THE HISTOLOGIC CHARACTERIZATION OF PERIANAL FISTULAS DURING TREATMENT WITH CYCLOSPORIN

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BYRON S. MISSEGHERS

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ABSTRACT

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Byron S. Misseghers
University of Guelph, 1999

Advisor:
Professor Allen G. Binnington

Canine perianal fistula is a chronic, progressive inflammatory and ulcerative disease involving the perianal region that most frequently affects German Shepherd dogs. The etiopathogenesis of perianal fistula has not been identified; however, the effectiveness of systemic immunosuppressive therapy using cyclosporin suggests that immune dysregulation plays an important role in the development and/or progression of perianal fistula. Currently there is no concensus on the identity of the initial target of the inflammatory process.

Biopsies from the zona cutanea of 15 dogs with naturally occuring perianal fistula were obtained before, and at various stages of healing following, treatment with oral cyclosporin in order to characterize the histologic lesions of perianal fistula, with particular attention to how they changed during an interval of clinical healing, and identification of the specific perianal structures most affected, as an indicator of the initial target of the inflammatory process.

The primary pattern of inflammation associated with perianal fistula was diffuse plasmacytic/lymphocytic inflammation, with frequent lymphoid nodules associated with glandular components of the dermis and subcutis. Sections directly adjacent to actual

sinus tracts showed a mixed inflammatory pattern, most likely due to associated opportunistic bacterial infection secondary to the ulcerated epidermis. Evaluation of paralesional histologic sections showed most of the inflammation associated with hepatoid circumanal gland lobules and evidence of gland destruction, with an impression of only secondary involvement of apocrine glands. The anal sacs were not primarily involved in the pathogenesis of perianal fistula. The anal sac abnormalities were most consistent with apocrine gland obstruction secondary to adjacent perianal inflammation, and did not support the presence of primary anal sac disease.

It is concluded that the clinical and histopathologic lesions of perianal fistula give supportive evidence to the primary involvement of the hepatoid circumanal glands in the pathogenesis of perianal fistulas.

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This thesis is dedicated to my family, especially my wife Laurelee, for their consistent and unfailing support throughout my training.

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DECLARATION OF WORK PERFORMED

I declare that, with the exception of the items listed below, all work reported in this thesis was performed by me.

Preparation of histologic sections using the microtome and subsequent staining with hematoxylin and eosin and Masson's trichrome stain were performed by the staff of the histology laboratory of the Department of Pathology

CHAPTER I

LITERATURE REVIEW

Introduction

Canine perianal fistula is a chronic, progressive inflammatory and ulcerative disease involving the perianal region of the dog. The German Shepherd breed is uniquely predisposed to this disease, with sporadic occurrences of perianal fistulas in other large and medium sized breeds[1-8] The disease is characterized clinically by the formation of single or multiple sinus tracts and severe ulceration and erosion of the perianal skin and deeper perianal tissue (Figures 1 and 2).[1,2,4,9] As the disease worsens, the extent of the sinus tracts progresses, with up to 360° perianal skin involvement.[4] Clinical signs associated with perianal fistulas commonly include tenesmus, dyschezia, malodorous mucopurulent discharge, excessive perianal licking, and constipation.[1,3] In severe cases dogs can also experience weight loss, lethargy, and rectal or anal bleeding. [1,3,5,6,10] In chronic cases, fibrosis of the rectal and perirectal tissues may result in worsening of tenesmus and constipation due to narrowing of the anal diameter.

Most reports indicate that male dogs are more frequently affected than females,[1,4,5,7,8,11-15] although some report no significant difference in gender distribution.[9,10,16,17] Sexually intact dogs, particularly males, also appear over-represented in many reports.[4,7,11-15] The disease most frequently affects middle-aged or older dogs, averaging 5-6 years of age, but has been documented in dogs as young as 6 months-of-age.[4]

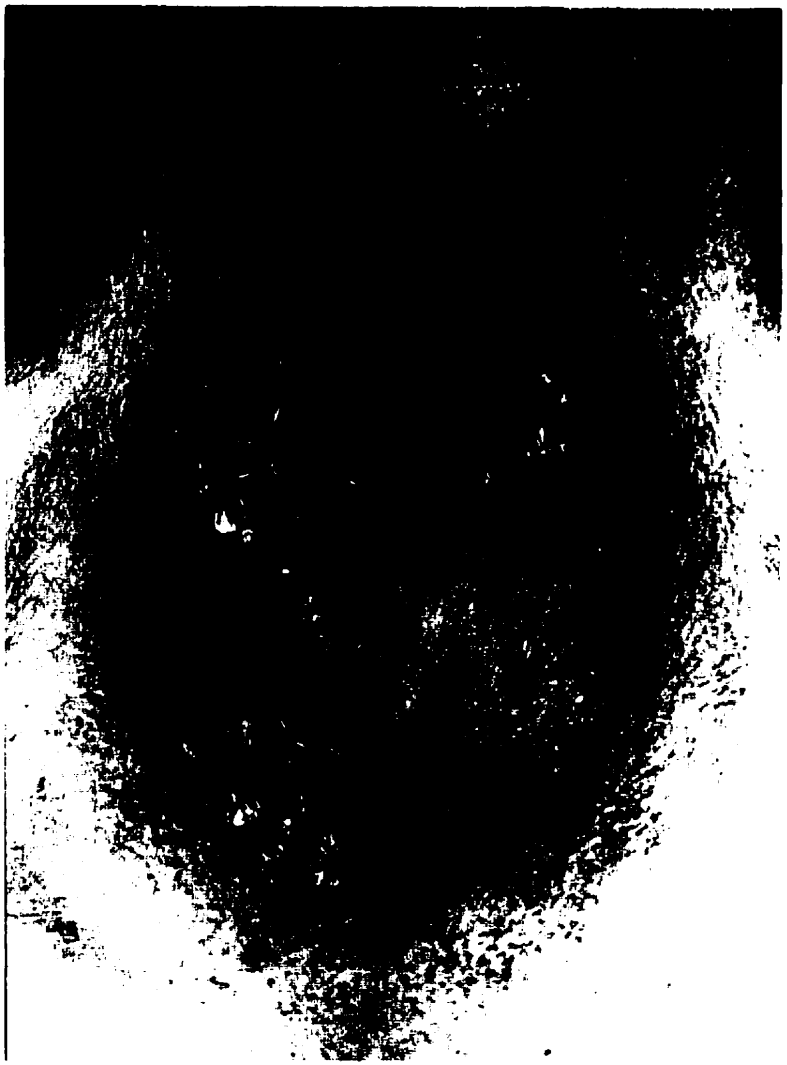

Figure 1. Clinical appearance of a moderate case of perianal fistula.

Figure 2 Clinical appearance of a severe case of perianal fistula.

The objective of this investigation was two-fold:

1) to characterize the histologic lesions of perianal fistula, with particular attention to how they change during an interval of clinical healing induced by chronic oral cyclosporin administration.
2) to help clarify the pathogenesis of this poorly understood syndrome by identifying the initial target of the inflammatory process.

Normal Perianal Anatomy

Perianal fistula is chronic inflammatory disease of the perianal skin and deep perianal tissues of the cutaneous zone, which is the most caudal portion of the anal canal. The anal canal is anatomically defined as the terminal portion of the alimentary canal, beginning at the termination of the rectum and extending caudally to the anus (Figure 3). This specialized segment of the digestive tract is divided, both grossly and microscopically, into three zones, the columnar zone (zona columnaris), the intermediate zone (zona intermedia), and the depilated cutaneous zone (zona cutanea) (Figure 4).[18,19] Some authors describe four zones by subdividing the zona cutanea into two zones, haired and non-haired.[19] The anal canal is supported by four prominent glandular components: anal glands, hepatoid circumanal glands, apocrine sweat glands, and sebaceous glands.[20] Additional structures surrounding the anal canal include the paired anal sacs and their associated apocrine glands, lymphoid tissue, the internal and external anal sphincter muscles, the rectococcygeal muscle, and associated neurovascular structures.[18,20] Motor function to the external anal sphincter muscle is provided from the caudal rectal branch of

the pudendal nerve and sensation to the perineum is supplied by innervation of the perineal branch of the pudendal nerve.[18]

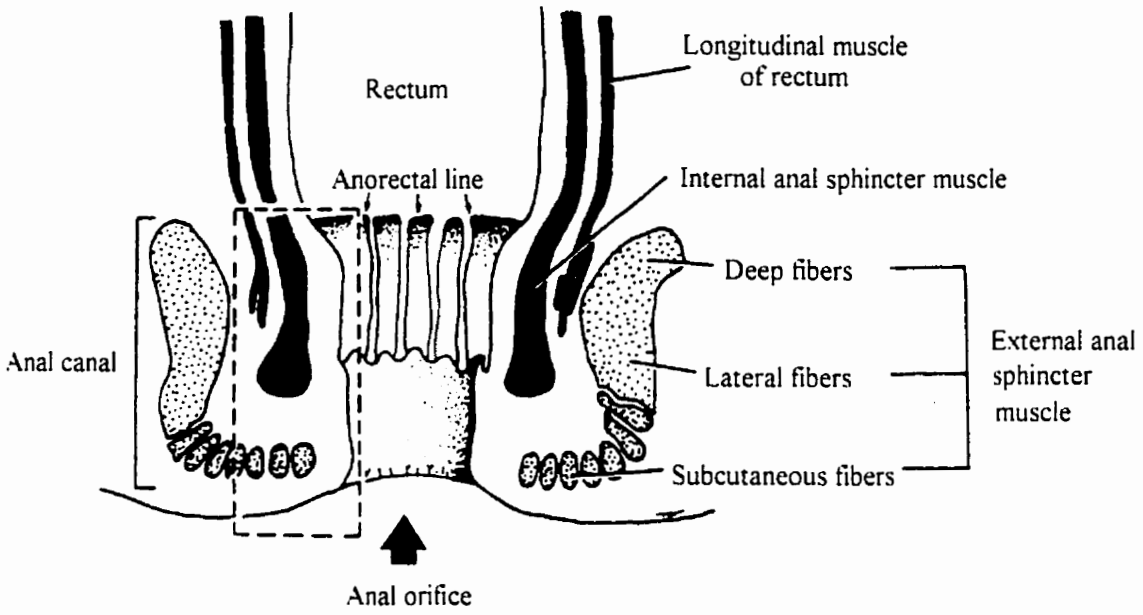

Figure 3: Diagrammatic illustration of a longitudinal cross-section through the anal canal. Rectangle indicates the region illustrated in Figure 4. (Adapted from Budsberg and Spurgeon[20])

Columnar Zone:

This is the most cranial of the 3 zones and averages 4.39 mm in length.[20] Its cranial boundary is the termination of the rectum, termed the anorectal line, and is characterized on a histologic level by the transition from the columnar cells and colonic crypts of the rectal mucosa, to the stratified squamous epithelium of the anal canal.[20] The zona columnaris is so named because of the characteristic longitudinal ridges, or anal columns, which run along its length (Figure 5).[18,19] Between these columns are shallow troughs, referred to as anal sinuses, that terminate caudally as blind-end pockets referred

to as anal crypts. These crypts are physically situated in the zona intermedia (Figure 4).[18,20]

Zona intermedia:

The intermediate zone, also known as the anocutaneous line, is situated between the zona columnaris cranially and the zona cutanea caudally, and averages between 1.76 mm in length.[20] The cranial boundary to this zone is characterized microscopically by the beginning of the anal crypts. Grossly this zone contains irregular, scalloped folds referred to as the anocutaneous line, created by four arches, one each of the smaller ones located dorsally and ventrally, and one each of the larger ones located laterally.[18] The glandular structures that reside in both the columnar and intermediate zones of the dog are the anal glands and their excretory ducts. These glands are modified sweat glands and are situated between bundles of muscle fibers of the internal sphincter muscle and in the connective tissue separating the internal sphincter muscle and longitudinal smooth muscle surrounding the rectum and proximal anal canal.[20] Budsberg & Spurgeon reported that anal glands rarely were noted extending into the cutaneous zone; however the excretory ducts of these anal glands always emptied into the intermediate zone.[20]

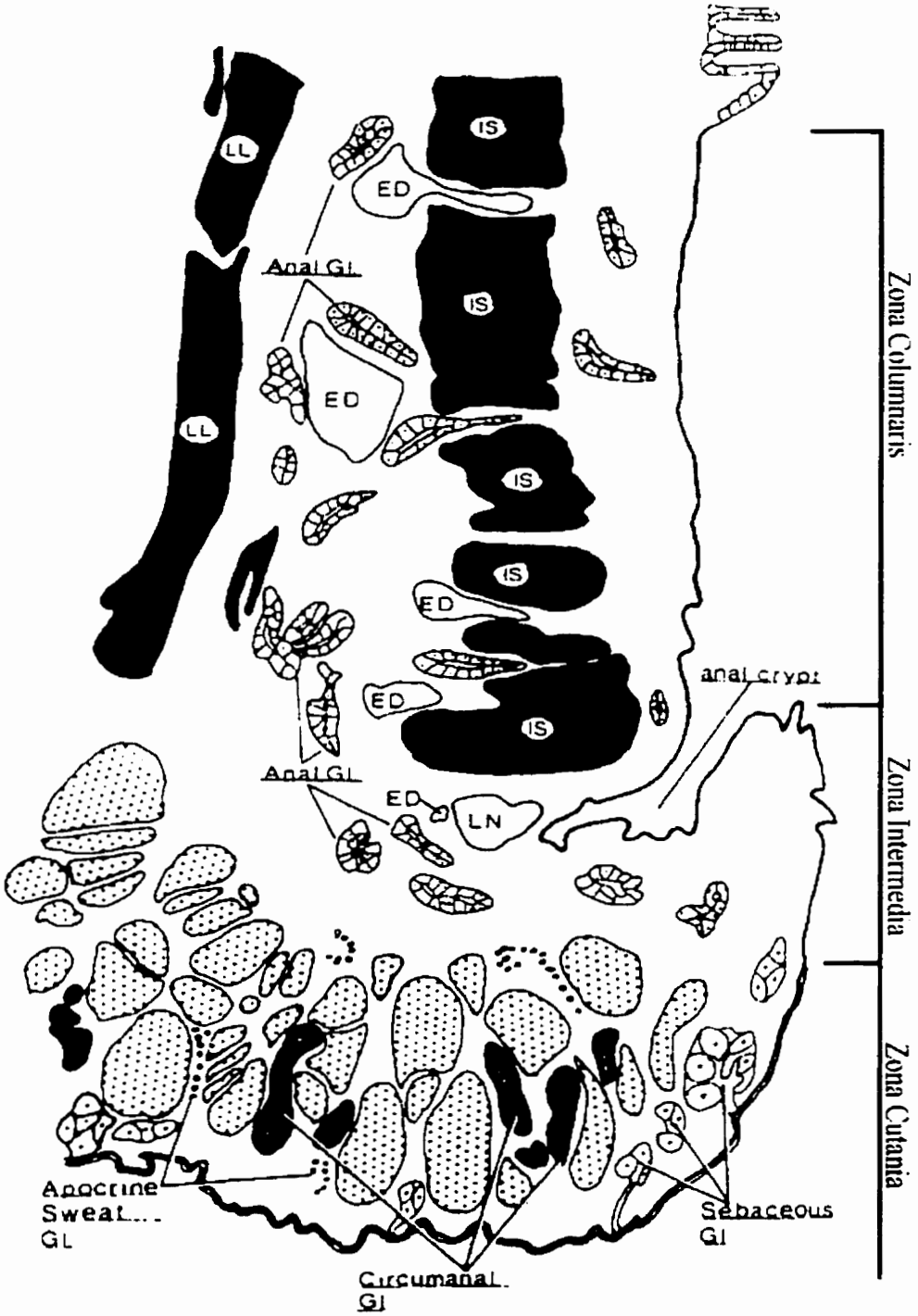

Figure 4: Detail illustration of the zones of the anal canal. ED = excretory duct of anal gland, LL = longitudinal layer of rectal smooth muscle, IS = internal anal sphincter muscle, LN = lymphoid nodule. (Adapted from Budsberg and Spurgeon[20])

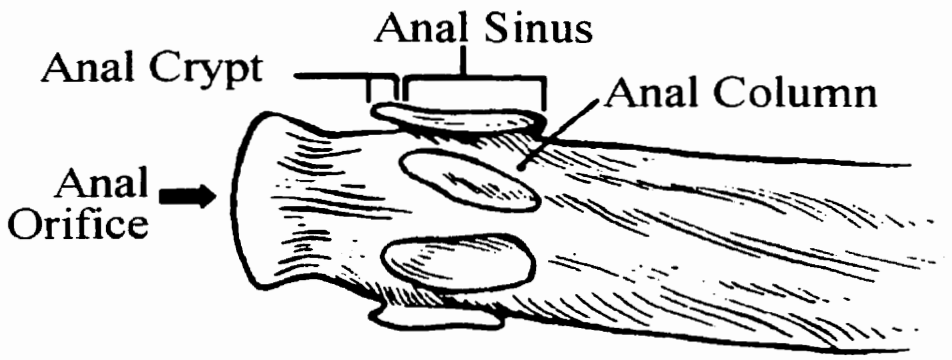

Figure 5: Illustration of a plastic cast of the anal canal depicting the anal columns, anal sinus', and anal crypts. (Adapted from Budsberg, Spurgeon, and Liggitt[21])

Zona cutanea:

This is the most caudal zone of the anal canal and is defined microscopically by keratinization of the stratified squamous epithelium. The caudal extent of this zone is vague, being characterized by the presence of normal thickness hair coat grossly, and disappearance of hepatoid circumanal glands microscopically.[20] The hepatoid circumanal glands are distributed in an irregular manner around the anus, with a diameter as great as 25 mm, and as small as 5 mm.[22]

Three different types of glands exist in the cutaneous zone: apocrine sweat glands, sebaceous glands, and hepatoid circumanal glands. The apocrine sweat glands and sebaceous glands are the same as those located in the skin elsewhere on the body and the sebaceous glands are clustered close to the anal orifice.[20] Some inconsistency exists in the literature on the precise classification of the glandular structures in the cutaneous zone, particularly with respect to the hepatoid circumanal glands. These glands are so

named because the cell morphology and arrangement resemble liver parenchymal cells.[20] Parks concluded that the circumanal glands are bipartite structures consisting of superficial sebaceous, and deep non-sebaceous hepatoid portions.[23] Baker also concluded that the circumanal glands are bipartite structures; however, he categorized them into major hepatoid and minor sebaceous elements rather than superficial and deep components since the sebaceous part, when present, was always surrounded by hepatoid circumanal glands.[22] Isitor and Weinman also found no basis for rigid distinction.[24] In evaluating the origin of the hepatoid circumanal glands they found that from birth the hepatoid circumanal gland is distinctly different in structural appearance to the sebaceous glands.[24] However, following birth they found development of a distinct subset of circumanal glands they termed transitional hepatoid glands (trHCG). These glands were located between the superficial sebaceous glands and the deep hepatoid circumanal glands and were the only glands to exhibit the bipartite nature described by Parks.[24] These glands are smaller than hepatoid circumanal glands and are thought to develop from the deeper portions of the superficial sebaceous glands.[24] Other authors denote only the deep hepatoid cells as circumanal glands.[18]

The hepatoid circumanal glands develop as buds from the external root sheath of hair follicles similar to the development of the sebaceous glands.[22,24] Early post-natal growth of the circumanal hepatoid glands is rapid and felt to be in response to growth hormone from the anterior pituitary gland (pars distalis), with ongoing growth throughout the remainder of life due to the effect of androgens.[22] Isitor and Weinman identified ducts communicating between the hepatoid circumanal glands, and found these ducts to be distinct and separate from the ducts of the sebaceous glands.[24] The ducts were solid

during the first 60 days postpartum and cysts developed in the ducts between days 60 to 152, both of which were interpreted as an indication that the hepatoid circumanal glands are functional exocrine glands.[24] Baker[22] and Maita and Ishida[25] both suggested a possible endocrine function. To date, the precise functional nature and status of the hepatoid circumanal glands is unknown.

