Acidophilic Protein Crystals in Lungs and Bile Ducts of Helminth-Infected Mice

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(Received 9 September 1987/Accepted 30 October 1987)


KEY WORDS: crystal, helminth infection, mouse.

Acidophilic protein crystals (PCs) are incidentally noticed in the epithelium-lined organs, such as tracheal glands, bronchi, bronchioli, gall bladder and bile ducts, and also in the alveolar macrophages of mice during long-term observations [10, 12, 14, 15]. A few reports [2, 3, 18] described accelerated occurrence of PCs in the lungs or bile ducts of mice with parasitic infections, and referred them as the Charcot-Leyden crystal. Recently, however, Kitamura et al. [9] demonstrated that the biliary PC was a product of abnormal epithelium showing high capacity of protein synthesis, and Shultz et al. [13] reported a close association between hemosiderin deposits and PCs in alveolar macrophages. The latter suggested that hemoglobin breakdown in impaired alveolar macrophages resulted in the formation of intracellular PC. Similar assumption has been made on the intracellular PC found in bone marrow and splenic macrophages of normal adult mice [6].

A histological survey was carried out on the mice infected experimentally with some parasites such as Schistosoma mansoni, Angiostrongylus siamensis, A. costaricensis, Toxocara canis and T. cati, and we found quite frequent incidence of PCs in the lungs and bile ducts of those mice.

1) Schistosoma mansoni-infected mice: Eighteen WCB6F1-(SI/SI4, +/+) mice at 5 or 9 month of age were infected subcutaneously with 100 cercariae of S. mansoni. Those mice were killed 6 months or 7 weeks after infection, respectively. All mice infected chronically (6 months) exhibited remarkable biliary lesions with PCs, while animals infected short-term (7 weeks) showed mild biliary lesions. The biliary lesions consisted of intraluminal and intraepithelial PCs, epithelial hyperplasia and hypertrophy with storage of eosinophilic, homogeneous or granular materials, and cuffings by plasma cells (Fig. 1). Hyperplastic epithelium was often infolded into crypt formations, and at the basal region of invaginations, goblet cells were increased. Occa-

Fig. 1. Intraluminal and intraepithelial biliary PCs and epithelial granular metaplasia of the interlobular bile duct. Mouse infected chronically (6 months) with S. mansoni. Hematoxylin-eosin. ×210.
those mentioned above except for the cuffings by eosinophils, lymphoid cells and macrophages in Angiostrongylus-infected mice. The lesion did not have apparent association with deposition and hatching of eggs, or larval migration in the liver.

Both alveolar and bronchiolar PCs (Fig. 2) were found only in 10 A. siamensis-infected mice between 60 and 94 DAI, all of which had the influx of eggs and/or heterotopic parasitism of adult worms in the pulmonary vasculature. Both alveolar and bronchiolar PCs were correlated well with hatching and migration of first-stage larvae into the alveolar spaces. In severe cases PC-laden cells filled up the alveolar spaces, and frequently fused each other to form bizarrely stellated giant cells, extending over more than one alveolus. The first-stage larvae were sometimes involved in PC-laden multinucleated giant cells. Perls’ Prussian blue stain failed to demonstrate a significant amount of pigments. Changes of bronchial and bronchiolar epithelia were essentially similar to those of the biliary epithelium, except for lack of subepithelial cellular reactions.

3) Toxocara-infected mice: Toxocara larvae migrate through the liver and lungs, and then are dispersed to the whole body. Fifteen 10-month-old ddY mice were orally inoculated with 1,000 or 3,000 fully embryonated eggs of T. canis and T. catti, respectively. In the lungs, numerous multinucleated giant cells laden with diffuse hemosiderin were distributed in the alveolar spaces, and frequently engulfed erythrocytes on 7 DAI. On 14 DAI, deposition of coarse hemosiderin granules and striation of the cytoplasm with slender PCs were apparent, and the latter was more prominent on 23 DAI. Biliary PCs and the related lesions were found in all mice on 23 DAI. Alveolar PC formation in Toxocara-infected mice was associated well with hemosiderin deposition. Electron microscopic examination of alveolar macrophages showed that both hemosiderins and PC rods were bounded by the same smooth-surfaced unit membrane in early phase of PC formation.

Neither intra- and extracellular PCs nor other significant lesions were found in the uninfected control mice for each experiment.

