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RAT CORONAVIRUSES

Sialodacryoadenitis Virus and Rat Coronavirus: History, Virus Classification, Clinical Disease, Pathology, Diagnosis, Control, Interference with Research and Virus Status of Experimental Rats

By altering the way in which animals respond to treatment, viral infections can adversely affect the quality and validity of experimental data. Viruses cause biochemical changes in the cells they infect, which in many cases, lead to histopathologic alterations called cytopathic effects (CPE) (7). Clearly, virus-induced CPE in an organ would make it difficult to evaluate the effect of an experimental treatment on that organ. Other common virus-mediated effects such as immunosuppression and transformation further cloud the interpretation of experimental data. Consequently, it is imperative that investigators consider the viral profile of their research animals and be able to recognize changes and diseases caused by viral infections.

The type and severity of disease caused by a virus infection are partially determined by the tendency of that virus to infect certain tissues and not others (7). Viral tissue tropisms help explain why some agents replicate in the upper respiratory tract and cause rhinitis whereas others are found in other locations, such as neural tissue, and cause encephalitis. Obviously, the consequences of a viral encephalitis are more severe than are those of an upper respiratory infection.

Coronaviruses, which are enveloped RNA viruses that infect many avian and mammalian species, are found in the upper respiratory tract of humans with colds, the gastrointestinal tract of pigs with transmissible gastroenteritis, and the central nervous system of mice with encephalitis (1). Thus, these viruses infect and cause disease in many types of tissues and organs. Two antigenically-related coronaviruses, sialodacryoadenitis virus (SDAV) and rat coronavirus (RCV), have been isolated from rat tissues. These viruses have dissimilar, although not totally distinct, tissue tropisms and, therefore, cause distinguishable diseases in rats (12).

SDAV, as its name indicates, infects the lacrimal and salivary glands of rats as well as their upper and lower respiratory tracts causing a transient (one week or less), highly contagious, non-fatal disease. The clinical manifestations of SDAV infection may include cervical edema, enlarged salivary glands, squinting, porphyrin-stained red nasal and ocular discharges, and ocular changes which persist and progress in small percentages of rats (16, 23).

RCV, in contrast to SDAV, is primarily pneumotropic causing little or no sialodacryoadenitis (SDA). Commensurately, Bhatt and Jacoby (2) demonstrated minimal amounts of virus in the lacrimal and salivary glands of RCV-infected rats. The pathogenicity of RCV is age and strain dependent. However, infection of rats 7 days or older is usually subclinical.

Historical Background

Linking coronavirus infection to clinical and subclinical disease in rats resulted from a sequence of observations and experimental findings typical of those linking most infectious agents to disease. Innes and Stanton (11) described two outbreaks of SDA in weanling rats. Jonas et al (14) were able to reproduce this disease by inoculating germfree rats intranasally with an ultrafiltrate of submaxillary salivary gland homogenates from rats with SDA. These findings, in addition to observation of virus particles in the ducts of submaxillary salivary glands of diseased rats, suggested that SDA was virally induced. This was corroborated by Bhatt et al (4), who were able to isolate this virus in tissue culture and induce SDA in rats inoculated with this agent. Physiochemical and immunologic characterization of SDAV showed it to be a coronavirus (4). Parker and associates (19) isolated another coronavirus, RCV, from lungs of normal rats. As stated in the introduction, infection with RCV could be differentiated from that with SDAV in that the former did not cause sialodacryoadenitis in experimentally infected rats (2, 13).

In summary, the association of SDAV and RCV with clinical and subclinical disease in rats occurred in several steps including 1) detection of disease, 2) demonstration that the disease was infectious, 3) *in vitro* isolation and characterization of an infectious agent from diseased tissue, and 4) reproduction of the disease in susceptible rats inoculated with the agent.