The sebaceous glands are the most numerous glands of the zona cutanea in the female dog.[20] In contrast, the hepatoid circumanal glands far outnumber sebaceous glands in male dogs.[20] It is well established that the growth, and neoplastic transformation, of the hepatoid circumanal glands is significantly influenced by androgens, particularly testosterone.[20]

The anal sacs are diverticula of the cutaneous zone and are lined by keratinized, stratified squamous epithelium.[19] Abundant apocrine glands within the wall of the sac secrete their products into the anal sac. The duct of each of the paired anal sacs opens onto the cutaneous zone just caudal to its cranial border.

Clinical Appearance of Perianal Fistula

Dogs with perianal fistula commonly present with one or more of the following complaints: tenesmus, dyschezia, malodorous mucopurulent discharge, excessive perianal licking, and constipation.[1,3] In the early stages of perianal fistula some dogs are relatively asymptomatic and mild clinical signs may go unnoticed by owners.[26] In severe cases dogs may also experience weight loss, pyrexia, lethargy, and rectal or anal bleeding.[1,3,5,6,10] In chronic cases, fibrosis of the rectal and perirectal tissues may result in worsening of tenesmus and constipation due to narrowing of the anal diameter. A

thorough examination of the perianal area usually requires sedation or general anesthesia due to the painful nature of the disease. In the majority of instances the sinus tracts and ulcerated perianal skin are located in the depilated portion of the cutaneous zone; however, once the hair is clipped from the entire perianal area, occasionally sinus tracts are identifed involving the peripheral haired region of the cutaneous zone. These sinus tracts may communicate with each other below the skin surface and may extend several centimeters cranially into the deep perianal tissue. Digital rectal palpation may reveal varying degrees of anal stricture, and also allows for palpation of the anal sacs. Many authors feel that anal sac disease is not the primary cause of perianal fistula;[5,10,27,28] however, they may become secondarily involved due to inflammation from adjacent sinus tracts or from dysfunction of the external anal sphincter muscle which impairs expulsion of the glandular secretion of the anal sacs.[19]

With this clinical appearance, differential diagnoses include localized cellulitis, secondary to infected and ruptured anal sacs, and perianal neoplasia, most notably hepatoid circumanal gland adenocarcinoma.[1,29]

Etiology and Pathogenesis

The earliest reports of perianal fistula in dogs was in 1945.[30] Lacroix and Lacroix noted the development of sinuses from the skin around the anus, with variable depth of extension cranially parallel to the rectum.[30] They thus designated the disease as Pararectal Fistula. Since this early report, perianal fistulas (also known as perianal sinus

and anal furunculosis in the veterinary literature) have been described in more detail, yet the exact etiology and pathogenesis of this disease have remained obscure.

The etiology of canine perianal fistulation is unknown and frequent recurrence with surgical treatment may reflect our ignorance of the underlying cause of the disease.[15] Many theories on the etiology of perianal fistula have been proposed, including immune deficiency, bacterial infection, and endocrine abnormalities (hypothyroidism). A conformational or anatomic predisposition to the disease has also been proposed.[3,7,10,31] The three most commonly suggested theories on the pathogenesis include:

1) extension from anal sac infection to the perianal skin.[30]

2) impaction of the anal crypts with minute fecaliths leading to microabscessation of the perianal skin.[32]

3) abscessation of the glandular structures in the cutaneous zone.[10,26]

These theories suggest a disease process involving specific structures of the anus or perianal skin, and additional detail regarding these theories is presented below.

Extension from Primary Anal Sac Disease: The earliest theory on the etiology of canine perianal fistula was that it represented extension of anal sac infection into the perianal skin.[30] In support of this theory it has been noted clinically that early cases of perianal fistulas often appear centered over the ducts of the anal sacs;[28] however, documentation of communications between the affected anal sacs and perianal sinus tracts is lacking. This, along with observations of frequent occurrence of perianal fistulas in the absence of any history of anal sac disease,[10] a very low prevalence of primary anal sac disease in German Shepherd dogs,[10] and a paucity of perianal fistulas in small dogs despite frequent and chronic anal sac disease, indicates that anal sac disease is probably

not a prerequisite for perianal fistulas.[10,27] Involvement of the anal sacs only as a secondary event is supported by recent histologic evaluation in a large number of German Shepherd dogs and non-German Shepherd dogs with perianal fistula.[9] The histopathologic changes associated with the anal sac were centered mostly on the duct of the anal sac, and when the body of the anal sac was significantly affected, these lesions were generally adjacent to inflamed sinus tracts.[9] These findings were interpreted as representing local extension of inflammation from the sinus tracts.[9]

Crypto-glandular theory: The crypto-glandular theory for perianal fistulation was first proposed in 1961.[32] The theory that anal crypt impaction results in fecal contamination of the anal glands of the columnar and intermediate zones and then spreads to create fistulous tracts is based largely on comparison to perianal abscess and fistula (fistula-in-ano) in people.[32,33] Based on this theory, the sequence of events involved in the pathogenesis of perianal fistula are as follows: Fecalith impaction occurs in the crypts of the anal sinuses of the columnar and intermediate zones of the anal canal; These impactions result in pressure necrosis, infection, and ultimately fistulation towards the zona cutanea by dissecting into the circumanal glands and penetrating the skin surface via the circumanal gland ducts.[32]

The crypto-glandular theory, however, has numerous weaknesses:

1) True rectocutaneous fistulas (communication between the rectum and skin surface of the zona cutanea) occur only rarely in dogs with perianal fistula.[4,10,34]
2) Contrary to what one would expect if sinus tracts formed from the deep and centrally located anal glands, early stages of sinus formation appear superficial

and extend to the deeper tissues as the disease progresses[10,26] and perianal fistulas are usually first seen quite a distance lateral to the anal opening.[10,26]

3) Additionally, as already mentioned, this theory is based largely on comparison to perianal abscess and fistula (fistula-in ano) in people. However, the sinus tracts in dogs with perianal fistula are lined with stratified squamous epithelium, instead of columnar epithelium as is seen in people.[10,26]

4) Finally, if the source of perianal fistula is dissecting abscessation from infected anal glands, then infection would be the primary component of the disease process. In fact, bacterial infection has not been shown to play a role in the initial development of canine perianal fistula. It is currently believed that it is initially a sterile disease, with bacterial contamination and secondary infection only after epidermal ulceration has occurred.[4]

Glandular Abscessation Theory: This theory proposes abscessation of the glandular structures in the cutaneous zone as being the likely primary lesion leading to perianal fistula in the dog.[10,26] This theory is substantiated by the histologic characterization of perianal fistulas in which the earliest stage of the disease is characterized by inflammation within and around the epidermal periadnexal structures, including sebaceous, apocrine, and hepatoid circumanal glands.[4] Identification of one particular glandular component of the zona cutanea as being the initial or primary target for this inflammation is lacking. Compared with normal non-German Shepherd dogs, Budsberg et al reported that normal German Shepherd dogs have a greater density of apocrine glands in the zona cutanea.[21] Unfortunately no supporting data regarding this difference was reported. Budsberg et al suggested that the predisposition of the German

Shepherd Dog to perianal fistula may be related to this greater density of apocrine glands.[21] This remains to be documented, however.

Other authors have taken a more holistic approach to the etiology of perianal fistula, focusing on issues of immune incompetence or endocrine dysfunction to explain individual or breed susceptibility to the development of perianal fistula. Although depressed peripheral lymphocyte counts, decreased serum immunoglobulins, and diminished in vitro lymphocyte-proliferation responses were noted in a small percentage of affected dogs in one study, these abnormalities resolved following successful treatment of the fistulas.[12] It was suggested by the authors that immunosuppression was a consequence, rather than a cause, of perianal fistulation.[12] Additional investigation into possible immune dysfunction has included immunohistochemical characterization of the inflammatory cell populations within perianal fistulas.[17] Using indirect immunoperoxidase staining, Day characterized the mononuclear inflammatory infiltrate of perianal tissues, both from German Shepherd dogs and non-German Shepherd dogs affected with perianal fistulas.[17] In an earlier report, Day and Weaver had noted that lymphoid cells infiltrating the perianal sinus tracts often appear as distinct perivascular aggregates or lymphoid follicles,[9] and Day subsequently showed that these lymphocytes were almost entirely CD3+ lymphocytes (T lymphocytes), suggestive that these aggregates or follicles are regions of T lymphocyte differentiation.[17] No evidence directly demonstrating an underlying immunologic abnormality has been published.[17] However, the recent discovery that the immunosuppressive drug cyclosporin is effective in the treatment of perianal fistula[2,5] has renewed interest in the possible role of immune dysregulation in the pathogenesis of the disease.

Endocrinopathy has been proposed as a possible cause of hidradenitis suppurativa in people and has also been investigated to a limited extent with regard to canine perianal fistula, specifically in regard to hypothyroidism.[12] However, no published evidence exists to support an association between hypothyroidism and canine perianal fistula. In one study, only one of 33 dogs with perianal fistula that were provocatively tested for hypothyroidism was truly hypothyroid.[12] Harkin et al [7] tested 27 dogs with perianal fistula for hypothyroidism, and all of those dogs were diagnosed as euthyroid.

An adequate explanation to account for the unique predisposition of perianal fistulas in German Shepherd dogs is still lacking. Early work in the characterization of canine perianal fistulas focused on identification of anatomic differences among normal dogs, both grossly and microscopically, to account for the marked overrepresentation of the German Shepherd breed with this disease. Proposed theories include tail conformation, with a broad-based tail and low tail carriage, and gross or microscopic anatomic differences associated with the anal canal. It has been suggested that the German Shepherd Dog tail conformation reduces perianal ventilation and predisposes to accumulation of moisture, fecal contamination, and anal sac secretion which results in infection of the local hair follicles and glands.[10,11] Consistent with this hypothesis, high caudectomy has been observed to result in healing of fistulas in approximately 80% of cases (Table 1), presumably secondary to the normalizing of local microenvironmental conditions;[11] however, tail conformation does not account for the paucity of perianal fistula in other breeds (like Belgian Sheepdogs, Gordon Setters, English Setters) with similar tail conformation.[4] Similarly, if perianal contamination due to tail conformation were responsible for fostering perianal fistula, then evidence of irritation of the perianal

skin, such as parakeratosis and epidermal hyperplasia, should be evident. These features are not present in early stages of perianal fistula.[4]

Budsberg et al utilized gross morphometric analysis to evaluate anal crypt dimensions of the anal canal of normal German Shepherd dogs, compared with normal non-German Shepherd breeds, to determine if there was any gross anatomic difference to account for the higher incidence of perianal fistula in German Shepherd dogs.[21] Although German Shepherd dogs had a larger variation (standard deviation) in crypt base width, no significant difference was noted between the two groups and no other gross anatomic differences have been identified.[21]

Microscopically, a significant difference has been found between these two groups of dogs. Budsberg et al found that normal German Shepherd dogs have a greater density of apocrine glands in the cutaneous zone than do normal non-German Shepherd dogs. They hypothesized that infection of the apocrine sweat glands, initially fostered by the moist and contaminated microenvironment caused by the broad based tail and low tail carriage, may promote infection of the deeper perianal region resulting in perianal fistula.[21] The main weakness of this theory, however, is that bacterial infection has not been shown to play a role in the initial development of canine perianal fistula, and it is currently believed that it is sterile disease initially, with bacterial contamination and secondary infection only after epidermal ulceration has occurred.[4]

In assessing healthy dogs unaffected by perianal fistula, Budsberg et al noted differences with respect to the prevalence and severity of inflammation of the anal glands of the intermediate and columnar zones between German Shepherd dogs and non-German Shepherd breeds.[21] Although there was only a small difference between groups with

respect to the percentage of inflamed anal glands seen (28%+/-21% in German Shepherd dogs vs 23% +/ 13% in non-German Shepherd breeds), subjectively the inflammation and fibroplasia were more severe in the German Shepherd dogs.[21]

Harkin et al [7] investigated the possible association between inflammatory bowel disease and perianal fistulas in German Shepherd dogs. They obtained colonic biopsies from 27 German Shepherd dogs with perianal fistula to determine the prevalence of colitis in this population. All 27 dogs showed histologic evidence of colitis, characterized by fibrous connective tissue proliferation in the large bowel lamina propria, with mild-to-moderate and diffuse plasmacytic/lymphocytic inflammation. Eosinophilic inflammation was also noted in some cases, being mild and diffuse in five cases, and focally severe in two cases. All 27 dogs were subsequently treated with immunosuppressive doses of prednisone, along with a hypoallergenic diet, to treat perianal fistulas.[7] Perianal fistulas resolved in 33.3% of cases and clinically improved in another 33.3% of cases; however, there was no significant change in the histopathology from the colonic biopsies. The authors suggested that an association between colonic inflammatory bowel disease and perianal fistula might exist.[7] Given the breed predisposition towards inflammatory bowel disease in the German Shepherd breed,[7,35] the lack of control animals in this study, including both German Shepherd dogs without, and non-German Shepherd dogs with, perianal fistulas, makes interpretation of the findings and conclusions difficult. Even if there is no direct association between perianal fistula and colitis it is interesting to note that even in cases for which the perianal fistulas failed to heal with prednisone, the clinical signs of tenesmus resolved in 70% of those cases.[7] The authors suggested that the coexistent inflammatory bowel disease contributed to the clinical signs attributed to

perianal fistulation (tenesmus)[7] This is not necessarily accurate since the anti-inflammatory and immunosuppressive effects of prednisone may simply have been sufficient to eliminate the tenesmus associated with perianal fistula, especially since no mention is made as to whether or not any of these dogs displayed clinical evidence of inflammatory bowel disease prior to treatment. However, the positive results with regard to healing of the perianal fistulas in response to immunosuppressive therapy using prednisone (one-third of cases) lends some support to an underlying immune-mediated etiology for canine perianal fistulation.

Histologic Appearance of Perianal Fistula

There has been very little published data on the histopathology of perianal fistula and all of the information presently known about perianal fistula in this regard comes from three published articles. Harvey was the first to publish the histopathologic findings from dogs with perianal fistula.[10] He noted epidermal ulceration, with acute or chronic inflammatory reaction, and sinus tracts extending into the subcutaneous tissue and muscle. These tracts were lined either by granulation tissue or stratified squamous epithelium. He also noted that the ducts of the sebaceous and apocrine glands were sometimes dilated with inflammatory cells and the glands themselves were often involved in, or surrounded by, inflammatory cells.

Killingsworth et[4] al evaluated tissue from all three anatomic regions of the anal canal in 44 dogs (51 biopsy specimens) with perianal fistula and concluded that the most severely affected region was the cutaneous zone. In this study, the histologic lesions were graded as early, intermediate, and late stages of the disease based on the severity

and nature of the inflammation, the amount of fibrosis, and the depth of the sinus tracts; however, it should be noted that the disease was, in reality, advanced even in lesions classified as "early" since sinus tracts were already present. In the study, the authors found that in early-stage lesions, the inflammation was focal, periadnexal, and pyogranulomatous in nature around the sinus tracts. In intermediate-stage lesions, in addition to the periadnexal inflammation, they observed epidermal ulceration, pyogranulomatous inflammation of the superficial dermis, and accompanying fibrosis, rupture of inflamed dilated apocrine glands, and destruction of sebaceous and hepatoid circumanal glands. Although the pattern of periadnexal inflammation in these intermediate-stage lesions involved all the glandular structures of the cutaneous zone, apocrine adenitis was the most constant feature, being present in 22 of the 51 biopsy specimens evaluated.[4] Late-stage lesions were characterized by fibrosis, formation of lymphoid nodules, and dissecting cellulitis, extending throughout the perirectal stroma in nodular-to-linear zones of pyogranulomatous inflammation. Bacterial organisms were not identified histologically in early-stage lesions, and were only infrequently identified in intermediate- and late-stage lesions. This was felt to be secondary to contamination following epidermal ulceration.[4] The authors concluded that the lesions of perianal fistula originate as periadnexal/glandular inflammation in the superficial cutaneous zone, and the inflammatory response produced by the disease is initially sterile and characterized by a mononuclear inflammatory infiltrate, most notably plasma cells and lymphocytes.[4]

Day and Weaver[9] histologically evaluated surgically resected tissue from 305 cases of perianal fistula. In this study they assessed the character and degree of

inflammation associated with the anal sacs, sinus tracts, non-sinus associated perianal skin, anal sphincter muscle, and the circumanal glands (collectively comprising the sebaceous, apocrine, and hepatoid circumanal glands).[9] Not surprisingly, the sinus tracts exhibited the most severe inflammation, which obliterated normal tissue architecture and was mononuclear to pyogranulomatous in nature.[9] Frequent aggregations of single populations inflammatory cells (plasma cells, lymphocytes) were also noted.[9]

With regard to the anal sacs, in the majority of cases inflammatory lesions were focal and consisted of mononuclear inflammatory cells. Interestingly, these changes were largely centered on the duct of the anal sac,[9] rather than the body of the anal sac. Additionally, anal sacs that showed inflammation or fibrosis that obliterated the normal architecture of the anal sacs were generally adjacent to inflamed sinus tracts.[9] Occasionally eosinophils were observed mixed in with the subepithelial mononuclear inflammation or forming distinct pustules within the duct epithelium.[9]

In the majority of cases Day and Weaver assessed the anal sphincter muscles and non-sinus associated skin as microscopically normal, but furunculosis was identified in a few cases. The collective assessment of the circumanal glands was abnormal in 51% of non-German Shepherd dogs and 64% of German Shepherd dogs, and these pathologic changes consisted of heavy focal to multinodular inflammation focused on the glands or the ducts of the glands.[9] The authors concluded that the notable histopathologic feature of perianal fistula was dense plasmacytic and lymphocytic inflammation, with lymphoid nodules.[9] In addition, based on the observation of a few instances of furunculosis in non-sinus associated (normal perianal) skin, the authors suggested that perianal fistula may be a manifestation of generalized cutaneous furunculosis as a consequence of deep pyoderma

seen in German Shepherd dogs.[9] This contrasts the findings of Killingsworth et al[4] who concluded that the initial inflammation is not associated with the hair follicles, but instead originates as inflammation of the periappendageal glandular structures.

