SIALODACRYOADENITIS VIRUS INFECTION OF RAT LACRIMAL GLAND ACINAR CELLS

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INTRODUCTION

The secretory immune system of the eye is designed to protect the ocular surface against microbial challenge and infectious disease.[1] This immunological role is mediated primarily through secretory IgA (sIgA) antibodies, which are produced by plasma cells in interstitial areas of the lacrimal gland and are selectively transported to tears by secretory component (SC), the polymeric IgA receptor.[1] After delivery to the eye's anterior surface, sIgA antibodies may act to prevent viral internalization, inhibit bacterial colonization, curtail parasitic infestation and attenuate toxin-related damage.[2]

However, despite the importance of this local immune protection, the mechanisms by which various antigens stimulate the secretory immune system of the eye have not been clarified. For example, active viral infection of the ocular, or even nasopharyngeal or enteric, surfaces, is known to elicit a striking tear IgA antibody response.[1] Yet, whether viruses actually gain access to the lacrimal gland, the principal effector tissue of ocular mucosal immunity,[1] and therein induce an IgA response, is not known. Indeed, it is quite possible that viruses first initiate an immune reaction in distant mucosae, resulting in the migration to lacrimal tissue of IgA-producing lymphocytes,[3] which then serve to provide ocular defense. Identification of the processes and specific sites involved in antigen-induced ocular immune responses is critical, in order to permit the development of optimal vaccination strategies for the effective immunization of the anterior segment.

Therefore, to begin to address this issue, we sought to determine first whether viruses may, in fact, invade, and replicate in, lacrimal tissue. Towards that end, we evaluated the

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capacity of sialodacryoadenitis virus (SDAV), an epitheliotropic, RNA coronavirus, to infect lacrimal acinar cells in vitro. Our rationale for the selection of this virus was two-fold: [a] SDAV is known to exert a profound impact on the lacrimal gland in vivo: when administered intranasally, SDAV causes a pronounced infiltration of plasma cells, lymphocytes and macrophages into lacrimal tissue, a distinct, non-suppurative periductular inflammation, extensive interstitial edema, degenerative, atrophic and/or necrotic alterations in acinar and ductal epithelia, reduced tear flow and keratoconjunctivitis sicca.[4-8] However, whether this viral action involves invasion and replication in lacrimal tissue is unknown; and [b] SDAV may serve as a model virus with which to examine antigen-immune interactions in the lacrimal gland, to explore the functional role of the ocular secretory immune system against viral challenge, and to develop vaccination strategies for immunization of the anterior surface of the eye. In this regard, it should be noted that sIgA antibodies appear to modulate coronaviral infections in other mucosal sites.[9] To complement these initial studies, we also: [a] assessed whether short term SDAV infection interferes with the viability and function of acinar cells in vitro; and [b] compared the infectivity of SDAV in acinar cells from submandibular and parotid glands, given that salivary tissues are highly susceptible to SDAV infection in vivo.[5-8]

MATERIALS AND METHODS

For the preparation of viral stocks, SDAV (L2-adapted strain; gift from Dr. Diane Gaertner, New Haven, CT, and originating from Dr. Dean Percy, Guelph, Ontario, Canada) was propagated in mouse L2 cells, according to reported procedures.[10] For the quantitation of SDAV levels in experimental samples, titers were accurately measured by a plaque assay, which involved the use of 4 day old L2 cell monolayers, application of defined overlay media containing Sea Plaque agarose (FMC Inc.), maintenance of inverted culture plates for 3 days at 37°C in a humidified atmosphere containing 5% CO_2, fixation of cells with 10% neutral-buffered formalin (Sigma Chemical Company) and staining with methylene blue.[11]

The isolation and culture of acinar cells from lacrimal, submandibular and parotid glands, as well as the exposure of acinar cells to SDAV, have been described in detail.[11-13] Briefly, tissues were obtained from pathogen-free, male Sprague-Dawley rats (6-7 weeks old, Zivic Miller Laboratories), rinsed in a soybean trypsin inhibitor (Worthington Biomedical) solution and disrupted through a series of incubations in EDTA (Gibco), or collagenase (Calbiochem-Behring), hyaluronidase (Calbiochem-Behring), and DNase I (Boehringer Mannheim) in DMEM- or HBSS (Gibco)-based buffers. The resulting digest was filtered consecutively through 500 μm and 25 μm Nitex meshes (Tetko Inc.), gently centrifuged and the cell pellet was resuspended in DMEM containing 20% heat-inactivated fetal calf serum (Hyclone). After centrifugation through a Ficoll 400 (Pharmacia) step gradient (2-4%), the final acinar cell pellet was resuspended in DMEM and plated at an average density of 1.5 to 2 x 10^6 cells/well on the reconstituted basement membrane, Matrigel (Collaborative Research) in 35 mm Primaria culture dishes (Falcon). Acinar cell viability, which was typically greater than 80%, was determined by trypan blue exclusion and cell numbers were enumerated with a hemocytometer. Following an overnight incubation at 37°C in a humidified incubator containing 95% air/5% CO_2, unattached acinar cells were