Virus Classification

Viruses are classified on the basis of physiochemical, immunological, and epizootiological characteristics (7). Both SDAV and RCV have the physiochemical characteristics of coronaviruses (2, 15, 19). Inactivation by lipid solvents and replication in the presence of DNA inhibitors indicate that coronaviruses are enveloped and contain RNA. Coronaviruses are assembled in cytoplasmic vesicles in which they accumulate. The virions are spherical, ranging from 80 to 160 nm in diameter, and are covered with 20 nm long projections which give the appearance of a crown hence, the name "coronavirus" (1).

Although SDAV and RCV are identical physiochemically, Bhatt et al (4) demonstrated antigenic differences between these viruses in reciprocal virus neutralization tests. Monovalent RCV-immune rat sera and monovalent SDAV-immune mouse sera contained higher homologous than heterologous antibody titers. Both viruses have been shown to be antigenically related to mouse hepatitis virus (MHV) which is also a coronavirus, in complement fixation (CF) tests (4), and enzyme-linked immunosorbent assays (ELISA) (20). This cross reactivity explained why Hartley et al (9) were able to detect antibodies to MHV in rat sera.

In addition to antigenic differences, SDAV and RCV, as previously noted, can be differentiated on the basis of tissue tropisms *in vivo. In vitro,* however, both viruses have been shown to replicate in primary rat kidney cell cultures (PRKC) (4, 19). Virus replication can be detected by 12 hours post-inoculation (PI) with immunofluorescence and CPE is apparent by 23 hours PI (4).

There is evidence to suggest that there are different strains of SDAV. Bhatt and co-workers (4) reported that mouse-brain adapted SDAV caused no clinical signs and less SDA then the virulent SDAV 681. Preliminary work by Bhatt and Jonas (3) suggested that strains of SDAV could be differentiated from one another on the basis of antigenicity and infectivity. Kojima et al (15) and, more recently, Maru and Sato (17) isolated SDAV-like viruses from the salivary glands of rats with SDA, which could be differentiated antigenically from the 681 strain of SDAV (4) in reciprocal virus neutralization and CF tests using monovalent antisera. Maru and Sato (17) named their virus isolate the causative agent of rat sialoadenitis (CARS). CARS, in contrast to SDAV-681, caused CPE in mouse-derived 3T3 cells but not in PRKC. These data strongly support speculation that there are coronaviruses of rats which differ from the prototypic strains of SDAV and RCV with regard to the various characteristics used to classify viruses.

Clinical Disease

Sialodacryoadenitis Virus

SDA can be seen either as an enzootic or epizootic finding in rats. In epizootics of SDA when non-immune rats are exposed to the virus, the clinical signs are usually obvious and morbidity is usually high (30-100 percent affected). The infection is self-limiting and generally no deaths are seen. Signs of infection may be present as early as five days post-exposure to SDAV (13). Clinical signs include nasal and ocular porphyrin-stained discharge, rubbing of the eyes and nose with forepaws, sneezing, rales, and swelling of the ventral cervical area. Signs of upper respiratory disease are usually present within 5-7 days of infection. In many outbreaks, the most prominent clinical change is cervical swelling which is usually seen within 7 days post-infection. Porphyrin-stained lacrimal secretions ("red tears") may also be present at this time or within a day or two of cervical swelling. In general, food consumption and water intake is significantly decreased during clinical disease (21, 22). A decrease in breeding efficiency has also been reported during epizootics of SDAV (21, 22). Occasionally, in epizootics of SDAV, only a few rats will have clinical disease (6).

When SDA virus is enzootic in rat colonies, few or no clinical signs may be observed (17). In some colonies, keratoconjunctivitis may be present, presumably due to decreased tear production from inflamed lacrimal glands (12, 16, 23). In this situation, some rats may develop acute corneal disease, including photophobia and corneal opacities. In a small percentage of rats, ocular disease may progress to panophthalmitis with megaloglobus.

There is clinical evidence that the tissues affected and the severity of the disease may be dependent upon the strain and age of the rats and possibly the strain of the virus. Therefore, clinical signs may vary among individual rats in the same outbreak. SDAV infection is found in many rat colonies worldwide and is highly contagious. Rats recover rapidly from the disease and there is no evidence of latent infection.