Comparison of Canine Perianal Fistula with the Pathogenesis, Pathology, and Treatment of Human Perianal Fistulas

The cause of canine perianal fistulas is not known. Similar pathology of the anus is seen associated with various skin and gastrointestinal conditions in people, including perianal hidradenitis suppurativa, cryptoglandular fistulas (fistula-in-ano), and perianal Crohn's fistulas secondary to Crohn's disease.

Hidradenitis suppurativa is a chronic and cictricial inflammatory disease that affects apocrine gland-bearing areas of the skin, including the axillary, perianal, inguinoperineal, and areolar region of the mammary gland.[36-39] The most commonly affected areas are the axillary and inguinal-perineal regions.[40] Like that of canine perianal fistulation, the etiology of perianal fistulas in people also remains unclear, and it is felt that several coexisting factors probably predispose to the disorder.[40] These factors include endocrine changes (puberty, menstruation, diabetes, disturbances in androgen metabolism), altered immune response, and follicular occlusion or occlusion of individual apocrine/sebaceous glands.[36,37,40] The pathogenesis of the inflammation of hidradenitis suppurativa in people has been investigated. Early reports, dating as far back as 1854, implicated the apocrine sweat glands as the cause of the inflammation, primarily as a result of poral occlusion with resultant obstruction, dilatation, leakage, inflammation, and

subsequent bacterial invasion of the apocrine duct.[38,40] More recent studies have shown that hidradenitis suppurativa is in fact a disease of follicular occlusion, rather than specifically apocrine duct occlusion, with apocrine inflammation being only secondary.[36-39,41]

There are many similarities between hidradenitis suppurativa in people and canine perianal fistulas, and indeed it has been stated that the clinical appearance of canine perianal fistulas most closely resemble the appearance of hidradenitis suppurativa in people.[4] Both diseases are rarely seen in patients prior to the onset of sexual maturity.[38] Numerous reports in the dog show an over-representation of males,[2,4,5,7,8,11,12-14] and men tend to be affected more frequently with perianal hidradenitis suppurativa.[38] In addition, many women with hidradenitis suppurativa report worsening clinical signs during menstruation,[38] and similar observations have been made regarding bitches in estrus.[42] Both diseases show a chronic course, lack of responsiveness to antibiotic therapy, and eventual formation of multiple sinus tracts, cicatricial fibrosis and scarring, and a tendency towards recurrence following surgical excision.[37,38,40]

In spite of these clinical similarities, the primary histopathologic lesions differ with respect to these two diseases. The most consistent histologic features seen with hidradenitis suppurativa include follicular hyperkeratosis and marked follicular occlusion, with apocrine gland inflammation occurring only as a bystander effect.[36-39,41] In contrast, follicular plugging with keratin is not a feature of canine perianal fistula.[4]

Crohn's disease is another disorder with many clinical similarities to canine perianal fistula. Crohn's disease is an inflammatory condition in people, primarily affecting the gastrointestinal tract, and an abnormal immune response has been implicated

as the cause of the persistent inflammation.[43] A cutaneous manifestation clinically similar to, and histologically indistinguishable from, perianal hidradenitis suppurativa frequently occurs in people with Crohn's disease and ulcerative colitis conditions.[36,44,45] Approximately 25% of all intestinal Crohn's patients present initially with cutaneous perianal lesions and disorders involving the perianal skin occur in 60-70% of Crohn's patients at some stage in their disease.[36] With this in mind it is interesting to note the well established predisposition of German Shepherd dogs to both inflammatory bowel disease and perianal fistulas.[7,35] Canine perianal fistulation may represent a cutaneous manifestation of immune-mediated inflammatory bowel disease, as it does in people with cutaneous Crohn's disease.

The etiology of inflammatory bowel disease is unknown. Multiple etiologic factors seem likely, and immunoregulatory abnormalities are felt to be one of these etiologic factors.[46,47] Similarly, although the etiology of Crohn's disease is also unknown, immune dysfunction has been implicated, regardless of whether it is a primary factor responsible for the onset of disease or whether it is secondary factor responsible for persistence of the inflammation.[43] In this regard, immunosuppressive therapy using cyclosporin has shown to be an effective treatment for perianal and enterocutaneous fistulas in people with Crohn's disease[43] but it's long-term use for this disease has been mainly limited by its side-effects, particularly nephrotoxicity.

A new therapy on the horizon for the treatment Crohn's perianal fistulas is infliximab (Remicade®), a chimeric IgG1k monoclonal antibody that binds specifically to human TNF-α (tumor necrosis factor-alpha) thus inhibiting TNF-α from binding with its

cell receptors.[48] As a result, this medication blocks the biologic activities of TNF-α, including the production of other pro-inflammatory cytokines.

Surgical Treatment of Perianal Fistulas

The treatment of perianal fistulas has consisted of both medical and surgical intervention. However, it remains a frustrating disease to manage as a result of poor response to historical treatments, high rates of recurrence, and morbidity associated with surgical therapy. Undoubtedly, effective treatment of perianal fistulas has been complicated by a lack of understanding of the underlying pathogenesis.[1,3] In the absence of adequate knowledge regarding the pathogenesis of perianal fistula, surgical management has been considered the treatment of choice.[1-3,7,8,10,11,13,14,16,26-29,49] Surgical treatment may include one or a combination of chemical cauterization, cryosurgery, deep excision, deroofing and fulguration, or tail amputation. The following paragraphs briefly describe the various surgical procedures currently used.

Deep Excision: Most surgical techniques are aimed at the complete removal (deep excision) or destruction (cryosurgery, deroofing and fulguration, laser excision) of the diseased tissue, the only exception being tail amputation in which the actual sinus tracts are left untouched. During deep surgical excision, all sinus tracts in the zona cutanea are excised, including affected areas of intermediate and columnar zones. In severe cases this may necessitate a complete rectal pull-through procedure and bilateral anal sacculectomy,

and may require removal of all or some of the external sphincter muscle.[8,10,15,27] The results of treatment for perianal fistula by surgical excision are included in Table 1.

Deroofing and Fulguration: Individual sinus tracts are opened by excision of the overlying tissue (deroofing) to expose the sinus lining. The exposed tissue is then fulgurated using an electrosurgical unit. Electrofulguration is distinct from electrocautery. Fulguration utilizes a muted high-voltage, high-frequency, electric current (spark gap current) that produces a potent dehydrating effect on the directed tissues, resulting in superficial destruction of the tissue.[14] The resultant wound is then left to heal by second intention. The results of treatment for perianal fistula using deroofing and fulguration are included in Table 1.

Cryosurgery: Cryosurgical treatment of perianal fistulas was first described by Borthwick[50] in 1971 as an alternative to conventional excisional techniques and a number of subsequent clinical trials have further defined the efficacy and limitations of this technique.[6,15,28,49,51,52] Cryogens typically utilized are liquid nitrogen or nitrous oxide. Following surgery, the cryotreated areas are allowed to necrose and heal by formation of granulation tissue. Several potential advantages of cryosurgery include relative analgesia of the treated tissue resulting from the destruction of sensory nerve endings, and the formation of very little fibrous tissue that theoretically results in less potential for anal stricture post operatively. A reduction in the prevalence of post operative anal stricture is not supported by the work of Vasseur.[15] The results of cryosurgical therapy for the treatment of perianal fistula are included in Table 1.

Tail Amputation: It has been hypothesized that a moist local environment in the perianal region, facilitated by a low tail carriage and broad tail base in the German Shepherd dog, may be a predisposing factor leading to the development of perianal fistulas.[11] Modification of the tail carriage temporarily using tail splints has been advocated by some. Van Ee and Palminteri[11] described the use of high caudectomy (tail amputation), at the level of the second or third caudal vertebrae, in 25 cases as a way to permanently alter this perianal microenvironment and facilitate resolution of the sinus tracts without the morbidity associated with conventional surgical techniques.[11] The results of tail amputation for the treatment of perianal fistula are included in Table 1. Interestingly a 20% recurrence was noted following initially successful resolution of fistulation. This highlights the probability that multiple factors are responsible for perianal fistulation.

Chemical Cauterization: Using this technique, superficial sinus tracts are first excised surgically, followed by chemical cauterization of the deeper fistulas. Chemical agents which have been utilized include 10% Lugol's solution,[13] 75% silver nitrate solution, or 80% solution of phenol.[28] The goal of chemical cauterization is to stimulate an acute inflammatory response resulting in second intention healing of the deep fistulas. The results of chemical cauterization for the treatment of perianal fistula are included in Table 1.

Laser Excision: The use of laser surgery to ameliorate perianal fistulation has only been recently reported. Ellison et al[16] described the use of a neodymium:yttrium aluminum garnet (ND:YAG) laser and synthetic sapphire scalpel to excise perianal fistulas in 20 dogs.[16] Using the laser scalpel, the affected tissues are excised via a process of thermal coagulation. This results in less thermal damage to surrounding tissue than that associated with traditional electrosurgical techniques while still providing excellent hemostasis.[16] In most of the cases in this study, the entire non-haired portion of the zona cutanea was removed, extending cranially into the intermediate zone. This approach frequently necessitated primary rectocutaneous closure and anal sacculectomy following fistulectomy. Although this technique is not readily available, it yielded excellent results, with lower morbidity rates compared with conventional surgical techniques. The results of laser excision for the treatment of perianal fistula are included in Table 1.

Unfortunately, all of these surgical techniques are invasive and painful, there is little agreement on which method of surgical treatment is most effective. None of the techniques is consistently curative, with multiple surgeries frequently required for successful treatment, especially in moderate to severe cases of perianal fistula. Surgical treatment is also associated with a high recurrence of perianal fistula (up to 70%) and a high risk of complications (up to 47%).[1,3,14] Complications frequently associated with surgery include intra operative and post operative hemorrhage, wound dehiscence, permanent fecal incontinence, anal stricture, and recurrence of fistulation.[29]

Medical Treatment for Perianal Fistula

Historically, medical therapy consisting of topical and systemic antibiotics, topical corticosteroids, topical antiseptics, local cleansing, anti-inflammatory medications, and the use of tail bracing has been generally ineffective, and palliative at best.[1-3,7,8,10,13] Results of successful non-surgical management of perianal fistula using oral cyclosporin, a drug that causes immunosuppression by suppressing T-cell mediated immune reactions,[53] were first published in 1997, and indicated full clinical healing of sinus tracts in 85-100% of cases, without any of the morbidity commonly associated with surgical management of perianal fistula.[2,5] The use of immunosuppressive therapy for the treatment of canine perianal fistula was prompted by the recognition of clinical similarities between canine perianal fistula and perianal fistula in people with Crohn's disease,[9] a chronic inflammatory bowel disorder of unknown etiology, and reports on the successful treatment of Crohn's-associated perianal fistula using cyclosporin.[43]

In a randomized, controlled clinical trial, Mathews et al demonstrated resolution of canine perianal fistula in response to immunosuppression using oral cyclosporin.[2,5] Dogs with naturally occurring perianal fistula were treated with oral cyclosporin[a] at an initial dose of 5 mg/kg every 12 hours. The whole blood trough cyclosporin concentrations (ie. concentrations 12 hours after the administration of cyclosporin) were measured weekly for the first three weeks, and oral cyclosporin dosages adjusted to obtain trough levels between 400-600ng/mL. Based on their findings, the authors suggested that lower oral dosages (between 1.75 and 3 mg/kg, every 12 hours) may be equally effective. These dogs were treated for 16 weeks and in 17 of 20 dogs the fistulas

[a] Sandimmune soft gel capsules, Novartis Pharma Canada Inc, Dorval, Quebec, Canada

were completely healed. In a clinical setting, the authors recommended that cyclosporin therapy be continued for as long as there is progressive improvement and for an additional four weeks after all of the fistulas have healed.[2,5]

At the dose used in that study, the side-effects of cyclosporin were mild, and included excessive shedding of guard hairs, transient lameness, and vomiting.[5] An increased risk of opportunistic infection is an adverse effect of cyclosporin in people.[54] Mathews and Sukhiani[5] reported no infections except in one dog that developed a pyometra while receiving cyclosporin. Nephrotoxicity, causing asymptomatic elevations in serum creatinine or manifested as acute renal failure, is one of the most limiting side-effects of systemic cyclosporin therapy in people. This adverse effect has not been documented in canine patients at the dosages used to treat perianal fistula.[5] Healing of the perianal fistulas occurred without any of the morbidity commonly associated with the surgical management of perianal fistula, such as fecal incontinence, anal stricture, or additional pain or discomfort.

The results of cyclosporin treatment for the treatment of perianal fistula are included in Table 1. The following section briefly introduces the origin of cyclosporin, its current role in the treatment of disease, and its pharmacodynamic and pharmacokinetic properties.

Table 1. Summary of results and complications after various techniques for the treatment of perianal fistulas (adapted from Matushek and Rosin[1])

Reference	Number of Cases	Successful Outcome (%)	Complications (%)		
			Recurrence[a]	Anal Stricture[b]	Fecal Incontinence[c]
Surgical Excision					
10	43	60	45	9	28
6	24	54	13	8	13
8	35	83	17	14	29
15	87	51	56	15	27
Deroofing and Fulguration					
14	30	60	70[d]	10	7
Cryosurgery					
51	40	88	10	3	0
6	46	50	7	0	7
49	35	97	0	6	0
15	64	88	45	47	16
Tail Amputation					
11	25	80	20	5	0
Chemical Cauterization					
28	28	96	17	0	0
13	20	87	15	5	20
Laser Excision					
16	20	95	20	not reported	20
Immunotherapy					
2	10	100	30	0	0
5	20	85	41	0	0
7	27	33 3	not reported	not reported	not reported

[a] Appearance of new lesions after resolution of the original fistulas

[b] Severe enough to require additional surgery

[c] Frequent incontinence or incontinence severe enough to result in euthanasia of the animal

[d] Any dog requiring multiple treatments was considered to be a recurrence

Cyclosporin

Cyclosporin is a lipophilic cyclic peptide (Figures 6 and 7) and was first isolated from the soil fungus *Tolypocladium inflatum* approximately 25 years ago.[55] Although

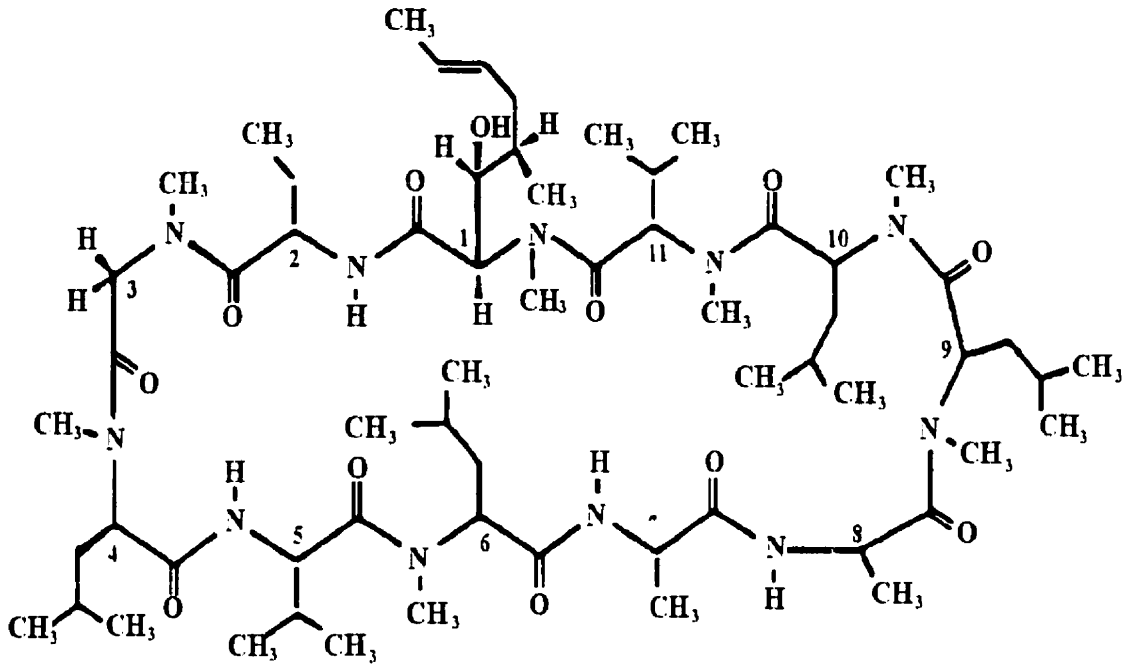

Figure 6: Chemical Structure of Cyclosporin (adapted from Mihatsch et al[56])

first studied to determine its potential for antibiotic properties, cyclosporin was found to possess potent immunosuppressive activity. It was introduced clinically in 1983 as a therapeutic agent to regulate immune rejection following renal allograft transplantation, and subsequently revolutionized the entire field of organ transplant therapy.[54,57] Since its introduction into clinical medicine, cyclosporin has been most widely utilized in the field of organ transplantation, but is now also widely used to modulate autoimmune diseases and allergic skin disease in people.[57,58]