removed and counted and attached cells (n = 5-10 wells/group) were inoculated with SDAV (1 to 3 x 10^4 plaque forming units [PFU]/well) or control L2 cell antigens. After a one hour adsorption, acinar cells were rinsed to remove residual inoculum, then cultured in serum-free modified Oliver's media containing defined supplements for 4 or 8 days; media was replaced on day 4. When indicated, media was aspirated, volumetrically measured, centrifuged and stored at -80°C. At experimental termination, attached cells were harvested from Matrigel by exposure to trypsin/EDTA (Gibco) and Dispase (Collaborative Research) solutions. Viral titers in culture supernatants or sonicated cell extracts were then measured by plaque assay.

To evaluate the impact of acute SDAV infection on acinar cell function, cells were challenged with SDAV or L2 cell antigen, then cultured for 4 days in the presence or absence of dihydrotestosterone (10^{-6} M DHT; Sigma). This androgen is known to stimulate the synthesis and secretion of SC by lacrimal gland acinar cells.[14] After a 4 day culture interval, media was processed for the measurement of SC levels by RIA.[15] Statistical analysis was performed by utilizing the unpaired, two-tailed Student's t test.

RESULTS [11]

Exposure of lacrimal gland acinar cells to SDAV, but not L2 cell control antigens, resulted in a definite viral invasion, as well as a dramatic, time-dependent increase in viral replication (Table 1).[11] Within 4 days after viral challenge, SDAV titers had risen significantly, such that total SDAV levels in culture wells averaged almost 5-fold higher than those present in the original inoculum. Moreover, during the following 4 day period, SDAV titers underwent an additional 3-fold increase (day 8). Throughout this 8 day time course, infectious progeny remained almost entirely within acinar cells, as compared to SDAV content in the incubation media. Thus, cell-associated SDAV typically accounted for over 96% of the total recovered virus per culture well (Table 1).

Table 1. SDAV infection in acinar epithelial cells from the rat lacrimal gland

Time Course (days)	(SDAV Titer in Total Cell Culture)/ (SDAV Titer in Initial Inoculum)	(Acinar Cell SDAV Titer)/ (Total Cell Culture SDAV Titer) - %
4	4.88 ± 1.05	97.5 ± 0.6
8	17.16 ± 0.58	96.3 ± 0.5

Rat lacrimal gland acinar cells (~ 2 x 10^6 cells/well; n = 5 wells/group) were inoculated with SDAV (3 x 10^4 PFU/well) or L2 cell control antigens on Day 0. After a one hour incubation period, residual virus was removed and cells were cultured for 4 or 8 days. The total SDAV titer in cell cultures equals the viral levels in both incubation media and sonicated cell extracts. No SDAV was detected in control cultures. Data from reference (11).

Acute SDAV infection had minimal impact on acinar cell viability. Following 4 or 8 days of viral exposure, the percentage of live cells was either similar to, or slighly below, that of control cells, whose viability often exceeded 94%.[11] In addition, short-term SDAV infection (i.e. 4 or 8 days) did not prevent a functional acinar cell SC response to DHT (10^{-6}

M). In fact, in certain experiments, the extent of androgen-induced SC production by SDAV-infected cells was almost identical to that observed in control cells.[11]

The infectious capacity of SDAV in acinar cells from the lacrimal gland appeared to differ from that in acinar cells from salivary tissues. Thus, significant variations existed in the kinetics and magnitude of SDAV infection in lacrimal, submandibular and parotid acinar cells.[11] Overall, these comparative studies demonstrated that parotid cells were most susceptible to SDAV replication under the experimental culture conditions.[11]

DISCUSSION AND ACKNOWLEDGMENTS

These studies show that the coronavirus, SDAV, invades, and replicates in, lacrimal gland acinar cells in vitro. Moreover, our recent preliminary research has indicated that ocular exposure to SDAV may result in lacrimal infection in vivo (Z Huang, LA Wickham, DA Sullivan, unpublished data). Given this information, SDAV may serve as a very useful model virus to: [a] explore the impact of viral infection on the capacity, function and role of the ocular secretory immune system; and [b] evaluate potential vaccination strategies for the effective immunization of the ocular surface.

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