DEVELOPMENT OF A CANINE MODEL FOR RECURRENT RESPIRATORY PAPILLOMATOSIS

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A canine model for recurrent respiratory papillomatosis (RRP) was developed with canine oral papillomavirus (COPV) inoculated into the buccal mucosa and supraglottic larynx of 5 beagles. The animals received systemic immunosuppression with daily oral prednisone at doses of 0, 1, 2, 3, and 4 mg/kg. Buccal papillomata developed at 6 weeks in all animals and regressed by 10 weeks in the animals that received 0 and 1 mg/kg. The other animals had continuous growth of their buccal papillomata for 26 weeks. The animal that received 2 mg/kg developed papillomata on the lingual surface of the epiglottis that continued to grow through 26 weeks. Systemic oral prednisone successfully maintained COPV-induced oral and laryngeal papillomata in beagles. Thus, COPV-induced oral and laryngeal papillomata that are prednisone-maintained may have utility as a model for RRP.

KEY WORDS — canine oral papillomavirus, immunosuppression, larynx, recurrent respiratory papillomatosis.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is a disease characterized by development of papillomas of the upper aerodigestive tract. The lesions frequently recur after treatment. The most common primary treatment is surgical laser ablation of the lesions to maintain a patent airway. The clinical course is variable, ranging from spontaneous remission to aggressive, rapid, and extensive papilloma growth. Malignant transformation has been reported.

The cause of RRP is the human papillomavirus (HPV). More than 80 different subtypes of HPV have been identified, although subtypes 6 and 11 are the most common in RRP. Viral DNA has been identified in areas of normal-appearing mucosa adjacent to papillomas in the upper aerodigestive tract. Clinically latent HPV may be responsible for the frequent recurrence of lesions following surgical ablation.[1]

Canine oral papillomavirus (COPV) is a mucosal papillomavirus that infects the oral mucosa of dogs to produce typical raised verrucous masses. The time course of infection follows a short incubation and proliferation, followed by immune-mediated regression. Systemic immunosuppression can result in extensive and persistent lesions in both HPV- and COPV-induced disease.[2]

The specific aims of this investigation were to 1) evaluate the efficacy of COPV in producing laryngeal papillomatosis with and without systemic immunosuppression, 2) determine the time course and resolution, if any, of COPV-induced papillomatosis with and without systemic immunosuppression, and 3) determine the optimal immunosuppressive dose required, if any, for development and maintenance of COPV-induced laryngeal papillomatosis.

MATERIALS AND METHODS

Five beagles were obtained from a colony with no history of COPV infection and housed in climate-controlled accommodations in compliance with UCLA Animal Research Review Committee guidelines. After a 10-day acclimation period followed by overnight withdrawal of food, each animal was examined and weighed before premedication with intravenous thiopental sodium (16 mg/kg). Anesthesia was induced by 1% isoflurane. Direct laryngoscopy was performed. A mouth gag was used to hold the jaws open, and the tongue was retracted with a gauze pad. The epiglottis was lifted with a ring forceps, and the larynx was inspected. One milliliter of COPV-induced papilloma homogenate was injected into the left supraglottis and buccal mucosa after scarification with a needle tip. Four of the animals began daily

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oral prednisone at doses of 1, 2, 3, and 4 mg/kg. All animals were kept in contact and respiratory isolation.

At 2-week intervals following COPV injection, direct laryngoscopy was repeated as described above. The presence and pattern of papillomatosis was recorded on a laryngeal diagram, and the larynx and buccal mucosa were video-recorded on VHS tape. At 32 weeks, the animals were painlessly sacrificed and the larynges and buccal mucosa were harvested, sectioned, and processed for histopathologic examination.

RESULTS

There was no papilloma formation in any of the animals until the 6-week examination. There was no endolaryngeal papilloma formation in any animal throughout the experiment. At 6 weeks, all animals showed evidence of buccal papilloma formation that appeared to be prednisone dose–dependent in regard to the number of lesions and their height above the surrounding mucosa. The papillomas appeared as multiple keratinized verrucous lesions. They were flat in the control animal, slightly raised in the 1-mg/kg through 3-mg/kg animals, and significantly raised in the 4-mg/kg animal. The number of lesions increased with the dose in the control through 3-mg/kg animals. The 4-mg/kg animal had a single confluent 2-cm mass of papilloma.

By 10 weeks, there was complete regression of buccal papillomatosis in the control animal and the 1-mg/kg prednisone animal. The buccal papillomatosis in the remaining animals continued to proliferate in a prednisone dose–dependent fashion through 26 weeks. The individual lesions increased in diameter and height above the surrounding mucosa, eventually forming a confluent area of papillomatosis involving the majority of the left buccal mucosa. The time to confluence was inversely dependent, and the size of the confluent area was directly dependent, on the prednisone dose.

After 26 weeks, the papillomatosis showed evidence of early regression, including loss of keratinization and decrease in size. The 2-mg/kg animal developed multiple papillomata on the laryngeal surface of the epiglottis at 12 weeks that increased in size through 26 weeks. The 4-mg/kg animal developed multiple papillomata on the lingual surface of the epiglottis at 18 weeks that increased in size through 26 weeks. The laryngeal papillomatosis in both animals showed signs of early regression after 26 weeks.

