Pathology of Experimental Chlamydiosis in Chicks

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ABSTRACT. Twelve one-day-old chicks were experimentally inoculated with Chlamydia psittaci derived from turkeys. Acute chlamydial septicemic lesions were induced by the inoculation into the air sac and trachea. No lesions were produced by the esophageal injection. Clinically, the affected chicks showed emaciation and mouth breathing, and were inactive while some birds died. Grossly, they had hepatomegaly, splenomegaly and airsacculitis. Histopathologically, fibrinopurulent airsacculitis, pneumonia and bronchitis, multiple fibrinous serositis in the hepatic and splenic capsules, peri- and epicardium, and mesenterium, focal endoarteritis in the aortae, activation of reticuloendothelial cells in the spleen, and hepatic necrosis were noted. Immunohistochemically, chlamydial antigen granules were present in the cytoplasm of epithelial cells of the respiratory system, hepatocytes, macrophages in the air sac, lung, serous membrane, liver, spleen, aortae, reticuloendothelial cells in the spleen, and mesothelial cells in various organs or tissues. Chlamydial multiplication in the cells of the organs or tissues involved was preceded to form the lesions.—KEY WORDS: chick, Chlamydia psittaci, chlamydiosis, immunohistology.

Avian chlamydiosis caused by Chlamydia psittaci is an important infectious disease in wild, cage or avairy, and domesticated birds because of public health hazards [11, 12, 21, 22] and because of economic losses in the poultry industry, especially in turkeys and ducks [11, 16, 21, 22]. Chickens show host resistance to C. psittaci [2, 11, 16]. For example, the infected chickens show inapparent or transient symptoms [11, 16], although the organism was isolated [4] and the antibody detected by serological survey [8, 13]. Experimentally, an age-based resistance to the development of chlamydiosis appeared to be more pronounced in chickens [1, 2, 23, 26] than in turkeys [5]. However, some acute cases of this condition induced experimentally have been described in young chickens [1, 2, 26]. The model of the systemic and acute chlamydiosis was established in chicks inoculated with C. psittaci derived from budgerigars [26].

As described above, chicks seem to have susceptibility to C. psittaci. However, pathological changes of chlamydiosis in chicks have not been described in detail. This paper induced acute chlamydiosis in chicks and described the histopathological and immunohistochemical findings referring to the histopathogenesis.

MATERIALS AND METHODS

Chlamydial strain and inoculum: The strain of C. psittaci used in this study was isolated from a naturally infected turkey flock [3]. Inoculum was propagated in the yolk sac of 7-day-old chicken embryos, and 10% suspension of the yolk sac was prepared in a mortar and pestle and stored at −80°C until used for the inoculation. The inoculum titer was $10^{1.37}$ to $10^{3.37}$ 50% egg lethal doses (ELD$_{50}$).

Experimental design: The chicks used were specific-pathogen-free and confirmed to be free from natural chlamydial infection.
Table 1. Induction of lesions by different inoculation routes and amounts of the inoculum

<table>
<thead>
<tr>
<th>Dose</th>
<th>Induction of lesions after inoculation into</th>
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<tbody>
<tr>
<td></td>
<td>air sac</td>
<td>trachea</td>
</tr>
<tr>
<td>$10^{1.37}b$</td>
<td>Yes</td>
<td>NE$^b$</td>
</tr>
<tr>
<td>$10^{2.37}$</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>$10^{3.37}$</td>
<td>No</td>
<td>No</td>
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</table>

a) ELD$_{50}$
b) Not examined.

The chicks were kept in an isolator, and given water and feed free from antibiotics, ad libitum.

To examine the effective routes of chlamydial infection, the following experiments were done. Every three 1-day-old chicks were inoculated with 0.3 ml of the inoculum size as shown in Table 1 into the right posterior thoracic air sac through an injector and the esophagus through a gastric feeding tube, and with 0.15 ml of it into the trachea. Three 1-day-old chicks injected with 0.3 ml of the yolk sac $10^{-3}$ diluted in PBS into the air sac served as the control. In addition, three age-matched chicks also served as the un inoculated control. After inoculation, clinical signs were observed, and surviving birds were sacrificed 7 and 14 days post inoculation (PI) for pathological examination.

