Analysis of Cytomegalovirus Infection and Replication in Acinar Epithelial Cells of the Rat Lacrimal Gland

Zhiyan Huang, Ross W. Lambert, L. Alexandra Wickham, and David A. Sullivan

Purpose. The objectives of this investigation were threefold: to advance understanding of the nature and impact of herpesvirus infection in the lacrimal gland; to determine the influence of gender and sex hormones on viral infectivity and replication capacity in lacrimal tissue; and to compare the susceptibilities of lacrimal, submandibular, and parotid cells to viral invasion.

Methods. Acinar epithelial cells were isolated from lacrimal or salivary glands from intact, orchiectomized, ovariectomized, or sham-operated rats, cultured on Matrigel in serum-free media, and briefly exposed to rat cytomegalovirus (RCMV). Cells were then incubated for varying time intervals, and RCMV titers and secretory component (SC) levels in media or cell extracts were measured by plaque assay or radioimmunoassay. Exocrine glands also were obtained from rats after RCMV inoculation in vivo and were analyzed for viral infection.

Results. These findings demonstrated that RCMV invades the rat lacrimal gland after intravenous or intraperitoneal viral inoculation; RCMV infects and undergoes a time-, dose-, strain- and gender-dependent replication in acinar epithelial cells from rat lacrimal tissue; the magnitude of RCMV replication in acinar epithelial cells in vitro may be altered by prior changes in the endocrine environment in vivo; viral challenge to acinar epithelial cells does not necessarily impair their functional ability or viability and may, in fact, induce an acute increase in the cellular production of SC; and the extent of viral replication in lacrimal and salivary gland epithelial cells displays distinct, tissue-specific variations.

Conclusions. The herpesvirus RCMV invades and replicates in acinar epithelial cells from the rat lacrimal gland. The magnitude of this viral infection may be significantly influenced by gender and alterations in the hormonal environment. Invest Ophthalmol Vis Sci. 1996;37:1174-1186.

One of the most frequent causes of keratoconjunctivitis sicca and xerostomia throughout the world is Sjögren's syndrome.1-5 This disorder is an extremely complex autoimmune disease that predominantly affects females and is associated with a marked lymphocytic accumulation in lacrimal and salivary glands, an immune-mediated dysfunction and/or destruction of acinar and ductal epithelial cells, and the consequent development of dry eyes and mouth.1-4 The precise etiology of Sjögren's syndrome is unknown, but it may well result from the interplay of several factors, including those of genetic, viral, endocrine, neural, and environmental origin.1-7

Recently, particular attention has been focused on the role of viruses, as well as sex hormones, in the pathogenesis and/or expression of this autoimmune disorder.4,7-10 Thus, a possible cause of Sjögren's syndrome may be a primary infection by, and/or reactivation of, Epstein–Barr virus, cytomegalovirus (CMV), or herpesvirus-6,7,8,11-17 These herpesviruses have been identified in epithelial cells or lymphocytes of lacrimal and/or salivary glands of patients with Sjögren's syndrome7,8,11-17 and may elicit the T- and B-cell activation, lymphocyte activation, and resultant inflammation apparent in affected exocrine tissues.1-5 However, whether invasion by these viruses, or even retroviruses16 or hepatitis C virus,19 serves to induce, perpetuate, or exacerbate Sjögren's syndrome remains to be

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Reprint requests: David A. Sullivan, Schepens Eye Research Institute, 20 Stanniford Street, Boston, MA 02114.
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It is also unclear whether variations in the magnitude of lacrimal and salivary gland pathology during Sjögren’s syndrome may represent differences between these tissues’ susceptibility to viral infection.

An additional consideration in the etiology of Sjögren’s syndrome is the striking prevalence among females in the incidence of disease. Autoimmune disorders often display a sexual dichotomy, with estrogens increasing disease severity in females and androgens decreasing autoimmune expression in males. Indeed, estrogen, as well as prolactin, action may be involved in the pathogenesis, acceleration, and/or amplification of Sjögren’s syndrome. In contrast, androgens may provide a protective effect: Androgen treatment of animal models or humans with this autoimmune disorder may lead to a dramatic reduction in the extent of immunopathologic lesions in both lacrimal and salivary glands and/or an apparent suppression of ocular signs and symptoms. Of interest, endocrine activity might exert a significant influence on viral infection, given that sex steroids may regulate the cellular invasion, transcription, and/or replication of various herpesviruses. However, whether these hormones modulate viral infection, latency, or reactivation in lacrimal or salivary tissues during Sjögren’s syndrome has yet to be elucidated. In fact, almost no information appears to exist regarding the invasive potential, replication ability, and functional impact of herpesviruses in lacrimal gland epithelial cells.