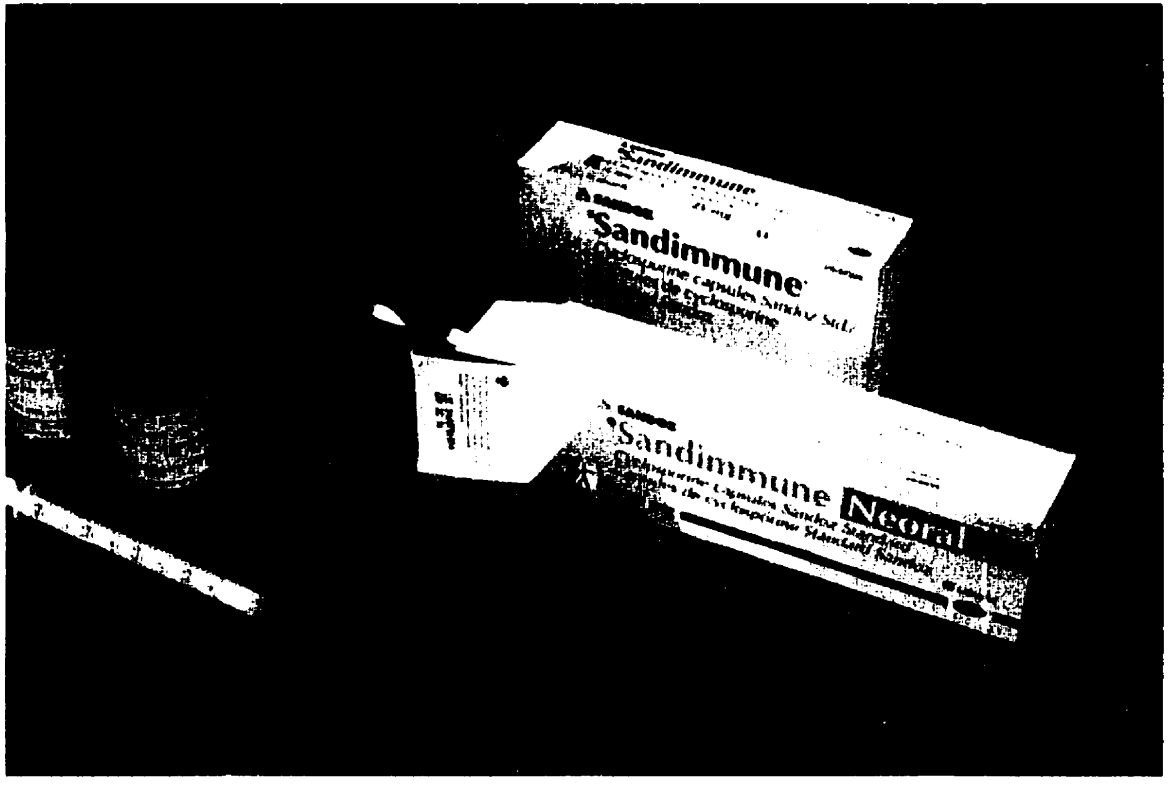

Figure 7. Oral suspension and capsules of cyclosporin

T lymphocyte function plays a crucial role in both rejection of transplanted allografts and in the pathogenesis of autoimmune disease.[57] Cyclosporin mediates immunosuppression by suppressing early events in T-lymphocyte activation, primarily by inhibiting the production of, and T lymphocyte responsiveness to, interleukin-2 (IL-2), as well as blocking other cytokines including interferon-γ, GM-CSF, TNF-α, and other interleukins (IL-3, IL-4).[53,55,57,59] Interleukin-2 is required for the activation and clonal expansion of T-helper and T-cytotoxic cells and for the maturation of other cell types involved in the cell-mediated immune response.[59] This action is most prominent in suppressing the activity of T-helper cells (CD4+ T-lymphocytes), which are the primary producers of IL-2 during an immune response as illustrated in Figure 8.[59,60] It does appears that T suppressor cell activation is unaffected by the action of cyclosporin.[59] As a result, the action of the T suppressor cells indirectly enhance the inhibitory effects of cyclosporin on T-cytotoxic cell functions.[59] As a result of this mode of activity, cyclosporin produces immunosuppression without causing myelosuppression or cytotoxicity, different from that of conventional immunosuppressive medications.[53] The adverse effects of cyclosporin are well documented in the human literature (Table 2).[53,54,56,59] The most severe adverse reactions are related to nephrotoxicity, which appears to be both dose dependent and cumulative in nature.[53,54] Although the

Table 2: Adverse Effects of Cyclosporin

Adverse Effect	Clinical Manifestation In People	Clinical Manifestation In Dogs
Nephrotoxicity	oliguria azotemia hyperkalemia hypertension metabolic acidosis	not documented
Hepatotoxicity	hyperbilirubinemia cholestasis elevated AST, ALT	not documented
Neurologic/ Orthopedic	fine tremors paresthesia generalized seizures encephalopathies	lameness
Dermatologic	hypertrichosis gingival hyperplasia acne	excessive shedding gingival hyperplasia
Gastrointestinal	anorexia gastric dilation nausea/vomiting diarrhea	 vomiting diarrhea
Immunologic	Increased risk of infectious disease Increased risk of lymphoproliferative disorders	not documented not documented
Hemostasis	thromboembolism	not documented

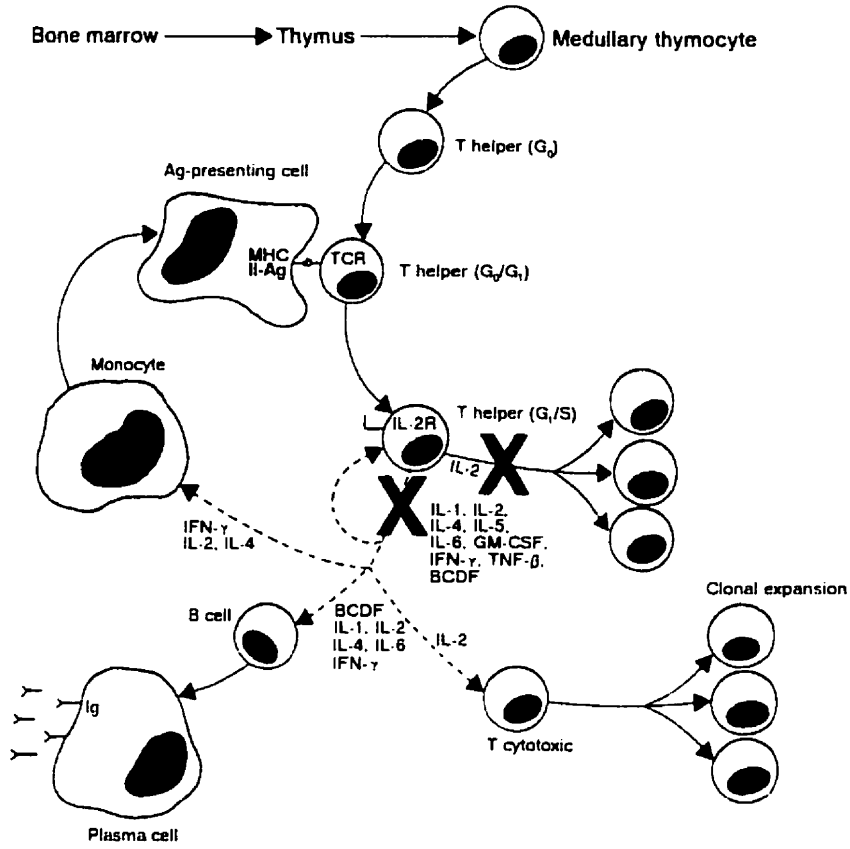

Figure 8: Schematic representation of the components of the immune response affected by cyclosporin. Abbreviations: Ag = antigen; BCDF = B cell differentiation factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN-γ = interferon-gamma; Ig = immunoglobulin; IL = interleukin; IL-2R = interleukin-2 receptor; MHC II = major histcompatibility complex; TCR = T cell receptor; TNF-β = tumor necrosis factor-beta (Adapted from Faulds, Goa, and Benfield [59])

mechanism of this nephrotoxicity has yet to be fully established, cyclosporin has an indirect renal vasoconstrictor effect, and cyclosporin-induced structural and functional changes occur in the proximal tubules and afferent arterioles, both of which may result in impaired renal function.[59] As already mentioned, this adverse effect has not been documented in canine patients at the dosages used to treat perianal fistula.[5]

With regard to cyclosporin for the treatment of perianal fistula, it may be recalled that cyclosporin mediates immunosuppression by suppressing the early events in T-

lymphocyte activation, primarily by inhibiting the production of, and T lymphocyte responsiveness to, interleukin-2 (IL-2). Interleukin-2 is required for the activation and clonal expansion of T-helper and T-cytotoxic cells; however, this action is most prominent in suppressing the activity of T-helper cells, which are the primary producers of IL-2 during an immune response as illustrated in Figure 8.[59] In his work, Day[17] showed that the lymphocytes from perivascular aggregates and lymphoid nodules in perianal fistula lesions were almost entirely CD3+ lymphocytes (T lymphocytes). The efficacy of cyclosporin in promoting the resolution of sinus tracts provides circumstantial evidence that these T lymphocytes may be T-helper lymphocytes (T_H cells). T_H cells are the principle regulators of the immune system since they direct and amplify the immune response and most autoimmune disease is promoted and perpetuated by T_H cells.[61] If perianal fistula is an immune-mediated disease, the efficacy of cyclosporin suggests that increased activation of T_H cells may be pivotal in its pathogenesis.

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CHAPTER II

THE HISTOLOGIC CHARACTERIZATION OF PERIANAL FISTULAS DURING TREATMENT WITH CYCLOSPORIN

INTRODUCTION

Canine perianal fistula is a chronic, progressive inflammatory and ulcerative disease involving the perianal region of the dog. The German Shepherd breed is uniquely predisposed to this disease, with sporadic occurrences of perianal fistulas in other large and medium sized breeds.[1-8] The disease is characterized clinically by the formation of single or multiple sinus tracts and severe ulceration and erosion of the perianal skin and deeper perianal tissue.[1,2,4,9] As the disease worsens, the extent of the sinus tracts progresses, with up to 360° perianal skin involvement.[4] Clinical signs associated with perianal fistulas commonly include tenesmus, dyschezia, malodorous mucopurulent discharge, excessive perianal licking, and constipation.[1,3] In severe cases dogs can also experience weight loss, lethargy, and rectal or anal bleeding.[1,3,5,6,10] In chronic cases, fibrosis of the rectal and perirectal tissues may result in worsening of tenesmus and constipation due to narrowing of the anal diameter.

Most reports indicate that male dogs are more frequently affected than females,[1,4,5,7,8,11-15] although some report no significant difference in gender distribution.[9,10,16,17] Sexually intact dogs, particularly males, also appear over-represented in many reports.[4,7,11-15] The disease most frequently affects middle-aged or

older dogs, averaging 5-6 years of age, but has been documented in dogs as young as 6 months-of-age.[4]

The etiopathogenesis of canine perianal fistulation has not been identified and less than optimal success, in conjunction with frequent recurrence, following surgical treatment may reflect failure to address the underlying primary cause of the disease.[7,8] Recent reports on the effectiveness of systemic immunosuppressive therapy using cyclosporin, a drug that suppresses T-lymphocyte action, suggests that immune dysregulation plays an important role in the development and/or progression of perianal fistulas.

The objective of this investigation was two-fold:

1) to characterize the histologic lesions of perianal fistula, with particular attention to how they change during an interval of clinical healing induced by chronic oral cyclosporin administration.
2) to clarify the pathogenesis of this poorly understood syndrome by identifying the initial target of the inflammatory process.

MATERIALS AND METHODS

This study was approved by the Animal Care Committee of the University of Guelph and adhered to the guidelines of the Canadian Council on Animal Care.

Fifteen dogs with naturally occurring perianal fistulas, representing consecutive cases referred to the Ontario Veterinary College-Veterinary Teaching Hospital, were included in the study. All dogs with clinical evidence of perianal fistulation were eligible for inclusion in the study, regardless of the severity of the lesions, duration of disease, or previous medical treatments. Exclusion criteria included previous anal sacculectomy or perineal surgery, and concurrent disease for which immunosuppressive therapy would likely compromise patient health. Owners of all dogs provided informed consent for immunosuppressive therapy prior to enrollment. The breed distribution included 12 German Shepherd dogs and one each of a German Shepherd cross, a Samoyed, and a Labrador Retriever.

Clinical Assessment and Sample Collection

A history, physical examination, complete blood count, and serum biochemical profile and urinalysis were performed at the time of enrollment into the study. All dogs were anesthetized for a rectal examination and biopsy acquisition. The depth of the sinus tracts, as determined using a blunt probe, and the surface area dimensions were measured directly. The severity of the perianal fistulas was then graded as mild, moderate, or severe (Table 3). Palpation characteristics of the rectum, anal canal, and anal ducts and sacs were recorded (See Table 4 for specific characteristics assessed).

Table 3. Clinical grading of perianal fistulas

Grade	Clinical appearance
Mild	one to two sinus tracts less than 1cm diameter **OR** depth
Moderate	3 or more sinus tracts in a multifocal distribution **OR** diameter and/or depth of individual tracts between 1-3 cm
Severe	greater than 3 sinus tracts **AND** diameter and/or depth greater than 3 cm

Biopsies of the zona cutanea, including normal (non-sinus) and sinus-associated skin, were obtained using a standard 6 mm dermal punch biopsy instrument[a] and the anal sac and duct from the clinically less severely affected side were surgically removed for histopathologic evaluation. Anal sacculectomy was performed using aseptic surgical technique and the surgical wound was closed in two layers. The subcutaneous tissues were apposed with interrupted suture of 4-0 glycolide dioxanone trimethylene carbonate,[b] and the skin apposed with interrupted sutures of 4-0 polypropylene[c]. All tissue biopsies were immediately fixed in 10% buffered formalin.

Following the initial evaluation and biopsy acquisition, all dogs were treated with oral cyclosporin[d] at an initial dose of 3-5 mg/kg per os every 12 hours. Whole blood samples were obtained 7-10 days following initiation of immunosuppressive therapy to determine trough blood cyclosporin concentrations (ie. concentrations 12 hours after the administration of cyclosporin) using a monoclonal radioimmunoassay[e]. The dose of cyclosporin was subsequently adjusted, if necessary, to ensure that trough levels were between 100-400 ng/mL (Table 5).

Dogs were re-evaluated 4 weeks after the initiation of cyclosporin therapy. For this evaluation dogs were sedated with oxymorphone hydrochloride[f] (0.1 mg/kg) and acepromazine maleate[g] (0.05 mg/kg) to evaluate the degree of sinus tract healing and obtain additional biopsies of the zona cutanea, including healed sinus-associated skin and sinus-associated skin. The healed sinus biopsies were obtained from regions previously

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[a] Acu-Punch® biopsy punch, Acuderm Inc, Ft. Lauderdale, Florida, USA
[b] Biosyn, United States Surgical Corporation, Norwalk, Conneticut, USA
[c] Surgipro, United States Surgical Corporation, Norwalk, Conneticut, USA
[d] Sandimmune soft gel capsules, Novartis Pharma Canada Inc, Dorval, Quebec, Canada
[e] Cyclotrak, Inkstar, Minneapolis, Minnesota, USA

affected by sinus tracts prior to immunotherapy. Each biopsy site was infiltrated with 0.5 mL of 2% lidocaine[h] to provide local anesthesia during the biopsy procedure.

Dogs were treated with cyclosporin until closure of perianal sinus tracts was documented. At that time dogs were anesthetized for examination of the perianal area and biopsy acquisition. Biopsies of the zona cutanea were obtained, as described above, and the remaining anal sac and duct were surgically removed for histopathologic evaluation. This final zona cutanea biopsy was obtained from a region previously affected with sinus tracts to most accurately represent the changes associated with sinus healing. Following acquisition of these final biopsies, cyclosporin therapy was then continued for an additional 4 weeks thereafter to reduce the risk of recurrence.[5]

Biopsy Processing and Histologic Technique

Formalin-fixed biopsies from the zona cutanea were bisected longitudinally using a scalpel blade and embedded in paraffin to obtain an orientation such that any hair follicles would be seen in sagittal section. Formalin-fixed anal ducts/sacs were sectioned transversely at multiple sites and imbedded in paraffin. The embedded tissues were cut using a Leitz microtome[i] to create 6 μm sections and stained with hematoxylin and eosin (H&E) and Masson trichrome stain for examination. Stained sections were evaluated by light microscopy.

