BRIEF NOTE

An Epizootic Outbreak of Sialodacryoadenitis in Rats

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Sialodacryoadenitis (SDA) in rats, which was first described by Innes and Stanton [3], is an acute infectious disease characterized by the epizootic pattern of outbreak and a marked swelling of the neck due to enlargement of the salivary glands, inflammation and edema of adjacent areolar tissues. The causal virus of the disease was isolated and partially characterized by Bhatt et al. [1]. It has now been classified into family Coronaviridae [8]. In Japan, an occurrence of a similar disease in rats was reported by Maru et al. [6].

Recently, authors encountered an epizootic outbreak of a disease resembling to SDA in a colony of Spague-Dawley rats. These rats, both sexes, 14-week-old, were obtained from a commercial source and used for a drug-toxicity test at authors’ laboratory. They were divided into seven groups (A-G) consisting of 120, 160, 15, 120, 120, 120 and 60 animals, respectively. These groups were housed in separate rooms with the barrier system. The first case of sick rats was found in group A. Then, the disease spread rapidly within the group and from this group to the other 6 groups. During a period of 3 weeks, the incidence of the disease reached 100% in groups A and C, 70% in group B, 43.3% in group D, 8.3% in group G, 5% in groups E and F. The condition was self-limiting and no death was seen among affected animals.

The symptom first observed in the affected animals was a soreness in the submaxillary region, which was manifested at the time when the drug was administered with a stomach tube. One or two days later, marked submaxillary swelling, exophthalmos and red tears, followed by decrease in feed-consumption and water-intake were observed in most rats. About 9% loss of body weight was recognized on the average. A peak of clinical manifestations characterized by submaxillary swelling was observed around 3 days after onset of the disease and the swelling remained palpable for 7 days. Male rats appeared to be more susceptible than females.

Two male rats were sacrificed 3 days after onset of the disease for pathologic studies. Grossly, the submaxillary salivary and lacrimal glands and neighboring lymph nodes were markedly enlarged. Gelatinous edema involving the periglandular tissues was also noted. No significant changes were detected in other organs and tissues. Tissues of the pharyngeal region, submaxil-
lary, parotid and sublingual salivary glands, Harderian and exorbital lacrimal glands, submaxillary lymph nodes, liver, spleen, heart, kidney and lungs were fixed in 10% neutral buffered formalin and processed by routine methods. Sections were stained with hematoxylin and eosin (H-E) and selected sections with periodic acid-Schiff (P.A.S.), Heidenhain’s azocarmine aniline blue (Azan) and Alcian blue methods.

Microscopically, significant changes were restricted to the pharyngolaryngeal region, submaxillary and parotid salivary glands, Harderian and exorbital lacrimal glands, and the regional lymph nodes. The mucous membrane of the larynx was markedly thickened. This was due to edema, congestion, dilatation of lumina of the mucous glands, and cellular infiltration in the lamina propria (Fig. 1). Degeneration and focal necrosis of the mucosal and glandular epithelia were prominent. In the submaxillary and parotid salivary glands, periglandular and interstitial connective tissues were markedly edematous and contained numerous infiltrates of lymphocytes, histiocytes, neutrophils and mast cells, resulting in wide separation of lobules from each other (Fig. 2). Parenchymal lesions consisted of hydropic degeneration and necrosis of ductular and acinar epithelial cells. In many parts, glandular architecture became obscured. The lumina of ducts were usually filled with a large amount of cellular debris and became narrowed (Fig. 3). In some of the interlobular excretory ducts, an early evidence of squamous metaplasia of the ductular epithelium was noted (Fig. 4). The similar changes were also seen in the excretory ducts and periductular glandular tissues of the Harderian glands (Fig. 5) and exorbital lacrimal glands. Many lumina of both tubuloalveolar glands were dilated and filled with a large amount of secretion containing cellular debris. The stromal tissue surrounding those lesions was edematous and infiltrated by lymphocytes and neutrophils with proliferation of fibroblasts. In the sublingual salivary glands, degeneration and necrosis of ductular epithelial cells were only occasionally seen (Fig. 6), although inflammatory edema accompanied by cellular infiltration was remarkable in periglandular tissue. The submaxillary lymph nodes were hyperplastic and contained many enlarged germinal centers with tiny focal necrosis of lymphoid cells. The capsular connective tissue was markedly edematous being involved in inflammatory processes of the adjacent submaxillary salivary glands.