Nevertheless, it is possible that viral infection in hormonally predisposed individuals may promote the onset and/or the development of lacrimal or salivary gland autoimmune disease in Sjögren’s syndrome. Therefore, to begin to clarify this viral–endocrine interrelationship, the objectives of the current investigation were threefold: first, to advance our understanding of the nature and impact of herpesvirus infection in the lacrimal gland; second, to determine the influence of gender and sex hormones on viral infectivity and replication capacity in lacrimal tissue; and third, to compare the susceptibility of lacrimal, submandibular, and parotid cells to viral invasion. To conduct these studies, we used rat cytomegalovirus (RCMV), which is an epitheliotropic and heat-labile DNA herpesvirus. Previous research has shown that RCMV invades the submandibular gland and induces a distinct, periductular lymphocytic infiltration, as well as a marked parenchymal inflammation. These immunologic sequelae are analogous to those observed in lacrimal and salivary glands of patients with Sjögren’s syndrome.

MATERIALS AND METHODS

Animal and Surgical Procedures

Male and female Sprague–Dawley rats (6 weeks old) were purchased from Zivic–Miller Laboratories (Allison Park, PA) and maintained in temperature-controlled rooms with light–dark intervals of 12 hours duration. When indicated, orchietomies, ovariectomies, or sham castrations were performed by surgeons at Zivic–Miller Laboratories on 6-week-old animals. Rats were allowed to recover for a minimum of 10 days after surgery before experimental use. All studies with experimental animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

General Procedures

At the time of sacrifice, tears were collected from both eyes, saliva and stimulated tears were obtained after the administration of pilocarpine, and blood was aspirated from the heart with a heparin-containing syringe, as previously described. Exorbital lacrimal, submandibular, and parotid glands, as well as globes, superior cervical lymph nodes, and trigeminal ganglia, were removed and processed for acinar epithelial cell isolation or viral titration protocols. In the latter instance, tissue vasculature was first perfused in situ with Hanks’ balanced salt solution (HBSS; Gibco, Grand Island, NY) to clear residual blood. Tissues were stored at −80°C until experimental use, whereas tears, saliva, whole blood, and plasma samples were analyzed for viral content on the day of collection. For acinar cell or tissue disruption, ice–alcohol-cooled samples were sonicated (50 W) for 20 seconds with a Branson Sonifier 250 (VWR Scientific, Boston, MA). This procedure did not interfere with RCMV recovery.

Rat Cytomegalovirus Strains and Plaque Assay Procedures

For the preparation of RCMV stocks for acinar cell infection, the English and Dutch strains of RCMV were generously provided by Drs. Gordon Sandford (Johns Hopkins University, Baltimore, MD) and Catharina A. Bruggeman (University of Limburg, Maastricht, The Netherlands), respectively. The English and Dutch viruses were maintained, propagated, and amplified in monolayer cultures of rat embryonic fibroblasts (REF, from Dr. Sandford) or Rat 2 fibroblasts (gift from Dr. Anthony Hayward, University of Colorado School of Medicine, Denver, CO), respectively, by following modifications of reported protocols. For use in certain studies in vivo, the English strain of RCMV was partially purified from the media of infected REF cultures by sequential centrifugations at 2000g for 10 minutes at 4°C (supernatant saved) and 45,864g for 3 hours at 4°C. The resultant pellet, which harbored infectious virus and contained more than 90% of the starting viral material, was resuspended in HBSS and stored at −80°C. For control purposes, media from uninfected REF and Rat 2 cells also were collected according to previously described
procedures. When indicated, RCMV was heat inactivated by incubating the virus at 56°C for 60 minutes.