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[f] Numorphone, DuPont Pharmaceutical, Mississauga, Ontario, Canada
[g] Atravet, Ayerst Veterinary Laboratory, Guelph, Ontario, Canada
[h] Lidocaine HCl 2%, Ayerst Veterinary Laboratory, Guelph, Ontario, Canada
[i] Ernst Leitz Wetzlar GMBH, West Germany

Descriptive Technique

1. Zona Cutanea

Qualitative analysis of the zona cutanea included characterization of the inflammatory infiltrate, in both sinus and non-sinus associated skin, and its association with hair follicles, periadnexal/glandular structures, and anal sphincter muscle. The overall severity of histologic changes at each stage of treatment was graded on a scale of 0 to 3; grade 0, normal tissue architecture and few to no inflammatory cells; grade 1, normal tissue architecture preserved, with heavy multifocal to diffuse inflammation; grade 2, normal tissue architecture lost and replaced with inflammatory cells; grade 3, normal architecture lost and replaced by fibrous connective tissue and residual foci of inflammation. The quantitative cellularity of the inflammatory infiltrate was graded on a scale of 1 to 3; grade 1, 0-5% inflammatory cellularity; grade 2, 5-25% inflammatory cellularity; grade 3, greater than 25% inflammatory cellularity. Periadnexal inflammation was specifically singled out for evaluation. It, too, was graded on a scale of 1 to 3; grade 1, mild; grade 2, moderate; grade 3, severe, and included individual assessments of each of the three glandular structures located in the cutaneous zone: apocrine, sebaceous, and hepatoid circumanal glands, as well as external anal sphincter muscle. A subjective assessment regarding the degree of fibrous replacement of normal tissue architecture was also made by assessing the trichrome stained sections. Differentiation of primary versus secondary inflammation was made based on the presence or absence of intraepithelial or intraluminal inflammatory infiltrate associated with the various glandular structures of the zona cutanea.[23]

2. Anal Sacs

Qualitative analysis of the anal sacs included characterization of the inflammatory infiltrate, and its association with the epithelium, subepithelium, and glands of the anal sacs. The overall severity of histologic changes prior to, and following completion of, treatment was graded on a scale of 0 to 3; grade 0, normal tissue architecture and few to no inflammatory cells; grade 1, normal tissue architecture preserved, with heavy multifocal to diffuse inflammation; grade 2, normal tissue architecture lost and replaced with inflammatory cells; grade 3, normal architecture lost and replaced by fibrous connective tissue and residual foci of inflammation. The quantitative cellularity of the inflammatory infiltrate was graded on a scale of 1 to 3; grade 1, 0-5% inflammatory cellularity; grade 2, 5-25% inflammatory cellularity; grade 3, greater than 25% inflammatory cellularity. The target for the inflammation was noted, including surrounding sphincter muscle, apocrine glands, and subepithelium, and was graded on a scale of 1 to 3; grade 1, mild; grade 2, moderate; grade 3, severe. A subjective assessment regarding the degree of fibrous replacement of normal tissue architecture was also made by assessing the trichrome stained sections.

RESULTS

A. Clinical Results

The age at presentation ranged from 2 to 13.5 years, with a mean of 6.2 years. Ten out of the 15 cases were male dogs (2:1 male-to-female ratio), and of these, nine were sexually intact. This contrasted with the 5 female dogs, all of which were spayed

(Table 6). The most common clinical signs of perianal fistula reported by owners were excessive perianal licking and tenesmus (11/15 and 10/15 respectively). Weight loss was described in 6/15 cases, dyschezia was reported infrequently (3/15), and lethargy was reported in only one case. Owners of two dogs reported a previous history of anal sac disease and 7 cases had history of recurrent skin or ear infections (Table 7).

The results of individual pre-treatment clinical examination findings are summarized in Table 4. Three cases were classified with severe, nine with moderate, and three with mild perianal fistulation, based on the three dimensional extent of sinus tract formation in the zona cutanea (See Table 3 for clinical grading criteria). Seven of the 15 dogs also had some degree of involvement of the zona intermedia, zona columnaris, and/or rectum based on the results of digital rectal examination, and four dogs subjectively had mild to moderate narrowing of the anal canal due to fibrosis at the time of initial examination. Clinical evidence of anal sac/duct involvement as evidenced by palpable fibrosis, whether primary or secondary, was present in 13 cases.

Of the 15 dogs initially enrolled in the study, only 12 dogs experienced full healing of the fistulas or were still actively enrolled by the end of the study period (Table 5). All of the dogs treated with the full cyclosporin protocol experienced full clinical healing of the sinus tracts (Figures 9-11). For these 12 dogs, the average duration of therapy in order to obtain full healing was 10.7 weeks, and ranged from four to 20 weeks (Table 5). In general, milder cases healed faster. Of the three dogs that did not complete the study, one dog (case 3) was unavailable for final assessment and biopsy at 16 weeks, one dog (case 8) developed hepatoid circumanal gland adenocarcinoma and was euthanized prior to completion of the study, and the third dog (case 14) had severe

gastrointestinal side effects secondary to oral cyclosporin at the standard dosage. In order to eliminate the adverse side effects in case 14, the cyclosporin dose was ultimately reduced to 2.3 mg/kg given only once daily, and the sinus tracts failed to heal at this low dose.

Side-effects or complications directly related to cyclosporin therapy were mild. The most frequent side-effect was generalized excessive shedding of guard hairs (without alopecia), noted in 6 dogs. Three dogs experienced a shifting weight-bearing lameness and three dogs showed gingival hyperplasia during treatment with cyclosporin. The lameness was always responsive to non steroidal anti-inflammatory medication and resolved following discontinuation of cyclosporin therapy. Obvious patient lethargy was reported by owners of two dogs, and one dog experienced severe vomiting and diarrhea in association with cyclosporin at the standard dosage.

Four of the 12 dogs that underwent full healing of the perianal fistulas experienced recurrence of sinus tracts following termination of cyclosporin therapy. The times to recurrence are listed in Table 5. There did not appear to be any correlation between recurrence (or time to recurrence) and any measurable variable from case to case, including gender, neuter status, oral cyclosporin dose, trough blood cyclosporin concentration, time to healing, or clinical severity or histopathologic severity of the disease.

Table 4: Pre-Treatment Perianal and Rectal Examination Findings in 15 dogs with naturally occurring Perianal Fistula

Case #	Cutaneous Zone	Extent of Perianal Fistula Involvement	Rectal Mucosa	Presence of Anal Stricture	Anal Sacs
1	:moderate hepatoid hyperplasia	0° - 360° s.a.[2], 11 mm depth[3] :severe[4]	smooth except at zona inter-media	mild	R[5]: severe periductal fibrosis L[6]: mildly distended and expressible
2	only PAF[1]	180° - 360° s.a. 20 mm depth :moderate bilaterally symmetrical	smooth and regular	none	:both anal sacs moderately distended and expressible
3	only PAF	90° - 360° s.a. 25 mm depth :severe	smooth and regular	none	R:moderately distended easily expressible L: normal
4	only PAF	320° - 30° s.a. 40 mm depth :moderate	smooth and regular	none	R: moderate fibrosis of duct and sac L: mildly distended
5	only PAF	90° - 270° s.a. 17 mm depth :moderate	smooth and regular	none	R: normal L: moderate fibrosis
6	only PAF	130° - 150° s.a. 2 mm depth :mild	smooth and regular	none	R: mild fibrosis of sac L:moderate fibrosis
7	only PAF	340° - 20° s.a. 12 mm depth :moderate	smooth and regular	none	R:severe periductal fibrosis L: normal
8	moderate hepatoid hyperplasia	no sinus tracts 0° - 360° erosive lesions :moderate	smooth but bled readily on exam	none	R: moderate periductal fibrosis L: mild fibrosis both sacs expressible
9	only PAF	90° - 270° s.a. 27 mm depth :moderate	smooth and regular	none	:moderate periductal fibrosis bilaterally

Table 4: Continued

Case #	Cutaneous Zone	Extent of Perianal Fistula Involvement	Rectal Mucosa	Presence of Anal Stricture	Anal Sacs
10	mild hepatoid hyperplasia	very little s.a. involvement, 4 multfocal regions with depths as great as 32 mm bilaterally symmetrical	moderately roughened and firm	moderate	R: moderate fibrosis L: moderate fibrosis
11	entire tail base & anal plate shows severe erythema and bruising	280° - 320° s a 5 mm depth mild	smooth, but submucosal fibrosis dorsally	mild	both anal sacs small bloody discharge expressed from right anal sac
12	only PAF	0° - 200° s.a. 16 mm depth moderate	mildly irregular	mild	R: moderate fibrosis of sac and duct L: mild fibrosis
13	only PAF	180° - 360° s.a. 5 mm depth moderate bilat.sym.	mild roughening	none	R: mild fibrosis L: mild fibrosis
14	only PAF	340° - 90° s.a. 27 mm depth moderate	mod. roughening	none	R: severe periductal fibrosis L: normal
15	only PAF	90° - 360° s.a. 3 mm depth moderate	smooth and regular	none	R: moderate fibrosis L: normal

[1] perianal fistulas the only abnormality
[2] perianal surface area involvement
[3] perianal sinus greatest measurable depth
[4] overall severity of perianal fistulation
[5] right anal sac
[6] left anal sac

Table 5: Case Clinical Treatment Data for 15 dogs with naturally occurring Perianal Fistula

Case Number	Time to PAF[1] Healing (weeks)	Complications/ Side Effects	Time to Recurrence (months)[3]	Disease-Free Interval[6] (months)	CsA[2] Dose (mg/kg, BID)	Trough Blood CsA[2] Level (ng/mL)
1	16	gingival hyperplasia.,shedding		30	4.5	NA[5]
2	20	shedding		30	3.9	215
3	NA[4]	shedding	NA[4]	NA[4]	3.1	230
4	16	gingival hyperplasia		28	4.3	150
5	12	pararectal abscess		27	4.9	240
6	4	gingival hyperplasia, lethargy		27	3.9	65
7	12	lameness	3.5		5	275
8	NA[4]	none	NA[4]	NA[4]	4.5	105
9	12	lethargy, lameness		25	4.4	NA[5]
10	12	shedding	11		3.7	375
11	4	lameness		23	5	110
12	12	shedding	8		4	NA[5]
13	4	none		20	5	NA[5]
14	NA[4]	severe vomiting/diarrhea, shedding	NA[4]	NA[4]	2.3[a]	NA[5]
15	4	none	1		4.3	440

[1] Perianal Fistula

[2] Cyclosporin

[3] for dogs with recurrence of perianal fistula during the 30 month observation period

[4] data not available; dog did not complete treatment protocol

[5] data not available

[6] for dogs with no recurrence of perianal fistula during the 30 month observation period

[a] administered only once daily due to gastrointestinal side effects

Table 6: Signalment and Perianal Fistula History for 15 dogs with naturally occurring Perianal Fistula

Case Number	Breed	Gender	Age (years)	PAF Duration prior to Immunotherapy (months)
1	German Shepherd	intact male	9.5	6
2	German Shepherd	intact male	3	8
3	German Shepherd X[1]	spayed female	13.5	2
4	German Shepherd	intact male	5.5	2
5	German Shephed	intact male	6	2
6	German Shephed	intact male	2	6
7	German Shepherd	spayed female	6	4
8	German Shepherd	spayed female	7	1
9	German Shepherd	spayed female	8	8
10	Samoyed	intact male	4.5	9
11	German Shepherd	intact male	4.5	36
12	German Shepherd	intact male	8	2
13	German Shepherd	spayed female	4	3
14	German Shepherd	intact male	4	14
15	Labrador Retriever	castrated male	7	24

[1] German Shepherd Cross

Table 7: Case clinical signs of 15 dogs with naturally occuring perianal fistula prior to treatment with oral cyclosporin

Case Number	Excessive Perianal Licking	Tenesmus	Dyschezia	Diarrhea	Weight Loss	Appetite	Lethargy	Previous History of Anal Sac Disease	History of Recurrent Skin/ Ear Infections
1	yes	yes	no	no	yes	decreased	no	no	yes
2	yes	no	no	no	no	normal	no	no	yes
3	unknown	no	no	no	yes	normal	no	no	no
4	no	no	no	no	no	normal	no	no	yes
5	no	yes	no	yes	yes	normal	no	yes	yes
6	yes	yes	no	no	yes	decreased	no	no	no
7	yes	yes	no	yes	yes	normal	no	yes	no
8	yes	yes	yes	yes	yes	normal	no	no	no
9	no	yes	no	no	no	normal	no	no	yes
10	yes	yes	yes	no	no	normal	yes	no	no
11	yes	yes	yes	no	no	decreased	no	no	yes
12	yes	yes	no	yes	no	normal	no	no	yes
13	yes	no	no	no	yes	normal	no	no	no
14	yes	no	no	no	no	normal	no	no	no
15	yes	yes	no	yes	no	normal	no	no	yes

Figure 9.1 Moderate perianal fistula in a six-year-old, intact male German Shepherd (case 5) prior to treatment with cyclosporin.

Figure 9.2 Same dog as in Fig. 9.1. Healed perianal fistulas following 12 weeks of oral cyclosporin.

Figure 10.1 Moderate perianal fistula in a six-year-old, spayed female German Shepherd (case 7) prior to treatment with cyclosporin.

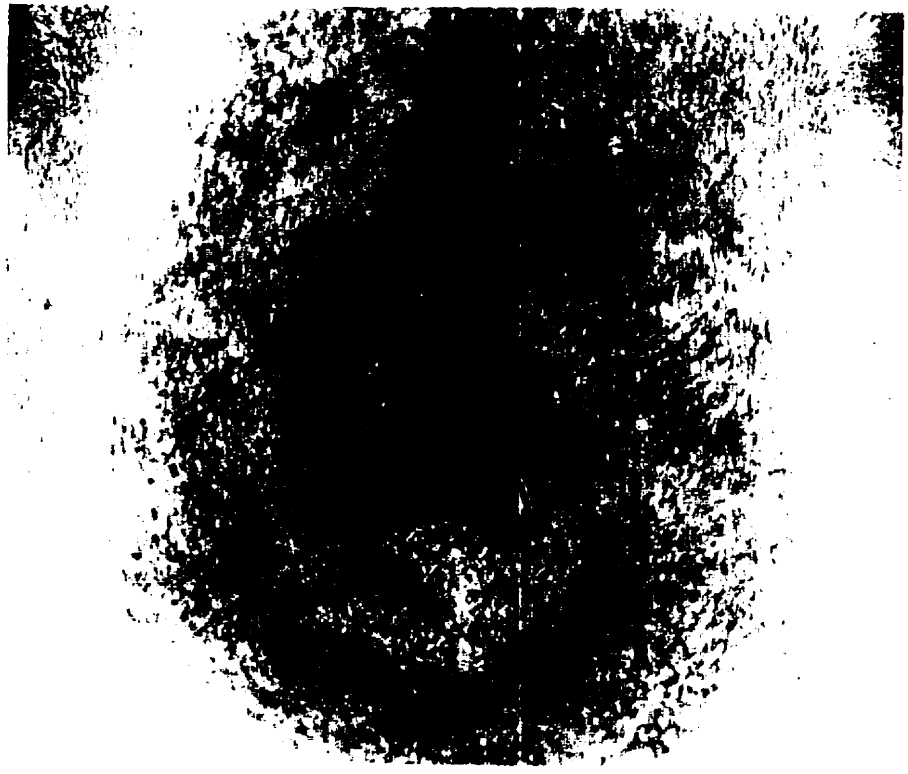

Figure 10.2 Same dog as in Fig. 10.1 Healed perianal fistulas following 12 weeks of cyclosporin.

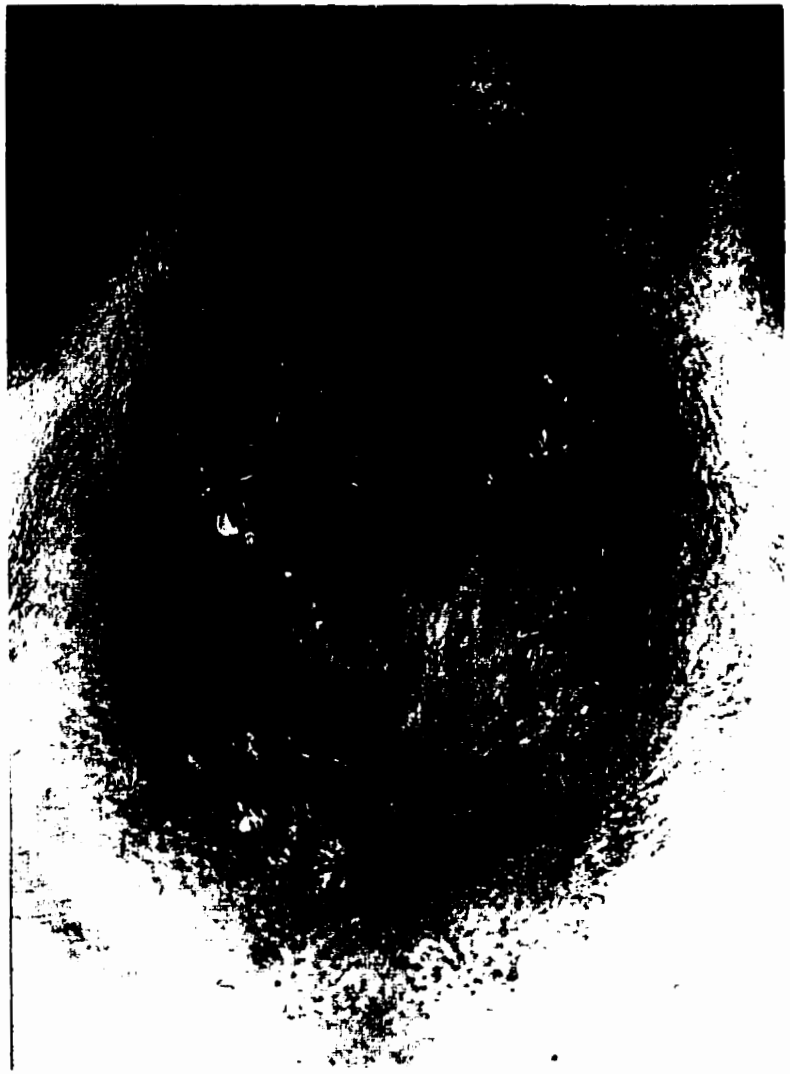

Figure 11.1 Moderate perianal fistula in an eight-year-old, intact male German Shepherd (case 12) prior to treatment with cyclosporin.

Figure 11.2 Same dog as in Fig. 11.1. Healed perianal fistulas following 12 weeks of cyclosporin.

B. Histologic Results

In total, 55 hematoxylin and eosin stained histologic sections, taken from the zona cutanea before and during treatment with oral cyclosporin, were examined.

1a. Non-sinus Associated Skin

The histologic appearance of normal, non-sinus associated skin from the zona cutanea of the anal canal was characterized by a keratinized stratified squamous epithelium (epidermis and dermis) superficially, and subcutis beneath. Five distinct structures occupied the dermis and subcutis, including variably present hair follicles, paratrichial sebaceous glands, apocrine sweat glands, external anal sphincter muscle fibers, and abundant hepatoid circumanal glands. Hepatoid circumanal glands were by far the predominant structures occupying the subcutis and were arranged as variably sized solid lobules of pink cuboidal "hepatoid" cells, with lobules grouped together in larger clusters (Figures 12 and 13). These clusters were situated throughout the entire subcutis, in superficial, mid-, and deep sections. Individual lobules typically had smaller, flattened cuboidal germinal cells as an incomplete ring at the periphery. Apocrine gland acini tended to be arranged in single linear columns with hepatoid circumanal gland clusters separating adjacent columns from one another. Occasionally, apocrine gland acini were arranged as small clusters, similar to the arrangement seen with the intramural tubuloalveolar apocrine glands of the anal sacs. The apocrine gland columns were generally associated with hair follicles, but not exclusively so.

