Immunological Properties of the Cell-associated Live Infectious Laryngotracheitis Virus

Akira TANENO, Takashi HONDA, Eishi SAKAI, Toru KAWAI, Yukio TOKUYAMA, Takuma HANAKI, and Masanobu ETO
Department of Animal Products, The Chemo-Sero-Therapeutic Research Institute, 668 Okubo, Shimizu-machi, Kumamoto 860, Japan
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Infectious laryngotracheitis (ILT), a herpes-virus infection of chickens, caused a highly localized infection of the respiratory tract. Tissue-culture-modified ILT virus vaccines, consisting of infected culture fluids, have been used to prevent this disease [2, 6, 8, 9, 11]. For prevention of Marek’s disease caused by another herpesvirus of chickens, the cell-associated vaccines of herpesvirus of turkeys (HVT) have been commercially used for day-old chickens. Chan et al. [1] reported that a bivalent cell-associated vaccine prepared by co-cultivating HVT and an attenuated infectious bursal disease virus had good efficacy. Therefore, cell-associated cultures of other attenuated viruses may be applicable to live vaccines.

This paper describes the efficacy of a cell-associated live ILT virus.

The cell-associated (C-A) virus consisted of chicken embryo fibroblast (CEF) cells infected with an attenuated live ILT virus. CEF monolayers were inoculated with the tissue-culture-modified strain CE [9] of ILT virus at a multiplicity of infection of 0.001 median tissue culture infective dose (TCID₅₀)/cell. After adsorption for 1 hr at 37°C, the monolayers were refilled with Eagle’s minimum essential medium (MEM) (Gibco) containing 100 units/ml of penicillin, 100 μg/ml of streptomycin, 10% calf serum and 5% tryptose phosphate broth (Difco) and incubated for 96 hr at 37°C. Then the cultured infected cells were harvested and collected by centrifugation at 900 × g for 5 min at 4°C. The sediments were adjusted to about 2 × 10⁷ cells/ml with MEM containing 10% dimethylsulphoxide and 15% calf serum. The cell suspensions were divided into ampoules and stored in liquid nitrogen until use. Infectious virus titers of the C-A virus, which were disrupted by sonication (Sonifier model 250, Branson), were measured using primary chicken kidney cell (CK) cultures.

The cell-free (C-F) vaccine, the infectious laryngotracheitis vaccine (live, dried) (The Chemo-Sero-Therapeutic Research Institute), was prepared from the cultural fluid of CEF cells infected with the strain CE. After centrifugation of the cultural fluid at 7,000 × g for 10 min at 4°C, the resulting supernatant was mixed with a stabilizer and freeze-dried. Infectious virus titers were measured using CK cultures.

Specific-pathogen-free chickens were obtained from The Chemo-Sero-Therapeutic Research Institute, Animal Supply Station (Kumamoto, Japan), and used throughout the present study.

Day-old or 20-day-old chickens were inoculated with 0.2 ml of dose of the inocula by the subcutaneous (s.c.) or intramuscular (i.m.) route, or with a 0.03 ml dose by the intraocular (i.o.) route immediately after reconstitution of the inocula. At 14 days post-inoculation (PI), chickens inoculated at 20 days old and uninoculated controls were challenged intratracheally with 2 × 10⁵ 50% chicken infective dose of the virulent strain NS-175, and observed for 10 days. Chickens inoculated at day old and controls were challenged at 14 to 70 days PI. Chickens which showed marked clinical signs (lacrimation, rales, coughing or gasping) were considered to be affected by ILT.

Twenty-day old chickens inoculated with the C-F vaccine by the i.o. or s.c. route had 40% and 90% protection, respectively, against the challenge 14 days PI. All the chickens inoculated with the C-A virus by the i.o., s.c. or i.m. route had complete protection. All controls showed marked clinical signs (Table 1).