To determine viral content in culture media and acinar cell and tissue sonicates, RCMV titers were measured accurately by plaque assay. In brief, this assay involved incubation of REF cells in 24 well plates (Nuncclone, Roskilde, Denmark) containing Earle’s minimum essential medium (Gibco) with 10% calf serum (Sigma Chemical, St. Louis, MO), streptomycin (100 μg/ml), penicillin (100 U/ml), and amphotericin B (0.25 μg/ml), until cell growth to confluence. After this period, culture media were aspirated, REF cells were exposed to one of a series of sample dilutions (3 wells/dilution) for 60 minutes at 37°C, and then monolayers were overlaid with 0.6% agarose (Gibco) dissolved in modified culture media (i.e., 5% calf serum with 0.22% NaHCO₃); a second agarose overlay was added on day 5. After a 10-day interval, REF monolayers were fixed in 10% neutral buffered-formalin (Sigma) overnight, stained with 1% methyl-gluces, and plaques were counted. Analysis of this plaque assay demonstrated that a linear relationship existed between viral dilution and plaque number.

Acinar Epithelial Cell Isolation, Culture, and Viral Infection Procedures

The isolation and culture of acinar epithelial cells from rat lacrimal, submandibular, and parotid glands were conducted by using previously described methods. Briefly, exocrine glands were rinsed in DMEM (with L-glutamine, 1000 mg D-glucose/L, 100 mg sodium pyruvate/L; Gibco) containing soybean trypsin inhibitor (0.1 mg STI/ml; Worthington Biomedical, Freehold, NJ), minced and washed with HBSS (without Ca²⁺ or Mg²⁺; Gibco). Both DMEM- and HBSS-based media contained gentamycin (25 μg/ml; Sigma Chemical, St. Louis, MO). Tissue fragments were then disrupted through a series of oscillating, 37°C incubations in EDTA (0.76 mg/ml; Gibco) or collagenase (200 U/ml; Calbiochem-Behring, La Jolla, CA), hyaluronidase (698 U/ml; Calbiochem-Behring), and DNase I (10 U/ml; Boehringer Mannheim, Indianapolis, IN) in DMEM- or HBSS-containing buffers. These incubations, which were performed in an atmosphere of 95% O₂ and 5% CO₂ for 17- to 25-minute periods, were interspersed with several wash procedures. The glandular digest was filtered consecutively through 500-μm and 25-μm Nitex mesh (Tetko, Briercliff, NY) and centrifuged at 50g for 10 minutes. The cell pellet was resuspended in DMEM and plated at an average density of 2 x 10⁶ cells/well on Matrigel (Collaborative Research, Bedford, MA) in 35-mm Primaria culture dishes (Falcon, Oxnard, CA). Acinar cell viability was evaluated by trypan blue exclusion, and cell numbers were counted with a hemocytometer.

After overnight culture at 37°C in a humidified incubator containing 95% air—5% CO₂, unattached acinar epithelial cells were removed and enumerated and attached cells (n = 5 wells/group) were challenged with varying amounts of RCMV or control REF cell antigens. After a 60-minute viral adsorption at 37°C, the inoculum was aspirated, and acinar cells were rinsed gently to remove remaining virus and were cultured in serum-free modified Oliver’s medium (SFMOM; 50% DMEM and 50% Ham’s nutrient mixture F-12 with L-glutamine, supplemented with 25 μg gentamycin/ml, 5 μg insulin/ml, 5 μg transferrin/ml, 5 ng selenous acid/ml, 10 ng dexamethasone/ml, 50 ng epidermal growth factor/ml, 100 ng fibroblast growth factor/ml, 1 mM putrescine, 25 μg L-ascorbic acid/ml and 10 μg reduced glutathione/ml) in the presence of vehicle or 10⁻⁶ M dihydrotestosterone (DHT) for for 4, 8, or 12 days. All SFMOM additives, with the exception of fibroblast growth factor (Collaborative Research), were purchased from Sigma Chemical. Culture media were replaced every 4 days, collected media samples were measured volumetrically and centrifuged at 10,000g for 4 minutes, and supernatants were stored at —80°C. At experimental termination, attached cells were harvested from Matrigel by treatment with trypsin (1 mg/ml HBSS)—EDTA (0.4 mg/ml HBSS) (Gibco) and Dispase (50 U/ml; Collaborative Research) solutions for 60 minutes at 37°C. Recovered cells were then counted, tested for viability, washed, and resuspended in a bovine serum albumin (Calbiochem—Behring, La Jolla, CA)-containing buffer (1 mg bovine serum albumin per 1 milliliter of 0.1 M phosphate-buffered saline, pH 7) and stored at —80°C until sonications. In studies using DHT, this steroid was dissolved in ethanol, and aliquots then were evaporated in sterilized vials before the addition of SFMOM. Steroid control media, in turn, were prepared by transferring SFMOM into vials containing the residue of evaporated ethanol.