Figure 12. Histologic appearance of the dermis and subcutis of normal zona cutanea. Note the abundance of hepatoid circumanal glands (large arrows) and the vertically linear arrangement of the apocrine glands (small arrows). Masson's trichrome, X 100

Figure 13. Magnified appearance of the hepatoid circumanal gland lobules. X 1000

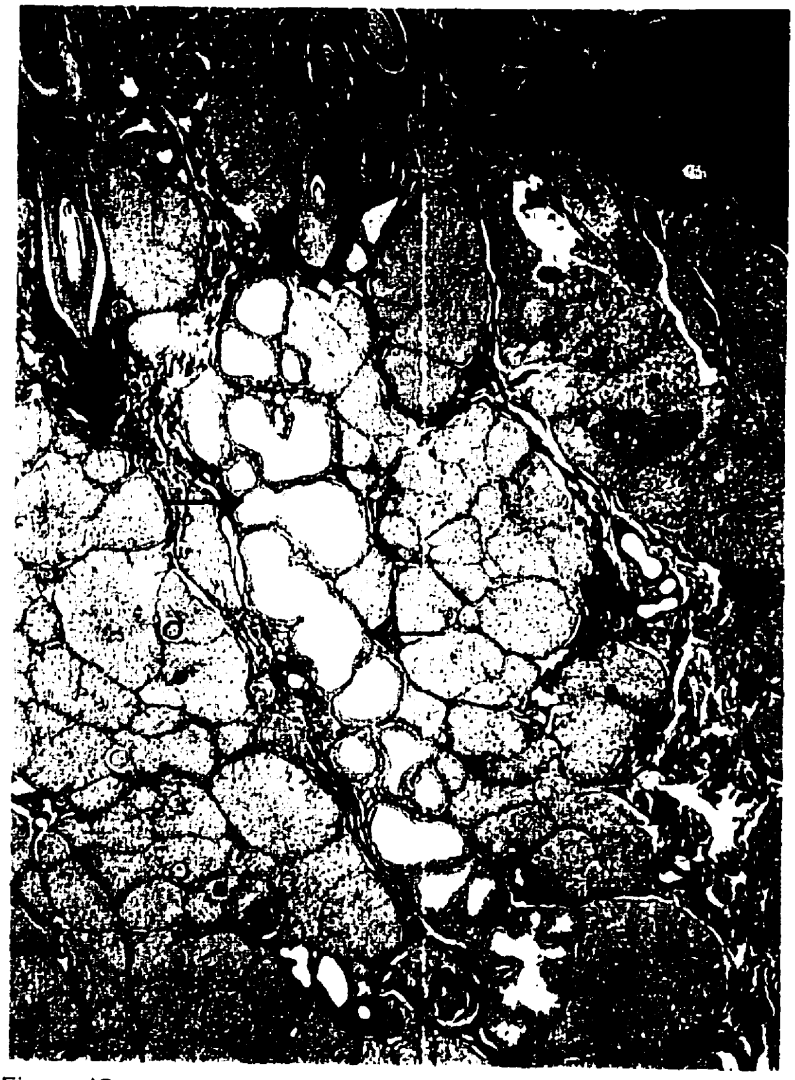

Figure 12.

Figure 13.

The pre-treatment epithelium and subcutaneous regions of sections from non-sinus associated zona cutanea were microscopically normal in the majority of cases (Table 8). Two cases exhibited a mild, non-specific, diffuse mononuclear inflammation of the subepithelial tissue, and associated superficial perivascular dermatitis, and one case exhibited a similar, but eosinophilic, inflammatory pattern. Four cases showed focal or multifocal mononuclear inflammation directly associated with hepatoid circumanal gland lobules, and this mononuclear inflammation tended to be superficial and perifollicular.

In histologic sections from healed sinus skin following 4 weeks of cyclosporin therapy, the epidermis, dermis, and superficial and deep subcutaneous regions were microscopically normal, with only mild differences when compared with the pre-treatment normal skin. Eleven of 12 sections evaluated showed mild persistence of mononuclear inflammation (Table 9). In all but one of these cases the inflammatory cells were most obvious around and involving the hepatoid circumanal gland lobules (Figure 14-16), with or without concurrent inflammation involving sebaceous or apocrine glands. In the one case in which hepatoid circumanal gland lobules were not affected, mild inflammation was associated with sebaceous glands. All sections evaluated showed either atrophy of hepatoid circumanal glands, with evidence of regenerating nests, and/or increased dissecting fibrosis among hepatoid circumanal gland lobules.

When histologic sections from healed sinus skin following 16 weeks of cyclosporin therapy were compared with normal pre-treatment samples to demonstrate differences between normal perianal skin and previously affected, but healed, regions of perianal skin, the differences were minor. With regards to inflammation, the epithelium and subcutaneous regions of post-treatment sections were microscopically normal in the

Table 8: Histology of Non-sinus Associated Zona Cutanea from 12 dogs with naturally occurring perianal fistula prior to treatment with cyclosporin

Case Number	SPD[fd]	Hair Follicles Present	Inflammatory Cellularity[a]	Predominant Inflammation[b]	Severity of Histologic Changes[c]	Epidermis	Dermis	Circumanal Gland Adenitis[d]	Squamous Metaplasia[d]	Apocrine Adenitis[d]	Sebaceous Adenitis[d]	Folliculitis/ Furunculosis[d]
1	1	yes	grade 1	P/L*	0	normal	mild edema	none	3	none	none	none
2	3	yes	grade 3 multifocal	P/L*	1	normal	mod. inflam.	3/focal	none	none	3/focal	none
4	0	yes	grade 1	none	0	normal	normal	none	2	none	none	none
5	2*	yes	grade 1	E	0	normal	SPD[f]/edema	none	none	none	none	none
6	0	no	grade 1	none	0	normal	normal	none	none	none	none	CE[g]
7	2	yes	grade 1	none	0	normal	normal	none	1	none	none	none
9	2	yes	grade 3 focal	L*	2 focal	normal	SPD[f]	2	1	none	2	none
10	0	yes	grade 1	none	0	normal	edema	none	none	none	none	none
11	2	yes	grade 1	mixed	1	normal	SPD[f]	none	none	none	2	none
12	0	yes	grade 1	none	0	normal	normal	none	none	none	none	none
13	1	yes	grade 1	mixed	2	normal	SPD[f]	2	none	none	1	none
15	1	yes	grade 1	L	1	normal	SPD[f]	1	none	none	2	none

* = large eosinophil component

[a] grade 1 = 0-5% inflammatory cells; grade 2 = 5-25% inflammatory cells; grade 3 > 25% inflammatory cells

[b] P = plasmacytic; L = lymphocytic; M = macrophage; E = eosinophil; N = neutrophil; mixed = pyogranulomatous

[c] 0 = normal architecture

1 = normal architecture but heavy multifocal to diffuse inflammation

2 = normal architecture replaced by inflammatory cells

3 = normal architecture replaced by fibrous connective tissue

[d] 1 = mild; 2 = moderate; 3 = severe

[f] superficial perivascular dermatitis

[g] cannot evaluate

Table 9: Histology of healed sinus associated Zona Cutanea from 11[h] dogs with naturally occurring perianal fistula at 4 Weeks post-treatment with cyclosporin

Case Number	SPD[af]	Hair Follicles Present	Inflammatory Cellularity[a]	Predominant Inflammation[b]	Severity of Histologic Changes[c]	Epidermis	Dermis	Circumanal Gland Adenitis[d]	Squamous Metaplasia[d]	Apocrine Adenitis[d]	Sebaceous Adenitis[d]	Folliculitis/ Furunculosis[d]
1	0	yes	grade 1	L	1	OHK[e]	mild edema	1	2	none	1	none
2	0	yes	grade 1	L	1	normal	normal	3/focal	none	none	3/focal	none
4	0	yes	grade 1	L/P*	1	normal	normal	none	none	none	1	none
5	0	no	grade 1	not applicable	0	normal	normal	none	1	none	none	CE[g]
6	0	yes	grade 1	P/L	1	normal	normal	1	1	none	1	none
7	1	yes	grade 1	L/P	1	normal	normal	1	1	none	none	none
9	1	yes	grade 1	L/P*	2	normal	SPD[f]	1	none	none	2	none
11	0	yes	grade 1	L	1	normal	normal	1	none	none	1	none
12	1	yes	grade 1	L	1	normal	SPD[f]	1	1	none	1	none
13	1	yes	grade 2	L/P	2	normal	SPD[f]	2	1	none	2	none
15	0	yes	grade 1	L	1	normal	normal	1	none	none	1	none

* = large eosinophil component

[a] grade 1 = 0-5% inflammatory cells; grade 2 = 5-25% inflammatory cells; grade 3 > 25% inflammatory cells

[b] P = plasmacytic; L = lymphocytic; M = macrophage; E = eosinophil; N = neutrophil; mixed = pyogranulomatous

[c] 0 = normal architecture
1 = normal architecture but heavy multifocal to diffuse inflammation
2 = normal architecture replaced by inflammatory cells
3 = normal architecture replaced by fibrous connective tissue

[d] 1 = mild; 2 = moderate; 3 = severe

[e] orthokeratotic hyperkeratosis

[f] Superficial Perivascular Dermatitis

[g] cannot evaluate

[h] case number 10 was unavailable for biopsy at 4 weeks

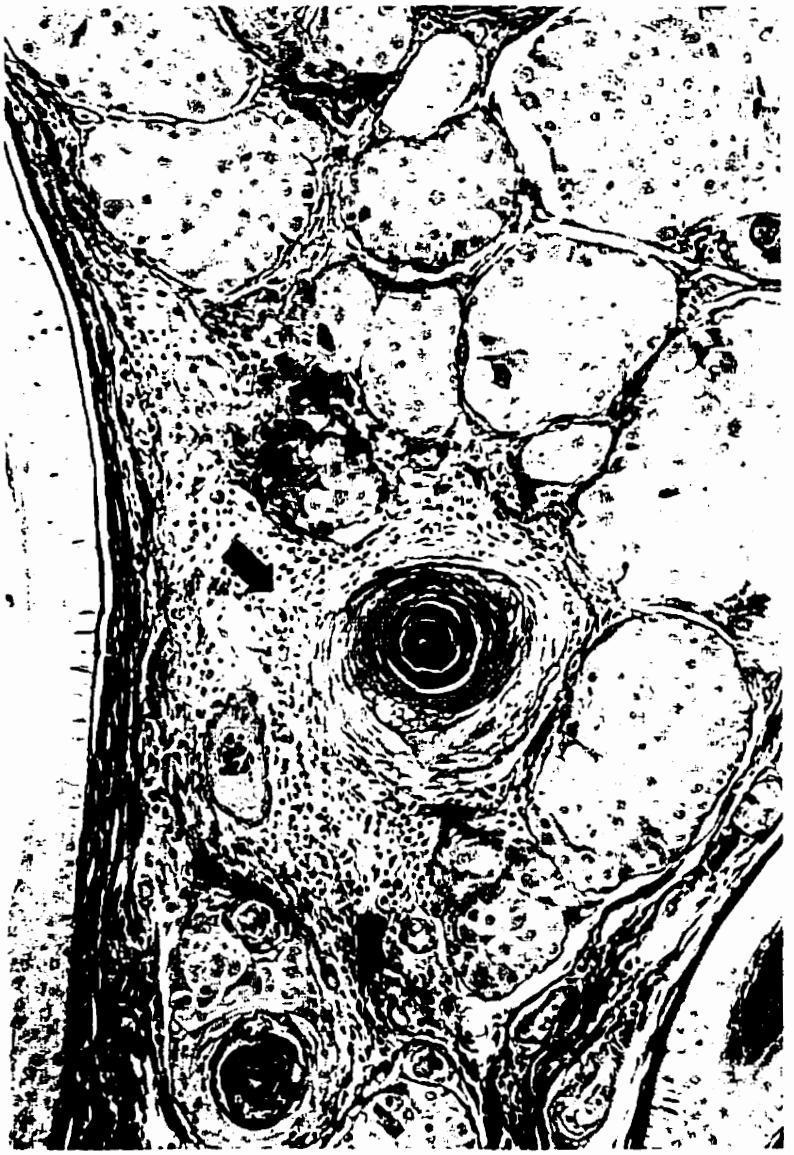

Figure 14. Typical healed sinus skin 4 weeks following initiation of treatment (case 13). Note the mild persistence of mononuclear inflammation around and through the superficial hepatoid circumanal glands (arrows). Masson's trichrome, X 400

Figure 15. Typical healed sinus skin 4 weeks following initiation of treatment (case 2). Note the mild persistence of mononuclear inflammation around and through the superficial hepatoid circumanal glands (arrow). Masson's trichrome, X 400

Figure 16. Typical healed sinus skin 4 weeks following initiation of treatment (case 6). Note the mild persistence of mononuclear inflammation around and through the superficial hepatoid circumanal glands (arrow). Masson's trichrome, X 400

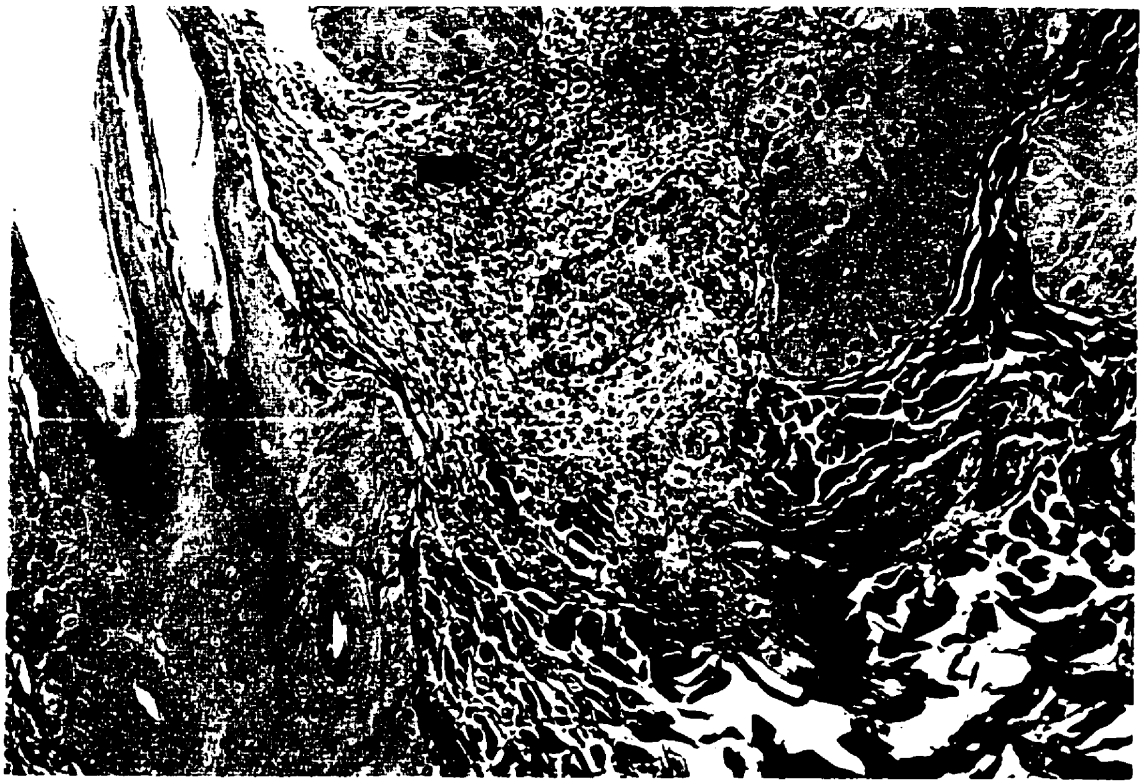

Figure 15.

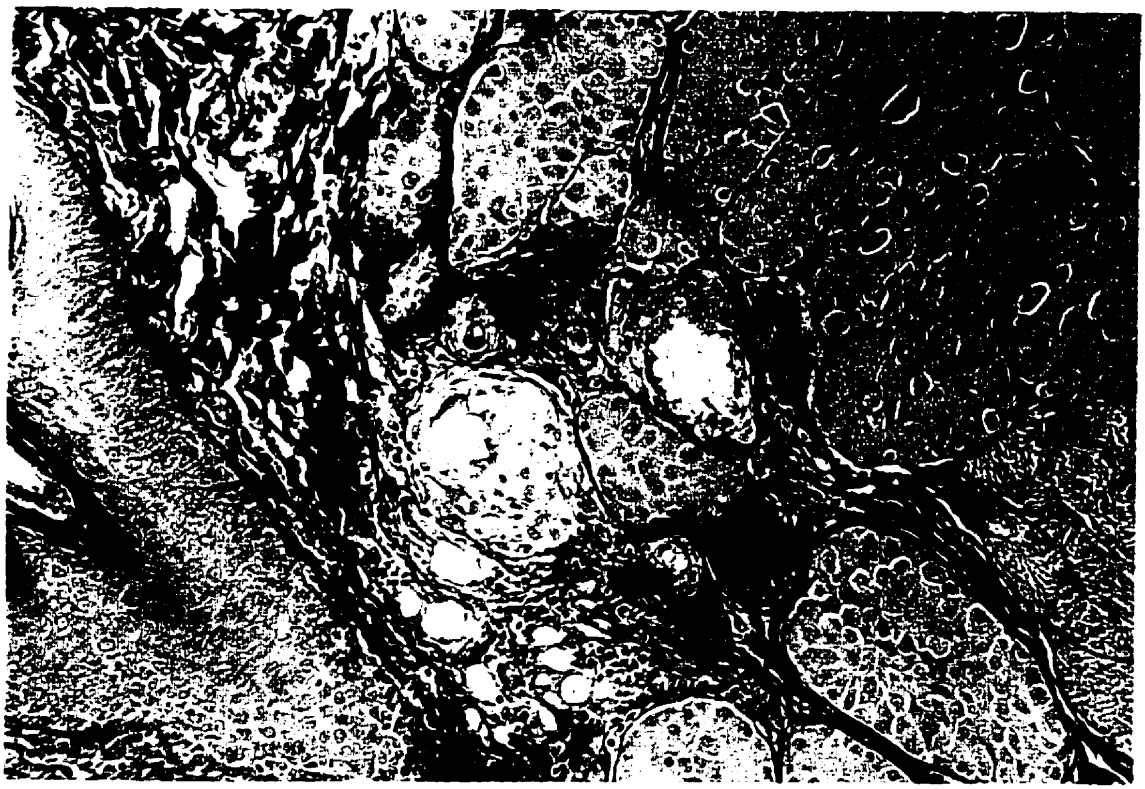

Figure 16.

majority of cases. Residual inflammation was sometimes associated with apocrine glands and hair follicles, but was most commonly associated with hepatoid circumanal glands. In the majority of cases the apocrine glands were normal. In several cases the hepatoid circumanal glands appeared more numerous than in comparable pre-treatment sections, but they were also smaller, with increased interlobular fibrosis. There was also a tendency for the hepatoid circumanal glands to be irregularly organized instead of being organized in the normal distinct multilobulated arrangements (Table 10).