To observe the chronological lesions, twelve 1-day-old chicks were inoculated with $10^{1.37}$ ELD$_{50}$/0.3 ml of the inoculum into the right posterior thoracic air sac. On 2, 4, 6 and 8 days PI, two chicks each were sacrificed and examined pathologically.

Chicks that died during the observation period were also examined pathologically.

Pathological examination: After necropsy, tissues were collected and fixed in 10% phosphate buffered formalin. Paraffin sections were stained with hematoxylin and eosin (HE). Immunohistochemical staining for the chlamydial antigen was performed by the indirect peroxidase method using anti-C. psittaci rabbit serum as the first antibody, as previously reported [25].

RESULTS

Induction of chlamydial lesions: The lesions related to the inoculated routes and doses are shown in Table 1. The lesions were induced by inoculation of $10^{1.37}$ and $10^{2.37}$ ELD$_{50}$ of the inoculum into the posterior thoracic air sac and of $10^{2.37}$ ELD$_{50}$ of it into the trachea. No lesions were reproduced by the esophageal inoculation. From the results, the inoculation of $10^{1.37}$ ELD$_{50}$ of the inoculum into the posterior thoracic air sac was made in the succeeding experiments.

Chronological observations: Clinical Signs The body weight in the inoculated chicks did not increased until the experimental terminal, 8 days PI. Emaciation, inactivity and mouth breathing appeared on and after 5 days PI and 5 out of 12 inoculated chicks died between 5 and 8 days PI.

Gross Lesions Mild hepatomegaly, splenomegaly and anterior or posterior thoracic airsacculitis were found on and after 6 days PI. The airsacculitis showed mild cloudy thickening of the wall, some with small amounts of fibrin exudation into the air sac cavity. Caseous exudates into the air sac were more abundant in the dead cases. The same lesions were seen in the abdominal air sac on and after 7 days PI. Consolidated lesions occurred in the abdominal side of the
Experimental Chlamydiosis in Chicks

Table 2. Chronological incidence of histopathological lesions by inoculation of the inoculum into the air sac

<table>
<thead>
<tr>
<th>Histopathological lesions</th>
<th>Days postinoculation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Fibrinous purulent airsacculitis&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fibrinous purulent pneumonia&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/2</td>
</tr>
<tr>
<td>Desquamative trachitis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/2</td>
</tr>
<tr>
<td>Fibrinous serositis&lt;sup&gt;c&lt;/sup&gt; (in spleen, liver, mesothelium and pericardium)</td>
<td>0/2</td>
</tr>
<tr>
<td>Endoarteritis or endocarditis&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/2</td>
</tr>
<tr>
<td>Hepatic necrosis or activated sinusoidal endothelia&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/2</td>
</tr>
<tr>
<td>Lymphocytic depletion from splenic follicles&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/2</td>
</tr>
<tr>
<td>Atrophy of bursa of Fabricius</td>
<td>0/2</td>
</tr>
<tr>
<td>Atrophy of thymus</td>
<td>0/2</td>
</tr>
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</table>

<sup>a</sup> Associated with CAG or CIB.
<sup>b</sup> No. of chicks with lesions/No. of examined chicks.

Lungs of both sides and after 7 days PI.

Histopathological findings: No significant lesions were present in the uninoculated or yolk sac inoculated chicks. The main lesions in chicks inoculated with C. psittaci are shown in Table 2.