Radioimmunoassay and Statistical Procedures

The levels of secretory component (SC) in cell culture media were measured with a double precipitation radioimmunoassay, according to reported methods. Reagents included purified rat free SC (from Dr. Brian Underdown, McMaster University, Hamilton, Ontario, and Dr. Jean-Pierre Vaerman, Universite Catholique de Louvain, Brussels, Belgium), rabbit anti-rat SC antiseraum (provided by Dr. Charles R. Wira, Dartmouth Medical School, Hanover, NH and prepared by Dr. Underdown), and goat anti-rabbit immunoglobulin G antiserum (Miles Laboratories, Elkhart, Indiana).
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IN). Rat SC was used in the radioimmunoassay as an unlabeled standard and as a radiolabeled tracer. Iodinations were performed by using IODO-GEN (Pierce Chemical, Rockford, IL) and 125NaI (Amersham, Arlington Heights, IL), as previously described. After iodination, radiolabeled SC was separated from free 125NaI by molecular sieve chromatography on columns containing Bio-Gel P6DG (Bio-Rad, Richmond, CA). Statistical analysis of data between two groups was conducted with the unpaired, two-tailed Student's t-test. Statistical comparisons between the means of multiple groups were performed by analysis of variance and Fisher's PLSD with a significance level of 95%.

RESULTS
Evaluation of the Rat Lacrimal Gland as a Target Tissue for Rat Cytomegalovirus Infection

Previous research has suggested that herpesviruses (e.g. CMV, Epstein–Barr virus) may play a critical role in the development of the immunopathology and dysfunction of lacrimal tissues in patients with Sjögren's syndrome. However, almost no information exists concerning the ability of herpesviruses to invade the lacrimal gland effectively and to replicate therein. Therefore, to address this issue, studies were designed to evaluate the rat lacrimal gland as a target tissue for RCMV infection in vivo and to assess the infectious and replicative potential of RCMV in acinar epithelial cells from rat lacrimal tissue in vitro. This focus on epithelial cells was prompted by the recognition that these cells are a primary site for RCMV invasion in other organs in vivo.

To examine whether the rat lacrimal gland and adjacent structures are susceptible to RCMV infection in vivo, the following experiment was conducted. Six-week-old male rats (n = 5 to 6 per treatment group) were challenged with 10^4 plaque-forming units (PFU) of RCMV (English strain, semi-purified) in HBSS by application to the ocular surface, by inoculation into the ocular surface (5 μl aliquot of 10^6 PFU RCMV/ml onto each eye after removal of residual tears); by inoculation into the intraperitoneal cavity (100 μl aliquot of 10^5 PFU RCMV/ml onto each eye after removal of residual tears); by inoculation into the femoral vein (100 μl aliquot of 10^5 PFU RCMV/ml). This dosage of RCMV had been reported to induce salivary gland infection within 1 week of intraperitoneal administration. Uninfected control rats were used to establish the absence of pre-treatment levels of RCMV. After 1, 2, and 3 weeks of viral exposure, the following samples were obtained and processed to be analyzed by plaque assay for RCMV content: lacrimal and submandibular (positive control) glands, superior cervical lymphatic tissues, trigeminal ganglia, and tears, saliva, whole blood, and plasma. Before collection, all tissues were perfused intravascularly with HBSS to remove residual blood-associated virus. Results demonstrated that RCMV infective capacity depended on the route of application and that the time course of, and susceptibility to, RCMV infection varied according to the tissue site (Fig. 1). Thus, 80% to 83% of all lacrimal tissues were infected with RCMV by 21 days after intravenous or intraperitoneal administration. In contrast, few lacrimal glands (i.e., 20% of intravenous group) were in-

![Graph](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933418/ on 04/02/2017)
fected after 14 days of viral exposure, and none were RCMV positive after 7 days. Of particular interest was the finding that RCMV treatment to the ocular surface did not induce viral infection in any tested site. With regard to other tissues, RCMV inoculation by the intravenous or intraperitoneal route led to rapid infection of the submandibular gland (e.g., 20% to 40% positive tissues by day 7), and all submandibular tissues were positive by days 14 and 21. However, RCMV could not be detected in tears, saliva, plasma, or whole blood, or in pilocarpine-induced tears (days 14 and 21 after infection), or in day 21 samples (n = 3 per group) of globes, superior cervical lymphatic nodes, or trigeminal ganglia.