1b. Sinus Associated Skin

Histologic lesions from pre-treatment sinus associated skin was characterized by moderate to severe, superficial and deep obliterative inflammation (grade 3), consisting mostly of lymphocytes and plasma cells, and varying degrees of replacement of normal architecture by fibrous tissue (Figure 17, Table 11). If the inflammation was not diffuse and uniform, the superficial structures were usually more severely affected than deeper structures. Glandular changes included inflammation and dilation of the apocrine glands and infiltration and obliteration of hepatoid circumanal gland lobules by inflammatory infiltrate, especially superficially. Occasionally mild sebaceous adenitis was noted. Hepatoid circumanal gland lobules were less densely organized and individual lobules appeared smaller than normal. As well, the apocrine glands appeared more densely clustered than normal (Figures 18 and 19). In the most severely affected sections, the only structures persisting in many instances were small clusters of collapsed apocrine glands surrounded by fibrosis and inflammatory infiltrate. No intraluminal inflammation was associated with the apocrine glands. Primary disease directly affecting the hair

Table 10: Histology of Healed Sinus Associated Zona Cutanea from 12 dogs with naturally occurring perianal fistula at full clinical healing following treatment with cyclosporin

Case Number	SPD[af]	Hair Follicles Present	Inflammatory Cellularity[a]	Predominant Inflammation[b]	Severity of Histologic Changes[c]	Epidermis	Dermis	Circumanal Gland Adenitis[d]	Squamous Metaplasia[d]	Apocrine Adenitis[d]	Sebaceous Adenitis[d]	Folliculitis/ Furunculosis[d]
1	1	yes	grade 1	P/L	1	normal	SPD[f]	none	mild	none	none	none
2	0	yes	grade 1	P/L	1	normal	normal	none	none	1/focal	1	none
4	0	yes	grade 1	L	1	normal	normal	2	1	none	1	none
5	1	yes	grade 1	L	1	normal	edema	none	2	none	1	none
6	1	yes	grade 1	P/L	1	normal	SPD[f]	none	1	none	none	none
7	0	yes	grade 1	L	1	normal	normal	none	none	none	1	none
9	1	yes	grade 1	L/P*	1	normal	SPD[f]	1	1	none	1	none
10	0	yes	grade 1	L/P	1	normal	normal	1	1	none	1	none
11	2	yes	grade 2	P/L	2	normal	SPD[f]	2	1	2	1	none
12	0	yes	grade 1	L/P	1	normal	normal	none	1	none	1	none
13	1	yes	grade 1	L/P	1	normal	SPD[f]	none	none	none	1	none
15	0	yes	grade 1	L	3	normal	normal	CE[g]	CE	2	2	none

* = large eosinophil component

[a] grade 1 = 0-5% inflammatory cells; grade 2 = 5-25% inflammatory cells; grade 3 > 25% inflammatory cells

[b] P = plasmacytic; L = lymphocytic; M = macrophage; E = eosinophil; N = neutrophil; mixed = pyogranulomatous

[c] 0 = normal architecture

1 = normal architecture but heavy multifocal to diffuse inflammation

2 = normal architecture replaced by inflammatory cells

3 = normal architecture replaced by fibrous connective tissue

[d] 1 = mild; 2 = moderate; 3 = severe

[f] Superficial Perivascular Dermatitis

[g] cannot evaluate

Table 11: Histology of Sinus Associated Zona Cutanea from 12 dogs with naturally occurring perianal fistula prior to treatment with cyclosporin

Case Number	SPD[fd]	Hair Follicles Present	Inflammatory Cellularity[a]	Predominant Inflammation[b]	Severity of Histologic Changes[c]	Epidermis	Circumanal Derma-titis[d]	Gland Adenitis[d]	Squamous Metaplasia[d]	Apocrine Adenitis[d]	Sebaceous Adenitis[d]	Folliculitis/ Furunculosis[d]
1	3	not visible	grade 3	P/L	2	FU[h]	3	4	CE[k]	3	4	4
2	0	no	grade 3	mixed	2	normal	1	1	none	2	none	none
4	3	yes	grade 3	mixed	2	SNP[i]	3	3 to 4	none	2	3	0 to 1
5	3	yes	grade 3	mixed	2	FU[h]	3	4	none	3	3	none
6	1	yes	grade 3	P/L	2	normal	1	2	none	1	4	none
7	3	not visible	grade 3	mixed	2	normal	2	3 to 4	1	1 to 2	1 to 2	not applicable
9	0	yes	grade 2	P/L	3	normal	1	1	none	2	1	none
10	3	yes	grade2	P/L	3	normal	3	2	none	1	CE	CE
11	3	yes	grade 3	mixed	2	normal	3	4	CE	2	1	none
12	3	yes	grade 2	mixed	3	OHK[e]	3	2 to 3	none	1	1	none
13	2	yes	grade 2	L/P	3	normal	1	3 to 4	2	1	2 to 3	none
15	2	yes	grade 3	mixed	2	normal	2	4	none	2	2	none

* = large eosinophil component

[a] grade 1 = 0-5% inflammatory cells; grade 2 = 5-25% inflammatory cells; grade 3 > 25% inflammatory cells

[b] P = plasmacytic; L = lymphocytic; M = macrophage; E = eosinophil; N = neutrophil; mixed = pyogranulomatous

[c] 0 = normal architecture
1 = normal architecture but heavy multifocal to diffuse inflammation
2 = normal architecture replaced by inflammatory cells
3 = normal architecture replaced by fibrous connective tissue

[d] 1 = mild; 2 = moderate; 3 = severe, 4 = obliterative

[e] orthokeratotic hyperkeratosis

[f] superficial perivascular dermatitis

[g] interface dermatitis

[h] focal ulceration

[i] subcorneal neutrophilic pustule

[j] pigmentary incontinence

[k] cannot evaluate

Figure 17. Typical sinus associated skin prior to treatment (case 1). Note the obliterative mononuclear inflammation with only apocrine glands persisting (arrows). Masson's trichrome, X 100

Figure 18. Sinus associated skin prior to treatment (case 4). Note clustering of the apocrine glands and disruption of their normal linear arrangement due to collapse of the supporting hepatoid circumanal glands (arrows). Masson's trichrome, X 150

Figure 17.

Figure 18.

follicles, such as keratin plugging, folliculitis or furunculosis, was not a feature of the inflammatory process. When present in the sections, the actual sinus tracts were lined with squamous epithelium and the inflammation directly adjacent to these tracts was mixed, suppurative to pyogranulomatous inflammation. The severity of inflammation and loss of normal tissue architecture (grade 2 histologic change) precluded identification of the origin of the sinus tracts in many sections; however, in regions with grade 1 histologic change, inflammation was seen surrounding and dissecting hepatoid circumanal gland lobules (Figures 20-24).

Histologic lesions from sinus associated skin following 4 weeks of cyclosporin therapy showed less inflammation (grade 2) compared with the pre-treatment samples (Table 12). Fibrous replacement of normal subepithelial architecture was a prominent feature, with less inflammatory cellularity (Figure 25). Other than decreased intensity, the composition of the inflammatory infiltrate was essentially the same as for pre-treatment sections, consisting mostly of lymphocytes and plasma cells. Although inflammation was associated with hepatoid circumanal glands, sebaceous glands, and to a lesser degree apocrine glands, the loss of normal structure and architecture of the hepatoid circumanal gland acinus was the most consistent feature (Figure 26).

2. Anal Sacs

The normal histologic appearance of the anal sac included a keratinizing inner layer of stratified squamous epithelium, usually 3-5 cell layers thick (Figure 27). A variable thickness subepithelium (ranging from 8-10 times the thickness of the epithelial layer), consisting of connective tissue collagen fibers, blood and lymphatic vessels, and

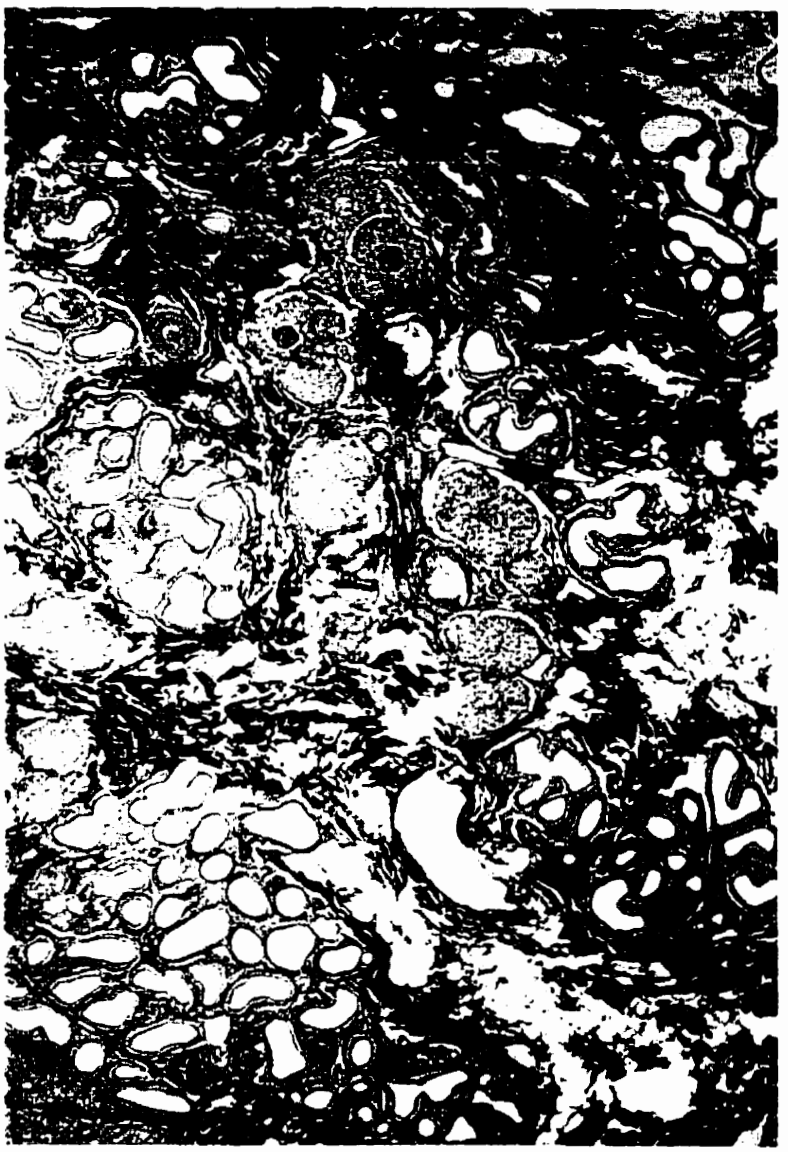

Figure 19. Sinus associated skin after 4 weeks of cyclosporin (case 13). Note the resolution of inflammation, but the presence of only small, infrequent hepatoid circumanal glands and the persistence of apocrine gland clustering. Masson's trichrome. X 100

Figure 20. Sinus associated skin prior to treatment (case 13). Note the selective destruction of hepatoid circumanal gland lobules (arrow). Masson's trichrome, X 1000

Figure 21. Sinus associated skin prior to treatment (case 5). Note the selective destruction of hepatoid circumanal gland lobules (arrow). Masson's trichrome, X 1000

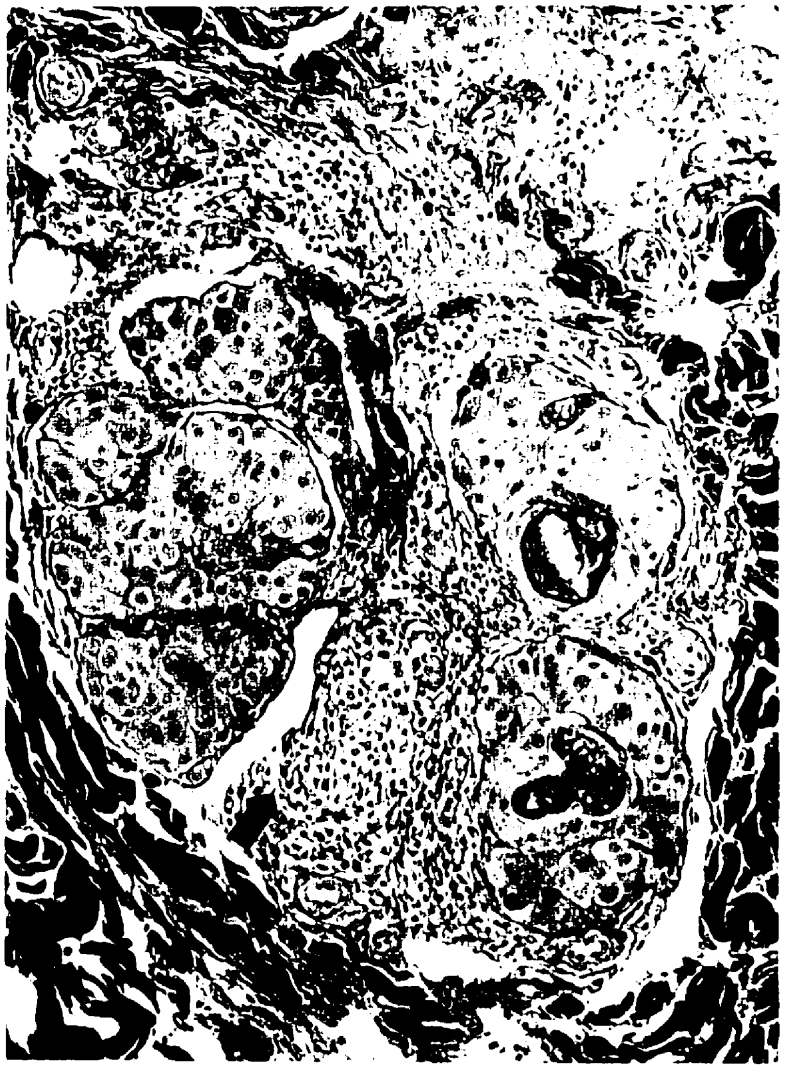

Figure 20

Figure 21

Figure 22. Sinus associated skin prior to treatment showing the junction between inflammed and unaffected tissue (case 2). Note the inflammation crossing the junction almost exclusively at the hepatoid circumanal glands (arrows). Masson's trichrome, X 150

Figure 23. Magnified region from Figure 22. Note the selective destruction of hepatoid circumanal glands (arrows). Masson's trichrome, X 1000

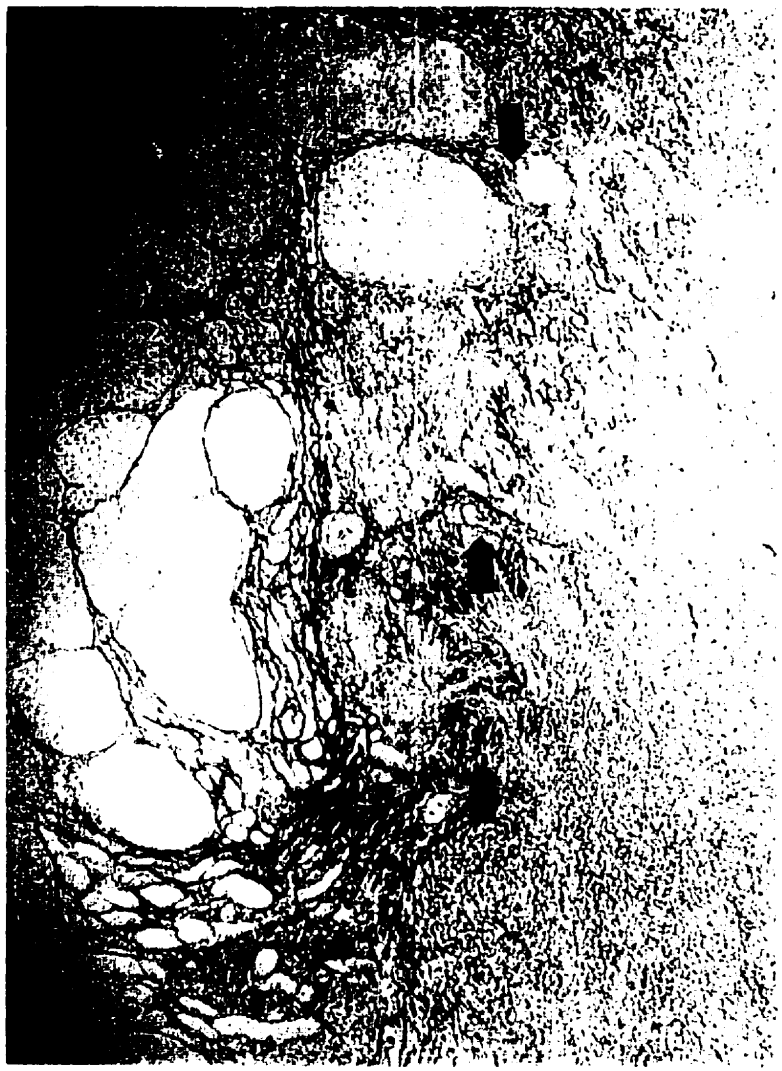

Figure 22.