Air sac The posterior thoracic air sac was affected firstly, extending to the anterior thoracic and abdominal air sacs. Chlamydial antigen granules (CAG) on sections stained immunohistochemically or basophilic chlamydial inclusion bodies (CIB) on sections stained with HE were noted in the cytoplasm of the epithelial cells 3 days PI as the initial change (Fig. 1a). CAG were small to large aggregations, sometimes occupying the cytoplasm. CIB, which were fewer in appearance than CAG, were 5 to 20 μm in diameter and finely granular in their interior. These were stained positively with the chlamydial antiserum. CAG were more numerous in the non-ciliated epithelial cells than in the ciliated. Non-ciliated epithelial cells with CAG were swollen and desquamated focally, resulting in erosion with exudation of fibrin, macrophages and heterophils (Fig. 1b). As the erosion advanced, the large area of the epithelial layer was lost and replaced with much of the exudate (Fig. 2). These extensive lesions were associated with reaction of macrophages, lymphocytes and fibroblasts in the subepithelial tissue,
and sometimes with mild lymphocytic aggregations around the blood vessels in the peripheral tissue. CAG were also found frequently in the macrophages.

**Lung** Fibrin, heterophils and macrophages were exudated in the tertiary bronchi, atria and air capillaries with swollen lining epithelial cells as airsacculitis advanced (Fig. 3). These lesions started from the lobules adjacent to the posterior thoracic air sac and extended to the surrounding lobules. In the advanced lesions which occurred in the dead cases, some lobules and secondary bronchi were filled with the exudate, associated with edema and lymphocytic infiltration around blood vessels in the interlobular connective tissue. CAG were observed in the exudated macrophages and lining epithelial cells of all the bronchi, atria and air capillaries from the early lesions. CIB were scattered in epithelial cells of the secondary bronchi. The same erosions as seen in the air sac were formed with CAG or CIB in the epithelial layer of the secondary bronchi.

**Trachea** On and after 3 days PI, CAG were detected in the epithelial cells, showing deciliation, swelling, hyperplasia and desquamation (Fig. 4). CIB were occasionally seen in the epithelial cells in the dead cases with marked airsacculitis and pneumonia.

Similar lesions were present in the larynx.

**Serous membrane (hepatic and splenic capsules, peri- and epicardium, and mesenterium)**: Initial lesions were similar to those in the air sac. CAG appeared in swollen mesothelial cells, followed by desquamation and exudation of fibrin, heterophils and macrophages having CAG (Fig. 5a). In the advanced lesions, extensive areas were covered with much of the exudate, associated
with fibroblastic reaction in the subserosal tissue (Fig. 5b). Such fibrinous serositis was more prominent in the hepatic and splenic capsules than in other serous membrane.

Liver On and after 5 days PI, fatty changes were prominent in some hepatic cells. Then, sinusoidal endothelia were swollen, associated with migration of a few lymphoid cells and heterophils in the sinusoids, and occasionally forming minute cell nodules. In some cases, on and after 5 days PI, sporadic or multiple small necrotic foci occurred with some fibrin and swollen sinusoidal endothelia (Fig. 6). In the periphery of these necrotic foci, CIB were seen in hepatocytes, macrophages and swollen sinusoidal endothelia (Fig. 6, inset). The hepatic cells with CIB showed acidophilic degeneration. CAG were present in the necrotic foci and in the hepatic cells and activated sinusoidal endothelia around them.

Spleen The normal development of lymphoid follicles with age was not seen. Lymphocytes were depleted from the lymphoid follicles on and after 4 days PI, associated with fibrin exudation and proliferation of reticular cells in them (Fig. 7). In the dead cases 8 days PI, lymphocytes were almost completely absent from the lymphoid tissues, and were replaced by macrophages containing CAG or CIB. There were activation of reticuloendothelial cells frequently having CAG and slight increase of lymphoid cells in the red pulp.

Blood vessels and endocardium Focal inflammation was observed in the descending and abdominal aortae and endocardium on and after 6 days PI (Fig. 8). These
lesions, endoarteritis and endocarditis, consisted of desquamation of the endothelia and infiltration of macrophages in the subendothelium. The macrophages contained CAG. Hyaline thrombi were present in the aortae involved.

CAG were scattered in the capillary endothelia of the kidney and bone marrow in some cases.

_Bursa of Fabricius_ Lymphocytic depletion from the cortex of lymphoid follicles with swollen reticular cells in the medulla was noted on and after 3 days PI. Thus, atrophy of this organ was evident with an irregular arrangement of the lining epithelial layer on and after 6 days PI.