The ability of RCMV to infect the lacrimal gland after systemic inoculation also appeared to depend on the source of virus. Thus, RCMV prepared from a media stock generated after one or more REF cell passages in vitro was almost entirely ineffective: Intraperitoneal injection of this latter virus (10⁵ PFU) into young adult male and female Sprague-Dawley rats led to lacrimal gland infection in only 4% (i.e., 1 of 24) of the animal population on day 21. In contrast, RCMV was detected in all submandibular glands in this experiment. Similarly, the extent of RCMV infection in lacrimal tissues of 4- and 6-week-old female rats (n = 3 per age group) was far less than that detected in submandibular glands after the intraperitoneal administration of RCMV (10⁴ PFU) prepared directly from virus-infected submandibular tissues.

To determine whether RCMV invades and replicates in acinar epithelial cells from the rat lacrimal gland in vitro, and to test whether viral infection is time, dose, and strain dependent, a series of studies was performed. To evaluate the kinetics of viral infection, acinar cells from male rat lacrimal glands were isolated, briefly exposed to RCMV (10⁴ PFU of English strain), heat-inactivated RCMV or REF cell control antigens for 1 hour, and then cultured for 12 days. As one additional control, wells (n = 5) containing Matrigel but without acinar cells, also were inoculated with RCMV and processed as described above. Media were changed every 4 days, and RCMV titers in all media and cell samples were analyzed by plaque assay. As shown in Figure 2, RCMV invaded rat lacrimal gland acinar cells and replicated in a distinct, time-dependent manner. By 4 days after RCMV challenge, viral titers in culture media and cells were almost undetectable, with viral content typically measuring less than 1 × 10⁵ PFU/well. However, within 8 days of RCMV application, viral yields increased significantly so that RCMV amounts per well often averaged more than 30-fold greater than those in the original inoculum. This RCMV replicative process continued to proceed through day 12 of the experimental time course, at which time titers frequently rose to more than 1 × 10⁶ PFU/dish. The distribution of these RCMV progeny was primarily cell associated, and no RCMV could be recovered from cultures treated with heat-inactivated RCMV or REF cell antigen or from wells without acinar cells. Of interest, RCMV infection also appeared to induce an alteration in acinar cell morphology, beginning in certain cells approximately 5 to 7
days after viral exposure: Affected cells seemed to enlarge, assume a more spherical form, lose cellular processes, and express atypical granular vacuoles. Despite these shape changes, RCMV infection had no consistent effect on acinar cell viability (data not shown).

To examine the influence of varying degrees of RCMV exposure on viral replication in acinar epithelial cells, cells from male rat lacrimal glands were inoculated with RCMV (10^1 to 10^4 PFU of English strain) or control antigen, then cultured for 12 days. Media were replaced every 4 days, and RCMV titers in all media and cell (day 12) samples were measured. As demonstrated in Figure 3, the extent of RCMV invasion and replication was dependent on the initial dosage of virus: In general, the greater the dosage, the higher the total recovery, at least within the RCMV concentration range tested. Challenge titers of at least 100 PFU of RCMV were required to induce a detectable infection. However, it is noteworthy that treatment of acinar cells with only 100 PFU resulted in at least a 1000-fold greater titer of RCMV/well after 12 days of cell culture (Fig. 3).

To explore whether the magnitude of RCMV infection in acinar epithelial cells is virus strain dependent, acinar epithelial cells were exposed to 10^4 PFU of either the English or Dutch strains of RCMV and then cultured for 8 days. Plaque assay analysis of resultant media and cell extracts showed that infection with the English, but not the Dutch, strain of RCMV resulted in the generation of high titers of infectious progeny (Fig. 4).