Rat Coronavirus

Asymptomatic infections occur in weanling and older animals infected with RCV. If rats are experimentally inoculated between 7 and 21 days of age, signs of respiratory disease often occur. Mortality may be seen in rats under 7 days of age that have been inoculated with RCV. The strain of rat has been reported to influence the mortality rate at this age (12).

Pathology and Pathogenesis

Sialodacryoadenitis Virus

There are numerous detailed accounts of lesions caused by SDAV (5, 6, 10, 11, 12, 14, 18). Only a brief description of the salient lesions will be given here.

The gross lesions present in SDAV infections involve primarily the salivary glands, lacrimal glands, and eyes. Among the most frequently observed external abnormalities are porphyrin-stained nasal and ocular discharges and swelling of the ventral cervical area (Figure 1). In early stages of the disease (7-10 days post-infection), the salivary glands are often swollen, gray/white, and edematous. Frequently, there is also edema of the surrounding tissues in the ventral cervical area (Figure 2). Harderian glands at this stage of the disease may contain numerous yellow/gray foci either unilaterally or bilaterally. Later in the disease there may be unilateral or bilateral corneal opacities which may progress to enlargement of the entire globe.

Those organs which have histological changes in SDAV infection include the extraorbital lacrimal glands, intraorbital lacrimal glands, Harderian glands, submaxillary and parotid salivary glands, cervical lymph nodes, thymus, upper respiratory tract, and, occasionally, the lung. In the lacrimal glands, salivary glands, and Harderian glands, the earliest changes seen are swelling and necrosis of the epithelial cells lining the ducts (Figure 3). There is edema of the interstitium of these glands and a rapid accumulation of inflammatory cells including neutrophils, macrophages, and lymphocytes (Figure 4). Lesions progress to include acinar necrosis of the lacrimal and salivary glands and necrosis of the tuboalveolar cells of the Harderian gland. As the inflammation subsides, there is squamous metaplasia of the ducts and tuboalveolar structures involved (Figure 5). Squamous metaplasia is usually most severe in the Hardefian gland and, in most cases, is not present more than 30 days post-infection in any of the affected glands. The cervical lymph nodes of affected animals exhibit varying degrees of reactive hyperplasia and, occasionally, sinus histiocytosis. The earliest histological lesion seen in SDA infection is necrosis and suppurative inflammation of the nasal mucosa. A non-suppurative tracheitis also occurs in some animals. Lung changes are not found in weanling or adult rats, but interstitial pneumonia has been reported to occur in some suckling rats. Focal necrosis of the thymus is also a frequently reported lesion of SDA virus, but is not seen in all outbreaks.

There are several reports of keratoconjunctivitis in rats which have been infected with SDAV (10, 12, 16, 23). Initial lesions are primarily in the cornea and conjunctiva and are characterized by edema and infiltrates of neutrophils. In most rats these lesions resolve in a few weeks. A small percentage of rats have progressive corneal lesions including diffuse keratitis and corneal ulcers (Figure 6). In some of these rats, the lesions progress to panophthalmitis with megaloglobus.

The pathogenesis of SDAV has been studied in gnotobiotic SD© rats by Jacoby, Bhatt, and Jonas (13). Their studies indicate that the virus replicates first in the nasopharynx. Subsequently, viral replication is found in the trachea, bronchi, parotid and submaxillary salivary glands, lacrimal glands, and Harderian glands. It has not been determined how the virus spreads from the nasopharynx and trachea to the salivary glands. It is of interest that in most natural and experimental infections with SDAV, there is considerable variation in the severity and location of lesions. In some rats, all of the above-mentioned lesions can be found at various stages of the disease, while in other rats in the same group, only one or two organs may be affected. It is also common to find unilateral involvement of the salivary or lacrimal glands in individual rats in an outbreak. The sublingual salivary gland has never been reported to be affected by SDAV.