_Thymus_ Depletion of thymocytes from the cortex was observed on and after 3 days PI, and was marked on and after 7 days PI.

Fig. 6. Liver. A focal necrosis consisting of fatty-changed hepatocytes, swollen sinusoidal endothelia, migrated cells and fibrin exudation. Died 8 days PI. HE. ×752. Inset: CIB (arrows) in the cytoplasm of hepatocytes showing acidophilic degeneration. Died 8 days PI. HE. ×712.

Fig. 7. Spleen. Depletion of lymphocytes, fibrin exudation and proliferation of reticular cells in two follicles. Died 8 days PI. HE. ×390.

Reticular cells were hyperplastic and Hassal's corpuscles increased in number in their medulla.

**DISCUSSION**

The respiratory tract is described to be the most important route of _C. psittaci_ infection in birds including chickens [11, 13, 16, 21, 22]. The present results support this view; the organism has multiplied actively in the epithelial cells of the respiratory system. Experimental inoculation of _C. psittaci_ into the air sac of chicks showed more constant recovery of the organism and better antibody response than in the oral injection [26].

Focal hepatic necrosis, lymphocytic depletion from splenic lymphoid tissues with activated reticular cells, and fibrinopurulent airsacculitis, pericarditis and conjunctivitis which were associated with CIB occurred in
to cell necrosis when the elementary bodies were released [27]. Inflammatory reactions by macrophages and reticuloendothelial cells were pointed out in chlamydia of turkeys [5–7, 9, 11, 16, 19, 22], ducks [11, 16, 19, 22] and pet birds [10, 11, 20, 22, 25]. Chlamydiae are able to grow within macrophages [28], by which they are transmitted to the whole body [12, 18, 25]. The macrophages and reticuloendothelial cells are considered to be important in the dissemination of chlamydiae within the body.

CIB in the affected tissue sections are pathognomonic for the diagnosis of chlamydiosis. Smear preparations from the tissues involved have been used for the detection of CIB [1, 5–7, 9–12, 14–21, 23–25], because it is difficult to find them out on routinely stained sections [10, 19]. The immunohistochemical staining method is useful and available for detecting chlamydial antigen. In immunohistochemical studies on spontaneous systemic chlamydiosis of pet birds, chlamydial antigen was detected in many cells of the whole body tissues [10, 14, 15, 17, 25]. CIB seem to be one of the developmental stages of the organism, probably a comparatively matured one. Further investigation is needed to clarify the relationship between CIB and CAG.

The histopathogenesis and significance of lymphocytic depletion from the spleen, thymus and bursa of Fabricius were unknown in the present study. Immunosuppression related to cellular immunity may be one of the factors which convert the latent to the septicemic form in chlamydiosis [11, 21].

REFERENCES


要約

鶏における実験的クラミジア症に関する病理学的研究：諏訪隆彦・安藤秀二11・橋本信夫11・板倉智敏（北海道大学獣医学部比較病理学講座）——初生雛にシチメンチョウ由来のChlamydia psittaciを各種ルートで接種した。その結果、クラミジア急性敗血症病変が心内および気管内接種で形成されたが、経口接種では形成されなかった。接種雛は、臨床的に発育不良、元気消失、開口呼吸を示し、一部が死亡した。肉眼的には、肝臓腫大、脾臓腫大、気囊炎の主病変であった。組織学的には、線維素性化膿性気囊炎、気管炎、肝および脾包膜・心膜および心外膜・腸間膜における多発性線維素性膿膜炎、大動脈の囊状動脈内潰瘍、脾臓の細網内皮系細胞の活性化、肝細胞壊死が認められた。免疫組織学的には、クラミジア抗原が呼吸器系上皮細胞、肝細胞、気囊・肺・腸膜・脾臓・動脈の大食細胞、脾臓の細網内皮細胞、肝および脾包膜・心膜および心外膜・腸間膜における中皮細胞の細胞質に存在していた。これらの臓器、組織の細胞におけるクラミジア増殖が、各病変形成に先行していることが注目された。