The formalin-fixed submaxillary and parotid salivary glands from one of the 2 rats, which were described above, were processed for electron microscopy by a technique previously reported [7]. Thin sections were stained with uranyl acetate followed by lead citrate. Ultrastructural examination of both glands confirmed the light microscopic observations described above. Numerous structures identified as viral particles were found most often in the ductular epithelial cells and occasionally in the acinar cells of both glands. The majority of the particles were located within cytoplasmic vacuoles and cisternae of various sizes and shapes (Fig. 7). Isolated viral particles were also seen in the ductular lumina and free in the cytoplasm of severely damaged cells. The particles had a circular outline and measured from 60 to 80 nm in diameter with a mean of 69 nm. Most particles had an electron-dense nucleoid with a transparent center although some were apparently hollow. The nucleoid was roughly spherical in shape, measuring approximately 58 nm in diameter. The viral limiting membrane, in which
trilaminar structure was sometimes discernible, was separated from the nucleoid by an electron-lucent zone. The outer surface of the viral particles was usually covered by a layer of moderately dense, fuzzy material suggestive of the presence of surface projections. Particles in the process of budding from the limiting membrane of the vacuoles or cisternae into lumina were occasionally observed (Figs. 8A and 8B).

In an attempt to isolate a causative agent, inoculation experiments were carried out. A 10% suspension was prepared from the Harderian glands collected from 5 rats approximately 1 week after the first sign of clinical illness. Twelve 3-day-old mice were inoculated intracerebrally with 0.02 ml of the suspension. None of them exhibited clinical signs and they survived until the 14th day, when they were discarded. The suspension was also inoculated onto primary monolayer cultures of rat kidney. In the absence of cytopathic effect, a blind passage was made 1 week after inoculation. The result was negative.

SDA in rats described by previous workers has many characteristic clinical and pathologic findings. Innes and Stanton [3] stated that SDA is specific in that the lesions are distinct and easily recognizable. On the basis of the epizootic pattern and results obtained by clinicopathologic observations, the disease described here appeared to be identical with SDA reported previously, even though causal agent was not isolated from the present material. This assertion was supported by the electron microscopic demonstration of viral particles in the salivary glands. Jonas et al. [5], in examining the submaxillary salivary glands of rats with experimentally induced SDA, have demonstrated virus-like particles only in the ductal lining cells. The morphology and intracellular location of viral particles observed in the present material agree well with theirs. It is of a further interest that the viral particles demonstrated here bore a striking resemblance, in many respects as observed by an electron microscopic technique of thin sectioning, to species of the Coronavirusidae [8].

Our failure to isolate causative virus in suckling mice and primary rat-kidney cultures may be explained by the fact that affected tissues obtained approximately 1 week after onset of the disease were used as inoculum. Jacoby et al. [4], in the salivary and lacrimal glands of rats with experimentally induced SDA, observed that titers of virus attained to the peak almost concurrently with the appearance of clinical signs and decreased rapidly within a few days. It is also known that coronaviruses are fastidious in their cultural requirement [2].

References

Explanation of Figures

Fig. 1. Larynx showing marked inflammatory degenerative changes in the mucous membrane. H.E. ×75.

Fig. 2. Submaxillary (under) and sublingual (upper) salivary glands showing edema and cellular infiltration in the periglandular and interstitial connective tissues. H.E. ×75.

Fig. 3. The submaxillary salivary gland showing degeneration and necrosis in the parenchyma. H.E. ×75.

Fig. 4. The interlobular excretory duct of the submaxillary salivary gland showing squamous metaplasia of the ductular epithelium. H.E. ×300.

Fig. 5. The Harderian gland showing parenchymal degeneration and marked inflammation in the interstitial connective tissue. H.E. ×75.

Fig. 6. Degeneration and necrosis can be seen in some ductular epithelial cells of the sublingual salivary gland. H.E. ×300.

Fig. 7. A portion of the cytoplasm of a ductular epithelial cell showing the double-membranous vacuoles and cisternae containing numerous viral particles in their lumina. ×30,000.

Figs. 8A and 8B. Portion of the cytoplasm of a ductular epithelial cell showing viral particles contained in cytoplasmic vacuoles. In 8A, the arrow designates a viral particles in the process of budding from the vacuolar membrane. Trilaminar limiting membrane can be seen in some of the viral particles, especially in those in Fig. 8B. ×100,000.