appropriate concentrations with phosphate-buffered saline (PBS) based on hemagglutination (HA) titers. A 2.5 volume of viral suspension with an HA titer of 1:256 was mixed with a 7.5 volume of oil adjuvant containing 3.9% anhydromannitol-octadecenoate-ether and sufficient light mineral oil to comprise the remaining volume. This mixture was emulsified using an ultra-homomixer (PRIMIX Corporation, Osaka, Japan) to produce a water-in-oil-type test vaccine. The virus concentration of the test vaccine was 640 HA units per dose [7]. SPF white leghorn chickens for this study were supplied by the Miyadu branch office of Kyoto Biken Laboratories, Inc., Kyoto, Japan. Healthy commercial layers of Julia and Boris-Brown were obtained from a common poultry farm for this study. Ten of each type of 10-week-old chicken, SPF, Julia and Boris-Brown, were respectively vaccinated intramuscularly in the lower thigh with 1 dose (0.5 ml) of the test vaccine. Blood samples were collected from all chickens each week for 6 weeks after vaccination and tested for antibody responses with hemagglutination-inhibition (HI) and virus neutralizing (VN) tests. The HI test was performed according to the Japanese Standards for Veterinary Biological Products [7, 8]. The HI antibody titers against Dk/Vac-1/04 were expressed as the highest dilution of the serum sample that showed complete inhibition of hemagglutination. The VN test was performed according to the WHO Manual on Animal Influenza Diagnosis and Surveillance [10]. The VN antibody titers against Dk/Vac-1/04 were expressed as the highest dilution of the serum sample that showed complete inhibition of cytopathogenic effect. Significant statistical differences in HI and VN antibody titer were observed between the SPF chickens and commercial layers by the t-test, respectively.

The changes in the HI antibody titers of the chickens after vaccination with the test vaccine are presented in Table 1. All chickens showed antibody responses 2 weeks after vaccination. The geometric means (GM) of the HI antibody titers reached the maximal value at 4 and 5 weeks after vaccination. The changes in the VN antibody titers of the chickens after vaccination with the test vaccine are presented in Table 2. The GM of the VN antibody titers rose continuously for the 6 weeks after vaccination. Significant differences in HI antibody titer between the SPF and Julia or SPF and Boris-Brown chickens were observed at 3 weeks or at 2 and 3 weeks after vaccination, respectively. However, no significant difference was observed in VN antibody titer between the SPF chickens and the commercial layers throughout observation period.

In the present study, the HI antibody responses of the chickens were statistically different during a limited period of time. The HI antibody responses of commercial layers might be delayed for a few days compared with SPF chickens. A possible reason for this is that SPF chickens are con-

### Table 1. Changes in HI antibody titers against Dk/Vac-1/04 until 6 weeks after vaccination

<table>
<thead>
<tr>
<th>Line of chickens</th>
<th>Number of chickens</th>
<th>HI antibody titers at the following weeks after vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPF 10</td>
<td>10</td>
<td>&lt;4 (&lt;4) 128–256 (181) 256–512 (338) 512–2,048 (1,176) 512–2,048 (1,024) 512–1,024 (891)</td>
</tr>
<tr>
<td>Julia 10</td>
<td>10</td>
<td>&lt;4 (&lt;4) 128–256 (169) 128–512 (223)** 512–2,048 (955) 512–2,048 (1,097) 512–1,024 (776)</td>
</tr>
<tr>
<td>Boris-Brown 10</td>
<td>10</td>
<td>&lt;4 (&lt;4) 128–256 (137)* 128–512 (208)** 512–2,048 (1,261) 512–2,048 (1,024) 256–2,048 (724)</td>
</tr>
</tbody>
</table>

a) The ranges of the HI antibody titers are shown. In parentheses: geometric means of the HI antibody titers.

### Table 2. Changes in VN antibody titers against Dk/Vac-1/04 until 6 weeks after vaccination

<table>
<thead>
<tr>
<th>Line of chickens</th>
<th>Number of chickens</th>
<th>VN antibody titers at the following weeks after vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPF 10</td>
<td>10</td>
<td>&lt;10 (&lt;10) &lt;10–10 (7) &lt;10–640 (35) 40–1,280 (485) 320–1,280 (970) 640–2,560 (1,470)</td>
</tr>
<tr>
<td>Julia 10</td>
<td>10</td>
<td>&lt;10 (&lt;10) &lt;10–10 (5) &lt;10–160 (14) 80–640 (226) 40–2,560 (557) 160–10,240 (1,280)</td>
</tr>
<tr>
<td>Boris-Brown 10</td>
<td>10</td>
<td>&lt;10 (&lt;10) &lt;10–40 (7) &lt;10–160 (32) 160–2,560 (422) 640–2,560 (1,114) 640–10,240 (1,810)</td>
</tr>
</tbody>
</table>

a) The ranges of the VN antibody titers are shown. In parentheses: geometric means of the VN antibody titers.
considered to be more sensitive than commercial layers because they feed constantly in a very hygienic environment. In addition, at 2 to 4 weeks after vaccination, their HI antibody responses showed a sharp increase. Thus, we believe that the statistical difference was observed during a limited period of time due to these factors. However, this presents no problems in vaccine practice for protection in common poultry farms because the HI antibody titers of all the test chickens from 2 weeks after vaccination were 1:137–1:181 in terms of the GM; these values are far higher than the minimum protective level of 1:16 shown in our previous report [7]. Therefore, commercial layers can be expected to show sufficient protection against HPAI virus. The VN antibody response to the vaccination tended to be delayed when compared with the HI antibody response. However, the SPF chickens and commercial layers showed equivalent levels of antibody response in their VN titers. HI antibody responses of all the chickens were confirmed 2 weeks after vaccination. However, VN antibody responses of all chickens were confirmed 4 weeks after vaccination. Thus, we think that the HI test is more advantageous than the VN test for earlier determination of infection and immunological response. In consideration of the results of previously conducted experimental challenges, it appears certain that the test vaccine is able to provide appropriate protection against HPAI virus even in commercial layers feeding in common poultry farms [4, 6–8]. In conclusion, this study clearly showed that the test vaccine induced good and sufficient antibody response in both SPF chickens and commercial layers.

REFERENCES