**Impact of Acute Rat Cytomegalovirus Exposure on the Functional Activity of Acinar Epithelial Cells In Vitro**

Theoretically, acute viral infection may alter significantly the functional capability of lacrimal gland acinar epithelial cells. To evaluate this possibility, the following experiments were conducted to determine whether RCMV infection interferes with the acinar cell response to DHT. The functional parameter tested was the synthesis of SC, which is known to be stimulated by DHT. Acinar cells from male rat lacrimal glands were first exposed to 10^5 PFU RCMV or REF cell antigen, and then, depending on the experiment, cultured for 4 to 12 days in SFMOM containing vehicle or DHT (10^{-6} M). Culture media and recovered cells were processed for the measurement of SC and RCMV titers. As shown in Figure 5, acute RCMV infection did not impair the androgen ability to augment SC production. In fact, RCMV exposure significantly elevated the DHT-induced output of SC. The extent of this viral-associated increase equaled 16.8%, 24.3%, and 52.5% in three separate experiments. In contrast, RCMV challenge did not typically enhance basal SC synthesis. This regulatory effect of RCMV on SC production by DHT-exposed acinar cells was observed on day 4 of culture but was not necessarily maintained beyond this time interval (data not shown).

Of particular interest, androgen treatment after viral infection had no consistent influence on the ex-
Influence of Gender and Castration on the Infectivity and Replication Capacity of Rat Cytomegalovirus in Acinar Cells of the Lacrimal Gland

Sjögren’s syndrome occurs almost exclusively in females, which supports the contention that gender, or more specifically sex hormones, play a role in disease etiology and resultant lacrimal tissue dysfunction. However, herpesviruses also have been implicated in the pathogenesis of Sjögren’s syndrome and the associated aqueous tear deficiency. Whether these hormonal and viral actions are independent or possibly synergize to accelerate the development of Sjögren’s syndrome is unknown. Therefore, to begin to examine whether the endocrine environment modifies viral infectivity and replication capacity in the lacrimal gland, the following experiments were designed to assess the effect of gender and castration on RCMV invasion in lacrimal acinar cells.

Acinar epithelial cells were isolated from lacrimal glands of age-matched intact, sham-operated, or castrated male and female rats, plated on Matrigel, exposed to $10^4$ PFU RCMV (English strain) or REF control antigen for 1 hour, and then cultured for 8 days. Media were replaced on day 4, and RCMV titers in media and cell (day 8) samples were measured by plaque assay. Results demonstrated that RCMV invaded and replicated in lacrimal gland acinar cells from male and female rats, but the extent of this viral infection appeared to be influenced markedly by gender and prior changes in the hormonal microenvironment in vivo. In two of three experiments, acinar cells from lacrimal tissues of female rats were far more susceptible to RCMV invasion and/or replication than cells from glands of males (Fig. 6). Moreover, these gender differences seemed to be modified by castra-
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A

![Graph A]

B

![Graph B]

**FIGURE 6.** Influence of gender and castration on rat cytomegalovirus (RCMV) infection in lacrimal gland acinar epithelial cells. Acinar cells were obtained from the lacrimal glands of 53-day-old male and female rats (n = 3 to 6 per group per experiment) that had undergone sham surgery or castration. Cells were plated (2 × 10⁶ cells/well) on Matrigel, challenged with RCMV (10⁴ plaque-forming units (PFU) of English Strain; n = 10 wells/group) or rat embryonic fibroblast antigen (n = 5 wells/group), and cultured for 8 days; all media were replaced on day 4. Viral titers in media and cell sonicates were determined by plaque assay. Columns and vertical bars represent the mean ± SE. Results are shown for two separate experiments (A and B) and are presented in terms of total RCMV levels per culture plate (i.e., media RCMV + cell RCMV). By analysis of variance, the RCMV amounts in acinar cultures from sham-operated males were significantly (P < 0.05) less than those in cultures from sham-operated females. Significantly (P < 0.05) greater* or less than† the value of the sham-operated control. In day 4 media, viral content ranged between 0 to 111 PFU in RCMV-exposed cultures. In contrast, no RCMV was detected in rat embryonic fibroblast-treated cultures.