Rat Coronavirus

The lesions present after infection with RCV are minimal compared to those seen with SDAV. The only gross pathological changes usually observed are multiple small red/gray foci in the lung. Histologically, these consist of areas of interstitial pneumonia, which are usually mild and subside rapidly (7-14 days). The other primary histological change in RCV virus infection is rhinotracheitis. Acute rhinitis may occur within two days of infection with RCV (2). Interstitial pneumonia and mild non-suppurative tracheitis have been reported five days after infection. In our experience, and in the study by Bhatt and Jacoby on the pathogenesis of RCV (2), salivary gland lesions similar to those seen in SDA were found in some rats.

The pathogenesis of RCV is similar to that of SDAV (2). Viral replication occurs primarily in the nasopharynx and, subsequently, in the trachea. The virus then spreads to the lung by six days post-infection.

Diagnosis

Diagnosis of virus infection can be made on the basis of 1) clinical signs, 2) gross and histopathological lesions, 3) virus detection, and 4) demonstrating an antiviral immune response. Of these methods, 1, 2, and 3 are particularly useful for diagnosing the acute phase of virus infection. In convalescent animals however, demonstrating specific antibodies by serological methods is frequently the only means by which the incidence of virus infection can be determined.

The clinical signs and pathologic changes which occur during the first and second weeks after exposure to SDAV (i.e., the acute phase of infection) are central to the diagnosis of SDA. Investigators not familiar with SDA have made diagnoses such as mumps and tracheal and thyroid tumors. In contrast, RCV infection, as previously noted, is predominantly a subclinical infection which causes mild pathologic changes in the upper and lower respiratory tract.

During the first week after virus exposure, SDAV can be demonstrated in affected tissues with fluorescein-labeled virus-specific antibodies (2, 12, 13) and by electron microscopy (14). Moreover, virus may be isolated from tissue homogenates inoculated onto PRKC or intracranially into suckling mice (4, 19). Samples collected for virus isolation should be stored at or below -60°C because at -20°C virus infectivity is rapidly lost (4).

The presence in rats of serum antibodies to SDAV and RCV strongly suggests prior infection with at least one of these viruses. In rats experimentally infected with SDAV or RCV, serum antibodies are detected by CF and virus neutralization (VN) tests as early as one week post-virus exposure, reaching peak titers at four or six weeks after exposure and persisting at detectable levels for up to six months. (12). SDAV/RCV antibodies can also be detected by ELISA assays with MHV antigen (20). It is difficult to differentiate between SDAV and RCV infections on the basis of serological assays although serum antibody titers against the infecting virus are reportedly higher than those against the heterologous virus (4).

In diagnosing SDAV infection, other agents which have to be considered include Sendai virus, murine respiratory mycoplasmosis, bacterial infections, and changes due to excessive environmental ammonia exposure. Characteristic changes in the salivary and lacrimal glands are not seen in any of these conditions and are reasonably pathognomonic for SDAV infection. Additionally, cytomegalovirus infection must also be differentiated from SDAV virus infection. Cytomegalovirus infections are asymptomatic and are histologically characterized by intranuclear inclusions in enlarged salivary duct epithelial cells (12). It is important to note that the detection of porphyrin-stained tears around the eyes or nose, while usually part of the SDA clinical picture, is not pathognomonic for SDAV. Other factors, such as stress from crowding in shipment, food or water deprivation, or major environmental changes, can produce similar clinical signs (8).

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Figure 1.Porphyrin-stained ocular discharge and swelling in the ventral cervical areas. (Figures 1 through 5 are from CD rats experimentally infected with SDAV.)

Figure 2. Ventral cervical area with skin reflected. There is extensive edema of the salivary glands and adjacent tissue.

Figure 3. Necrosis and inflammation of a duct within a lobule of the parotid salivary gland. Numerous neutrophils are in the lumen and between degenerating epithelial cells.