All the PCs and materials stored in the hypertrophic bronchial and biliary epithelia showed similar staining reactions as reported previously [4, 17]. Gmelin’s reaction, Stein’s iodine stain for hematoidin and periodic acid-Schiff failed to stain those crystals, and eosin, acid fuchsin, phloxin, toluidine blue (pH 7.2) and Heidenhain’s iron-alum hematoxylin stained them positively. All of them exhibited neither autofluorescence nor birefringence. Green [4], and Yang and Campbell [17] examined in detail the nature of those crystalline materials in the lungs and gall bladder of mice, and demonstrated that those were quadrangular crystalline plates of proteinaceous nature. Designation of them as a variant of Charcot-Leyden crystal as well as hematoidin crystal is inadequate because latter two are formed extracellularly and have different nature from intracellular PC in mouse alveolar macrophages [5, 11].

Intraluminal PCs found in the bronchi, bronchioli and bile ducts are shown to be a product of abnormal lining epithelium, designated as glandular metaplasia [10], because the abnormal epithelium is bulged with stored materials show-
Table 1. Incidence of PCs in lungs and bile ducts of mice infected with *A. siamensis* and *A. costaricensis*

<table>
<thead>
<tr>
<th>Infection</th>
<th>Days after infection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-10 -20 -30 -40 -50 -60 -70 -80 -94</td>
<td></td>
</tr>
<tr>
<td><em>A. siamensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mice examined</td>
<td>4 4 5 5 5 8 4 7 5 47</td>
<td></td>
</tr>
<tr>
<td>mice with PCs in bile ducts</td>
<td>0 0 1 0 2 6 3 4 1 17</td>
<td></td>
</tr>
<tr>
<td>mice with PCs in bronchioli</td>
<td>0 0 0 0 0 1 2 6 1 10</td>
<td></td>
</tr>
<tr>
<td>mice with PCs in alveoli</td>
<td>0 0 0 0 0 1 2 6 1 10</td>
<td></td>
</tr>
<tr>
<td><em>A. costaricensis</em></td>
<td></td>
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<tr>
<td>Number of</td>
<td></td>
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</tr>
<tr>
<td>mice examined</td>
<td>2 3 5 4 7 8 4 6 5 44</td>
<td></td>
</tr>
<tr>
<td>mice with PCs in bile ducts</td>
<td>0 2 3 3 4 7 3 3 3 28</td>
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<tr>
<td>mice with PCs in bronchioli</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>mice with PCs in alveoli</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
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</table>

ing similar staining reactions to PCs [3, 12, 17] and occasionally includes intracytoplasmic PCs in the vicinity of the dilated rough-surfaced endoplasmic reticulum [9]. As one of the interaction between the helminth and the biliary epithelium, an amino acid produced by *Fasciola hepatica*, proline, has been used to induce biliary hyperplasia in experimental fascioliasis [8]. In biliary granular metaplasia induced by repeated intraperitoneal injections of swine serum into mice [7, 9], a clear strain difference in the degree of lesions, associated with production of anti-swine serum antibodies has been detected, although the pathogenesis of the lesion has not been elucidated [7]. Concerning these problems, we consider to conduct experimental study in the near future.

The origin of alveolar intracellular PC is a subject of controversy. The frequent occurrence has been reported in mice under varied experimental conditions as the followings; parasitic invasion into the lungs [2, 18], exposure to grain dust [1] or high concentration of oxygen as well as inheritable pulmonary disease of motheaten and viable motheaten mice [13, 16]. Mice with pulmonary tumor [4, 15] or bronchopneumonia [17] have also been reported to show the lesion with alveolar PC. In the present study, it is revealed that PC in alveolar macrophages appeared following the damage of pulmonary vasculature by the parasite, i.e., migration of larvae into the alveoli and embolism by masses of eggs. It seems reasonable to presume that the intracytoplasmic PCs of mouse macrophages result from breakdown of erythrocytes within their phagosomes, because a close association between hemosiderin deposits and PCs was observed.

REFERENCES


要約

各種蠕虫感染マウスの肝および胆管に形成された好酸性蛋白結晶（短報）：佐藤 宏・川瀬史郎1）・奥祐三郎・神谷正男・大林正士（北海道大学獣医学部家畜寄生虫病学講座，1）北海道立衛生研究所実験動物室）——Schistosoma mansoni, Angiostrongylus siamensis, A. costaricensis, Toxocara canis, T. cati 各感染マウスの肝および胆管を組織学的に観察した。蠕虫感染と関連して、胆管および細気管支では異形成性上皮に由来する蛋白結晶が、肺胞マクロファージでは赤血球に由来すると考えられる蛋白結晶が高率に認められた。