**DISCUSSION**

The current investigation demonstrates that RCMV invades the rat lacrimal gland after intravenous or intraperitoneal viral inoculation; that RCMV infects and undergoes a time-, dose-, strain- and gender-dependent replication in acinar epithelial cells from rat lacrimal tissue; that the magnitude of RCMV replication in acinar epithelial cells in vitro may be altered by prior changes in the endocrine environment in vivo; that viral challenge to acinar epithelial cells does not necessarily impair their functional ability or viability and may, in fact, induce an acute increase in the cellular production of SC; and that the extent of viral replication in lacrimal and salivary gland epithelial cells displays distinct, tissue-specific variations.
of anterior segment barriers (e.g., mucus, tight junctions), the apparent absence of retrograde transport of antigens through the lacrimal duct, the rapid clearance of RCMV from the ocular surface through the nasolacrimal passage to the intestinal tract, and/or possible viral inactivation by nonspecific, mucosal immune defense mechanisms. It is also of interest that the capacity of RCMV to invade the lacrimal gland after systemic inoculation was variable and possibly dependent on the source of virus. Thus, intraperitoneal injection of infected REF cell media into young adult rats led to a pronounced viral uptake into submandibular, but minimal RCMV accumulation in lacrimal, tissues. Furthermore, the administration of RCMV from salivary gland isolates resulted in a distinct viral infection in submandibular tissues but reduced RCMV titers in lacrimal glands. It may be that the limited lacrimal infectivity of RCMV after one or more passages in vitro may relate to viral attenuation, as has been described. In addition, it may be that RCMV prepared from salivary gland homogenates preferentially targets salivary tissues in vivo. Further research, particularly with RCMV obtained from infected lacrimal glands, may help to clarify the invasive capacity of this herpesvirus for lacrimal tissue in vivo.

Our results also showed that RCMV invades and replicates in lacrimal gland acinar epithelial cells in vitro. This finding suggests that the acinar cells may express surface receptors for CMV and may be infected independently of other cell types. In contrast, tracheal epithelial cells in mice lack functional CMV binding sites and appear to require cell-to-cell contact with fibroblasts for a productive epithelial infection in vivo. It should be noted that the capacity of RCMV to replicate in acinar epithelial cells may have been enhanced by the presence of dexamethasone in the culture media. Previous research has shown that glucocorticoids can cause an earlier appearance of CMV antigens and DNA, as well as an increase in the number of cells able to produce infectious progeny. This effect appears to relate to a hormone-induced rise in CMV-specific immediate early antigens and DNA, as well as an increase in the number of cells able to produce infectious progeny. However, whether dexamethasone played a stimulatory role in the current investigation is uncertain, given that the media concentrations of this hormone were approximately 4000-fold lower than those used in the human CMV studies. The ability of RCMV to invade and/or produce progeny in acinar epithelial cells was strain dependent. Thus, the English, but not the Dutch, strain caused a pronounced infection in these cells. It is unclear why this discrepancy exists, given that both strains are known to infect rat salivary glands in vivo and to cause distinctive cytopathic effects within REF cells in vitro. It may be that the Dutch strain
is more restricted than the English variant in terms of potential tissue targets (e.g., lacrimal gland). Alternatively, it is possible that these strain variations may be related to differences in their genomic structure.

A particularly striking observation was that the magnitude of viral production in acinar epithelial cells appeared to be influenced by gender, ovariectomy, or orchietomy. Thus, acinar cells from female lacrimal tissues were often far more vulnerable to RCMV infection than cells from male glands. In contrast, this gender-associated difference in viral susceptibility seemed to be lessened by castration, suggesting that androgens may possibly provide a protective effect against RCMV invasion and/or replication in the lacrimal gland. The possibility that the endocrine system may modify the extent of RCMV infection would not be surprising. Previous experiments have shown that distinct differences may exist between males and females, as well as between their cells in vitro, to viral infection and activity. In turn, these sexually dimorphic responses to viral exposure may be erased by castration and restored by the administration of sex steroid hormones. Indeed, these hormones are known to exert a profound effect on the expression, replication, or infectious potential of a wide array of viruses, including human CMV, hepatitis B virus, Coxsackievirus, human T-lymphotropic virus type 1, herpes simplex virus type II, adenovirus types 7 and 12, equine encephalomyelitis virus, mammary tumor virus, human papillomavirus, or vaccinia virus. The precise nature of the sex steroid action appears to depend on the specific hormone, virus, species, and cell type and may result in potentiation, suppression, synergism, or no effect on viral function. Moreover, the mechanism(s) underlying sex steroid–virus interactions may be direct or indirect. Thus, steroid action may be mediated through a hormone-receptor association with specific steroid response elements in the viral genome, thereby leading to an alteration in the rate of viral transcription. Alternatively, hormone effects may be elicited through changes in viral receptor expression, viral adsorption, cellular metabolic pathways, tissue barriers, or the immune system, which is uniquely sensitive to sex steroid regulation.