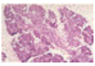

Figure 4. Parotid salivary gland with intralobular and interlobular edema and inflammation. Numerous inflamed ducts are in the center of the lobule.

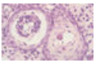

Figure 5. Harderian gland. Squamous metaplasia of tuboalveolar structures. Necrotic debris and inflammatory cells are also present in the lumen.

Figure 6. Keratitis and a central corneal ulcer in a rat from a colony with enzootic SDAV.

Controlling the Spread of Infection

The two principal approaches for controlling the spread of a virus infection are preventing exposure of susceptible non-immune animals to the virus and/or reducing their susceptibility by passive or active immunization. To prevent exposure of rats to SDAV/ RCV, as well as other viruses, they should be transported between facilities in filtered crates. When these crates arrive at their destination they should be wiped with a disinfectant before being opened in a clean area such as a laminar flow hood or barrier room. Rats can be maintained free of SDAV/RCV in barrier rooms, in filter top cages, or in conventional rooms in facilities where the disease is not present.

It is also possible to keep susceptible rats free of coronavirus infections in colonies that contain rats previously exposed to these viruses because neither SDAV nor RCV persist in the tissues of infected rats once virus-specific immunity develops during the second week after virus exposure. It has been reported that by five weeks after the onset of clinical signs all rats in a colony should be immune to SDAV/RCV and not be shedding virus provided that during this time no litters are born and no susceptible rats are introduced into the colony to perpetuate the infection. To ensure that they are no longer a source of infectious virus, infected rats should be quarantined without breeding for six to eight weeks after the onset of clinical signs before being mixed with susceptible rats.

SDAV and RCV do not remain infectious at 37°C for more than three hours, after being heated to 56°C for 30 minutes, nor when exposed to detergents or lipid solvents. The lability of SDAV and RCV guarantee that these viruses will not remain infectious in the environment for long periods of time nor on equipment that is washed with detergent and/or autoclaved.

Active immunization against SDA can be accomplished by natural exposure to SDAV. The disadvantage of this approach is that a percentage of infected rats will develop the chronic eye lesions described earlier. Unfortunately, no effective vaccine that would actively immunize rats without inducing SDA and ophthalmic lesions has yet been reported.

Interference with Research

SDAV and RCV may not have a significant impact on all research protocols and should be evaluated in terms of specific research by each investigator. The upper respiratory lesions in the acute stages of SDAV and RCV infections predispose animals to other. respiratory pathogens (12, 12, 19). This can be a potential problem in inhalation studies. Studies involving the morphology of the lung may be compromised by the short and long-term effects of both of these viruses. The secondary eye lesions of SDAV infection can interfere with toxicological studies where the eye is a target organ (11, 16) and behavioral research where visual cues are important. Rats may also be irritable during acute outbreaks which may affect numerous behavioral experiments. Feed consumption and water intake is decreased during clinical disease which may have a significant impact upon toxicological studies, particularly those in which the compound is given via the feed. There is a report of decreased breeding efficiency during outbreaks of SDA virus infection (22) which may impact reproductive research and teratology studies. Also, studies involving infectious diseases and immune responses may be affected by these viruses.

Virus Status of Experimental Rats

As stated in the previous section, SDAV and RCV infections cause pathological changes which may adversely affect various types of research. This can be avoided by using barrier-raised rats which have not been exposed to these and other viral pathogens as shown by the absence of virus-specific antibodies. Virus antibody free (VAF™) rats are routinely available from Charles River

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VAF™ rats housed in facilities in which exposure to SDAV cannot be prevented will usually experience epizootics of SDA. Outbreaks of SDA in these facilities can be reduced by requesting that the supplier send immune rats with significant antibody titers to SDAV. Alternatively, if immune rats are not available, susceptible rats may be brought into an SDAV-contaminated facility and used after they have recovered from SDA. Virus exposure and recovery from infection by rats in a colony should occur rapidly since SDAV infections are highly contagious, transient, and usually non-fatal.

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