Histopathologic examination of the larynges and buccal mucosa demonstrated no koilocytosis or other signs of viral change in the endolaryngeal injection sites. Buccal and epiglottic papillomas demonstrated fibrovascular stalks beneath redundant epithelial fronds. There was acute and chronic inflammation at the mucosa-submucosal junction of the oral papillomata. No lymphocytic infiltrate was noted in either the laryngeal or buccal papillomata.

DISCUSSION

Animal models of mucosal papillomatosis have been developed in rabbits,[3] cows,[4] and dogs.[5] These models use conjunctival or oral mucosa as the primary site of infection, and there is rapid regression of virus-induced lesions. A model of laryngeal involvement in RRP is important, since the primary treatment method currently is surgical extirpation. The fine layered structure of the vocal folds is very susceptible to trauma. Surgical ablation of laryngeal papillomatosis carries the risk of alteration of normal laryngeal anatomy and function secondary to scarring from cold instrument and/or laser manipulations. Derangements of laryngeal anatomy that affect phonatory and respiratory function can have a tremendous impact on the acquisition of communication skills and concomitant mental development of children affected by RRP. An ideal animal model for RRP would involve papilloma growth in the oral and/or laryngeal mucosa that is resistant to regression.

Canine oral papillomavirus infects closely related canids, including domestic dogs (*Canis familiaris*), coyotes (*Canis latrans*), and possibly wolves (*Canis lupus*). The primary site of infection is the oral mucosa; however, the conjunctiva and planum nasale can also be involved. Canine oral papillomavirus typically produces single or multiple raised, verrucous masses on the buccal mucosa or tongue. Grossly, the masses appear white to pink and are firm. Microscopically, the lesions appear as epithelial fronds with an orderly maturation sequence supported by fibrovascular stalks. In experimentally induced COPV infection, there is a typical incubation period of 4 to 8 weeks, followed by immune-mediated regression within an additional 4 to 8 weeks. Persistent or neoplastic progression of COPV-induced papillomata is not common, but has been reported.[6]

There is a spectrum of responses to papillomavirus infection ranging from rapid clearance of virus with no clinically evident disease to rapid and extensive proliferation of papillomata. Individuals with persistent and recurrent florid papillomatosis such as that seen in RRP may have an underlying and specific inability to mount an effective immune response to certain HPV subtypes. The importance of host im-

munity in regulating the extent of clinical manifestation of papillomavirus infection is demonstrated in immunosuppressed individuals. Systemic immunosuppression from either human immunodeficiency virus or immunosuppressant medication administered after solid organ transplantation can result in extensive and persistent papillomata that are often refractory to therapy.[7,8]

Studies of regressing oral papillomata induced by COPV demonstrate a predominantly CD4+ and CD8+ T-cell infiltrate at the dermoepidermal junction. This infiltrate appears just before clinical regression and is maximal during the period of rapid regression. This pattern of immune-associated regression is similar to that seen in regressing human anogenital warts.[9] Systemic glucocorticoids have long been used for their immunosuppressive properties in solid organ transplantation and autoimmune disease. Glucocorticoids affect leukocyte traffic and function by multiple methods. They induce lymphopenia by redistribution of T-lymphocytes from the circulation to the lymphoid tissue and bone marrow. In addition, they decrease eosinophils and basophils and inhibit polymorphonuclear leukocytes and monocytes by altering normal chemotaxis, complement receptor regulation, interleukin secretion, and arachidonic acid production.[10] Systemic administration of glucocorticoids may interfere with the T-cell infiltrate associated with papilloma regression in COPV-induced papillomata. Prednisone administration in this experiment may have facilitated the persistence of oral and laryngeal papillomatosis induced by COPV injection. Indeed, the lack of lymphocytic infiltrate normally associated with regressing papillomata in the buccal and laryngeal specimens suggests prednisone-mediated suppression of cell-mediated immunity.

The lack of papilloma growth at the endolaryngeal injection site may be related to the virus site specificity. Papillomaviruses can have specific tissue tropism. It has been suggested that corticosteroid-induced immunosuppression can expand the tissue tropism of papillomaviruses. The growth on the lingual surface of the epiglottis with sparing of endolaryngeal sites may be related to the differing embryological origins of laryngeal and pharyngeal mucosae.

The incidental growth of papilloma on the epiglottis of two of the immunosuppressed animals may be related to local trauma during direct laryngoscopy. Canine oral papillomavirus requires scarification or mucosal disruption at the site of inoculation to facilitate mucosal infection. Scarification was performed at the buccal mucosa and endolaryngeal injection sites. During direct laryngoscopy, the epiglottis was routinely grasped with a ring forceps and lifted to enable visualization of the larynx. This maneuver resulted in minor mucosal trauma to the epiglottis and may have facilitated infection by running of virus-laden saliva from the oral mucosal infection sites down onto the traumatized epiglottic mucosal sites.

CONCLUSIONS

Canine oral papillomavirus–induced oral and laryngeal papillomatosis can be maintained beyond the normal incubation-regression period by systemic administration of prednisone. It is likely that COPV has a tissue tropism that limits its extent to the oral mucosa and lingual surface of the epiglottis. Experimentally induced oral and laryngeal papillomata in which natural regression can be delayed by steroid immunosuppression may allow study of soft tissue effects of current treatment methods, including laser technology, microdebrider debulking, and antiviral therapy. Canine oral papillomavirus–induced oral and lingual papillomata that demonstrate delayed regression due to systemic prednisone maintenance may have utility as a model for RRP.

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