However, if androgen action is associated with a suppression of RCMV infection in vivo, how might this effect be reconciled with the apparent lack of DHT influence on RCMV replication in vitro? One explanation might be that if androgens do provide relative protection against RCMV infection, that this effect may be imparted to acinar epithelial cells before their isolation from the lacrimal glands of intact or sham-operated male rats. Thus, for example, if androgens act to reduce the number of cellular receptors for RCMV, thereby limiting subsequent invasion and eventual replication, this hormonal action would be induced in vivo and would possibly be maintained during the initial stages of cell culture. Consequently, the administration of DHT to acinar cells after viral challenge in vitro may have been either unnecessary (i.e., androgen effect already present) or too late. In support of this hypothesis are two observations. The first is our finding that acinar cells from orchietomized rats were significantly more susceptible to RCMV infection than those cells from sham-castrated male rats. The second is the knowledge that certain sex steroids delay the onset and/or markedly decrease the cellular replication of adenoviruses and herpesviruses in vitro, but that these effects are observed only if cells are exposed to hormones for 24 to 48 hours before, not after, viral inoculation. Nevertheless, definitive clarification of the role of androgens in regulating RCMV dynamics in vivo and in vitro awaits further experimentation.

In contrast to the effect of orchietomy, ovariectomy led to a diminished replicative potential for RCMV in lacrimal gland acinar epithelial cells compared to that found in cells from sham-operated females. This difference was found in two separate studies but not in a third experiment that used castrated and intact female rats. These inconsistent results may have been caused by cycle variations among control groups. Thus, depending on the stage of the estrous cycle, cellular susceptibility to herpesvirus infection may be increased significantly or reduced dramatically. Moreover, cycle-associated fluctuations in cellular responsiveness to viral challenge may be related to circulating sex steroid concentrations, given that estrogens may enhance and progestrone may inhibit human CMV replication. Therefore, to determine the impact of gender and castration on RCMV infection in the female lacrimal gland, future studies are required that control for serum hormone levels during the estrous cycle.

Despite the vulnerability of acinar epithelial cells to RCMV invasion, the resultant infection did not seem to impair cell function or viability significantly. In fact, viral infection often caused an acute rise in the production of SC. This finding was of particular interest in that SC controls in vivo the transport of immunoglobulin A antibodies to the ocular surface, whereupon immunoglobulin A protects against viral infection. The mechanism underlying this RCMV effect is unknown, but it may be virus specific given that sialodacryoadenitis virus infection does not induce this SC response in rat lacrimal gland acinar epithelial cells. It is tempting to speculate that the RCMV modulation of SC may relate in part to this virus' ability to stimulate the synthesis and secretion of tumor necrosis factor-α (TNF-α). This cytokine's mRNA has been identified in acinar epithelial cells of the rat lacrimal
gland,73 and TNF-α is known to augment the acinar cell output of SC.74 However, whether RCMV regulates TNF-α production in these lacrimal gland cells remains to be elucidated.

Lastly, our studies showed that RCMV invaded salivary gland acinar epithelial cells in vitro and that the extent of the ensuing RCMV replication was considerably greater in parotid than in submandibular or lacrimal acinar cells. This capacity of RCMV to infect acinar epithelial cells of salivary tissues is consistent with the findings of previous studies,20,75 which demonstrated that herpesviruses may successfully invade human submandibular and parotid acinar cells in vivo. However, an explanation for the differential RCMV replication within various cell types is not readily apparent. In the very least, this nonuniform infection may reflect a heterogeneity among exocrine acinar cell populations with regard to RCMV susceptibility.

Overall, our research has shown that RCMV invades the lacrimal gland and that the magnitude of viral replication in this tissue may be dependent on gender and sex steroids. These findings support the hypothesis that herpesvirus infection in hormonally predisposed individuals may promote the development of lacrimal gland autoimmune disease in Sjögren’s syndrome.

Key Words
androgen, cytomegalovirus, gender, lacrimal gland, secretory component

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