Day-old chickens were inoculated with 3 lots of the C-A viruses containing 10⁴.7, 10⁵.0 and 10⁵.1 TCID₅₀/dose by the s.c. route, or with the C-F vaccine containing 10⁴.7 TCID₅₀/dose by the i.o. route. With the C-F vaccine, the protection rate in the inoculated chickens was 60% at 14 days PI, but decreased rapidly (Table 1). However, in the chickens vaccinated with the C-A viruses, the protection rate was 85 to 95% at 14 days PI and
Table 1. Protective potency of the cell-associated virus and cell-free vaccine against a virulent infectious laryngotracheitis (ILT) virus

<table>
<thead>
<tr>
<th>Age of chickens</th>
<th>Inoculum</th>
<th>Route</th>
<th>Virus titer</th>
<th>Day of challenge after immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>C-A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Intraocular</td>
<td>4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10/10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>5.2</td>
<td>10/10</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td>5.2</td>
<td>10/10</td>
<td>N.T.</td>
</tr>
<tr>
<td>20-day-old C-F&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Intraocular</td>
<td>4.7</td>
<td>9/10</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>5.5</td>
<td>4/10</td>
<td>N.T.</td>
</tr>
<tr>
<td>control</td>
<td>—</td>
<td>—</td>
<td>0/10</td>
<td>N.T.</td>
</tr>
<tr>
<td>Day-old C-A</td>
<td>Subcutaneous</td>
<td>5.1</td>
<td>19/20</td>
<td>18/20</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>5.0</td>
<td>18/20</td>
<td>16/20</td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td>4.7</td>
<td>17/20</td>
<td>14/20</td>
</tr>
<tr>
<td>C-F</td>
<td>Intraocular</td>
<td>4.7</td>
<td>12/20</td>
<td>6/20</td>
</tr>
<tr>
<td>control</td>
<td>—</td>
<td>—</td>
<td>0/20</td>
<td>0/20</td>
</tr>
</tbody>
</table>

a) Cell-associated live ILT virus.
b) log<sub>10</sub>TCID<sub>50</sub>/chicken.
c) Number of protected chickens/Number of chickens tested.
d) Not tested.
e) Cell-free live ILT virus vaccine.

maintained more than 60% until 70 days PI.

No clinical signs attributed to the C-A viruses or the C-F vaccine were shown in any chickens listed in Table 1 (data not shown).

In the present study, it was demonstrated that protective potency of the C-A live ILT virus was superior to that of the C-F vaccine which was administered by the i.o. route (Table 1). When the C-A virus was administered by the i.o. route to 20-day-old chickens, the protective efficacy against a virulent ILT virus was almost equal to that of the C-F vaccine. However, the s.c. administration with the C-A virus gave better protection against the challenge exposure than with the C-F vaccine. Day-old chickens did not respond fully to the C-F vaccine, but did so sufficiently to the C-A virus. Furthermore, 60 to 70% of day-old chickens vaccinated with the C-A virus maintained immunity for 70 days PI.

In general, tissue-culture-modified ILT vaccines have defects as follows: (a) Chickens less than 2 weeks of age cannot respond well to vaccination as do adult birds [3, 4]. (b) Duration of immunity induced by vaccination is not so long in younger birds [7]. (c) The eyedrop method of vaccination is labour-consuming.

The present results clearly demonstrate the usefulness of the s.c. administration of the C-A virus to day-old chickens and the establishment of long-lasting protection against ILT. It is considered that the C-A virus is a candidate for the live virus vaccine overcoming the defects mentioned above.

The protective mechanism against ILT is mainly dependent on cell-mediated immunity, but is not a humoral one [3, 5, 12]. The high efficacy of the C-A virus in the present study may be attributed to the following: (a) The injected cells as a source of the C-A virus in vivo may have the ability to produce and release the progeny virus for a while. (b) The C-A virus in vivo may be transmitted to neighboring cells by cell-to-cell contagion, which may be resulted in rapid and more effective establishment of the infection. (c) Far more viral proteins may be present in virus-infected cells than in virion [10]. Hence, immunogenicity of the C-A virus is superior to that of the C-F vaccine.

In further experiments, the safety and efficacy of the C-A live ILT virus in the field remain to be confirmed.
REFERENCES


要 約

鶏伝染性喉頭気管炎弱毒生ウイルス (ILT) 感染細胞の免疫学的性状（短報）：種子野 章・本田 隆・酒井 英史・河合 透・徳山幸夫・花木琢磨・江藤正信（財団法人化学反有効利用研究所）——ILT 感染細胞を20日齢鶏に皮下、筋肉内及び点眼投与したところ、強毒株による攻撃に耐え、投与後14日目でいずれの投与経路においても、細胞フリーの現行ワクチンと同等以上の防御効果を示した。また、初生鶏に感染細胞を皮下投与したところ、投与後14日目で85-95％、70日目でも60-70％の効果が認められた。このことから、ILT 感染細胞は、免疫原性に優れた新しいタイプのワクチンとして応用できることが示唆された。