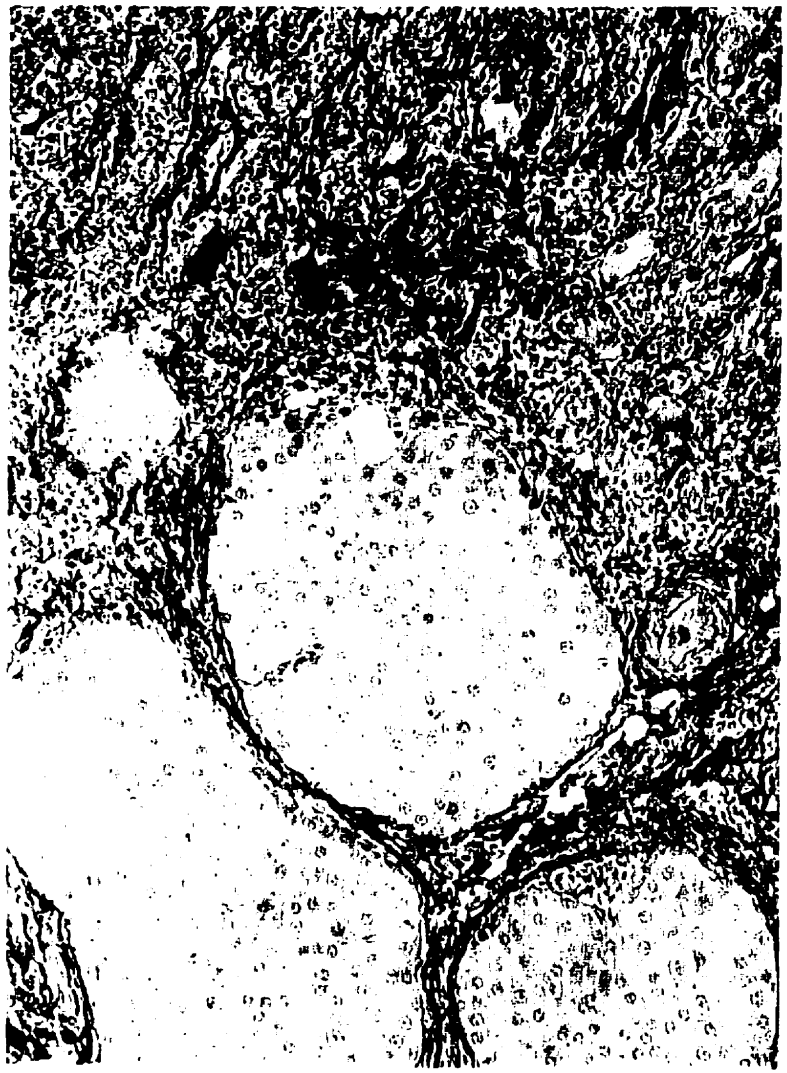

Figure 23

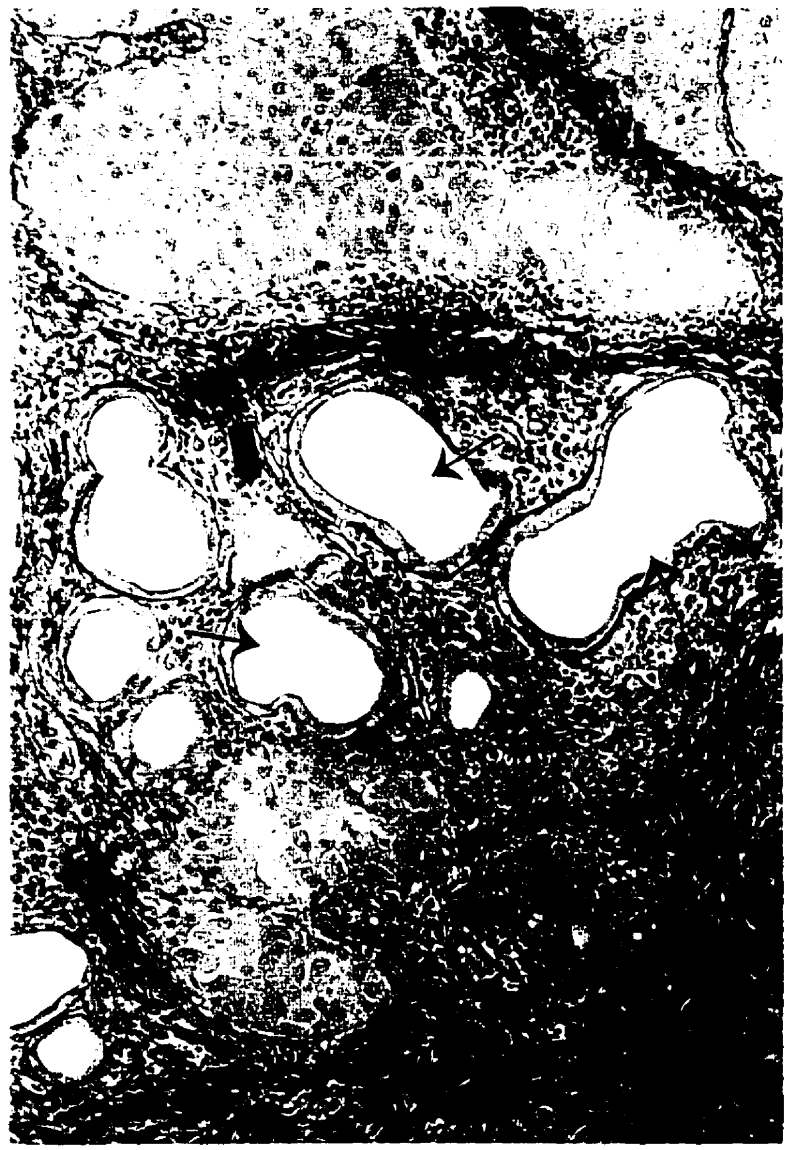

Figure 24. Sinus associated skin prior to treatment (case 2). Note selective destruction of hepatoid circumanal glands (large arrows). Also note the absence of any intraluminal inflammation associated with the adjacent apocrine glands (small arrows).
Masson's trichrome. X 1000

Table 12: Histology of Sinus Associated Zona Cutanea from 8 dogs[1] with naturally occurring perianal fistula after 4 weeks of treatment with cyclosporin

Case Number	SPD[fd]	Hair Follicles Present	Inflammatory Cellularity[a]	Predominant Inflammation[b]	Severity of Histologic Changes[c]	Epidermis	Derma-titis[d]	Circumanal Gland Adenitis[d]	Squamous Metaplasia[d]	Apocrine Adenitis[d]	Sebaceous Adenitis[d]	Folliculitis/ Furunculosis[d]
1	1	no	grade 2	L/P/N	3	normal	1	1	1	1	none	none
2	2	not visible	grade 2	N	3	FU[h]	SPD[f]	4	none	4	4	not applic.
4	3	not visible	grade 2	mixed	3	normal	SPD[f]	3	none	2	3	CE
5	2	yes	grade 2	P	1	normal	SPD[f]	3	none	2	3	none
7	1	yes	grade 2	mixed	3	OKH[e]	1	2	none	1	1	none
9	3	yes	grade 2	P/L	3	normal	3	3 to 4	none	2	2	none
12	2	not visible	grade 2	mixed	3	FU	2	CE[g]	CE	CE	CE	CE
13	1	yes	grade 2	L/P	3	normal	1	4	CE	1 to 2	2	none

* = large eosinophil component

[a] grade 1 = 0-5% inflammatory cells; grade 2 = 5-25% inflammatory cells; grade 3 > 25% inflammatory cells

[b] P = plasmacytic; L = lymphocytic; M = macrophage; E = eosinophil; N = neutrophil; mixed = pyogranulomatous

[c] 0 = normal architecture
1 = normal architecture but heavy multifocal to diffuse inflammation
2 = normal architecture replaced by inflammatory cells
3 = normal architecture replaced by fibrous connective tissue

[d] 1 = mild; 2 = moderate; 3 = severe; 4 = obliterative

[e] orthokeratotic hyperkeratosis

[f] superficial perivascular dermatitis

[g] cannot evaluate

[h] focal ulceration

[1] cases 6, 10, 11, and 15 were clinically healed at 4 weeks

Figure 25. Healed sinus skin 4 weeks following initiation of treatment (case 1). Note the abundance of obliterative fibrosis surrounding clustered apocrine glands (large arrows) and residual atrophic hepatoid circumanal glands (small arrows). Masson's trichrome, X 100

Figure 26. Healed sinus skin 4 weeks following initiation of treatment (case 9). Note the persistence of intraepithelial inflammation affecting the hepatoid circumanal glands (small arrows), but the absence of intraluminal inflammation affecting the apocrine glands (large arrows). Masson's trichrome, X 560

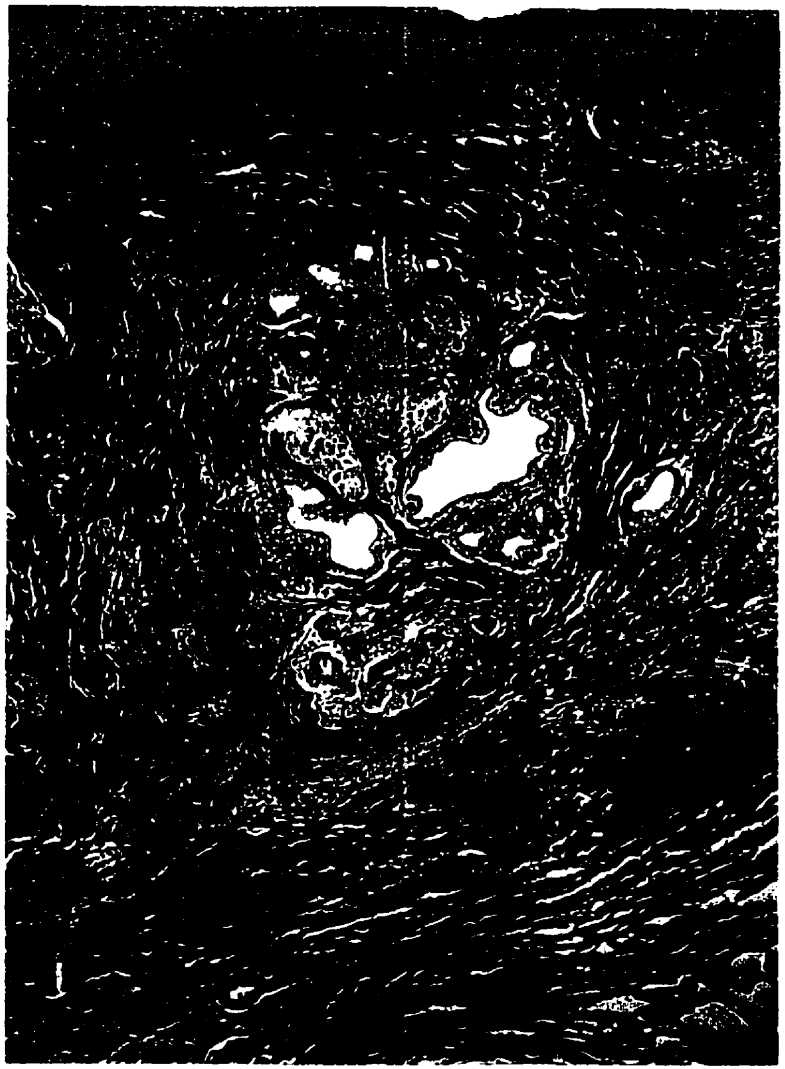

Figure 25.

Figure 26.

draining ducts of the intramural anal sac apocrine glands, was interposed between the epithelium and anal sac apocrine glands. Prominent apocrine glands surrounded the periphery of the anal sac; they were less prominent, even absent, in the region of the anal sac duct.

The apocrine gland acini themselves were arranged in a classical tubuloalveolar structure, with a small amount of connective tissue between individual acini, and slightly more connective tissue separating acinar groups. Normally the lumens of the acini either appeared empty or contained sparse amounts of pale basophilic to slightly eosinophilic acellular material.

Histologic evaluation of the pre-treatment anal sacs (and where possible the ducts of the anal sacs) revealed consistent multifocal to diffuse mononuclear inflammation (grades 1-2), most prominently in the subepithelium of the anal sac/duct wall, superficial to the apocrine glands themselves (Table 13). In a few cases similar multifocal mononuclear inflammation and lymphoid aggregates were seen associated with the apocrine glands, however, the most prominent feature of the apocrine glands themselves was moderate to marked dilation with associated collapse of the epithelial lining of glandular acini (Figure 28). No evidence of inflammation was present deep to the apocrine glands and all surrounding sphincter muscle was assessed as normal

Evaluation of the post-treatment anal sacs and ducts showed a similar inflammatory pattern but with less intensity of inflammation (grade 1) than seen in the pre-treatment samples (Table 14).

Figure 27. Normal histologic appearance of an anal sac. Anal sac lumen (small arrow). Note the small, uniform apocrine gland acini (large arrows). Masson's trichrome, X 150

Figure 28. Typical appearance of an anal sac from a dog with perianal fistula. Note the marked dilation of the tubuloalveolar apocrine glands (arrows). Masson's trichrome, X 150

Figure 27

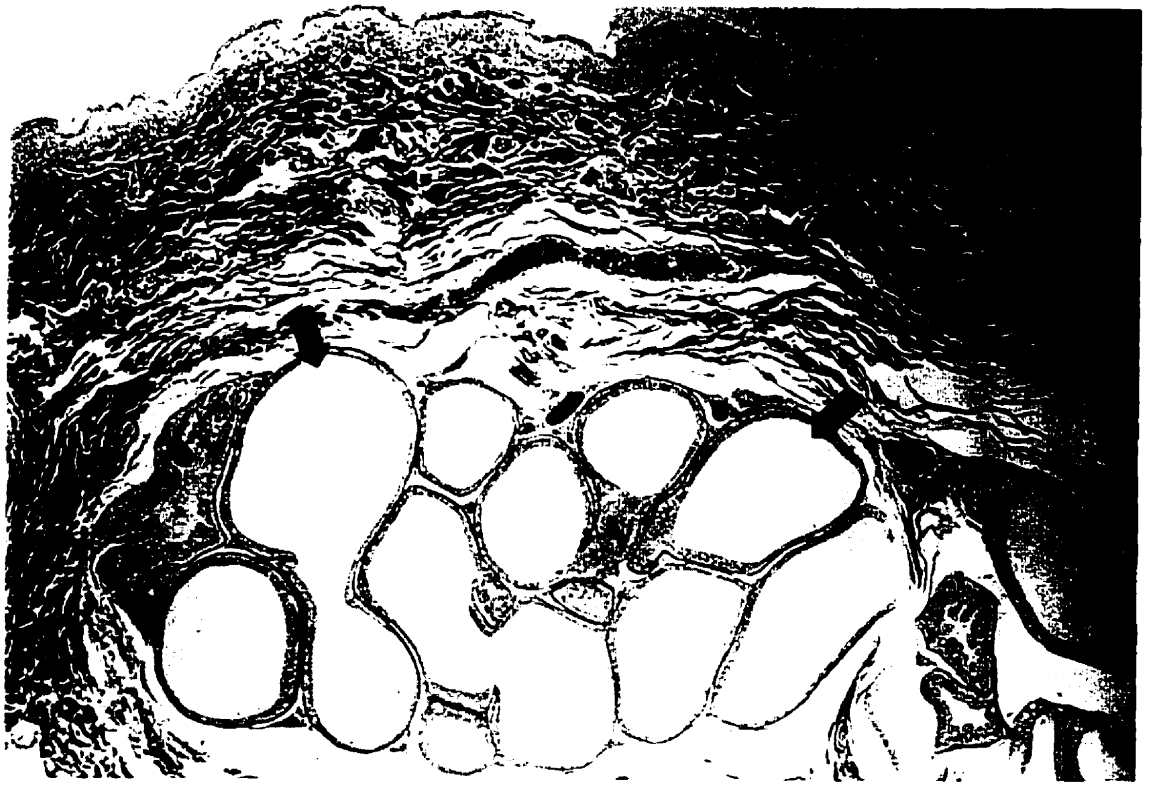

Figure 28.

Table 13: Histology of anal sacs (least affected side) from 12 dogs with naturally occurring perianal fistula prior to treatment with cyclosporin

Case Number	Inflammatory Cellularity[a]	Predominant Inflammation[b]	Severity of Histologic Changes[c]	Epithelium	Subepithelial Inflammation[d]	Apocrine Adenitis[d]	Apocrine Glands[e]
1	grade 3	L	2	absent	3	1	D,SM
2	grade 2	L*	2	HK[f]	2	2	D,SM,A
4	grade 2	L	2	normal	1	2	D,SM
5	grade 2	P/L*	1	normal	2	1	D
6	grade 2	P/L*	1	absent	3	0	normal
7	grade 1	none	0	normal	0	0	D
9	grade 1	L	1	normal	1	1	D
10	grade 1	L	0	normal	0	0	D
11	grade 3	L	2	focal loss	3	2	D
12	grade 1	none	0	normal	0	0	D
13	grade 2	L	2	normal	2	1	D,SM
15	grade 2	L/P	1	normal	1	0	D

* = large eosinophil component

[a] grade 1 = 0-5% inflammatory cells; grade 2 = 5-25% inflammatory cells; grade 3 > 25% inflammatory cells

[b] P = plasmacytic; L = lymphocytic; M = macrophage; E = eosinophil; N = neutrophil; mixed = pyogranulomatous

[c] 0 = normal architecture

1 = normal architecture but heavy multifocal to diffuse inflammation

2 = normal architecture replaced by inflammatory cells

3 = normal architecture replaced by fibrous connective tissue

[d] 1 = mild; 2 = moderate; 3 = severe; 4 = obliterative

[e] D = dilated; SM = squamous metaplasia; A = atrophic

[f] HK = hyperkeratosis

Table 14: Histology of anal sacs (most affected side) from 12 dogs with naturally occurring perianal fistula following treatment with cyclosporin

Case Number	Inflammatory Cellularity[a]	Predominant Inflammation[b]	Severity of Histologic Changes[c]	Epithelium[f]	Subepithelial Inflammation[d]	Apocrine Adenitis[d]	Apocrine Glands[e]
1[g]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	grade 2	L	1	normal	1	2	normal
4	grade 1	L	1	normal	1	0	D
5	grade 1	L	1	normal	0	1	D
6	grade 1	none	0	normal	0	0	D
7	grade 2	L	1	absent	2	0	D
9	grade 1	L	1	normal	1	1	D
10	grade 1	L	1	normal	0	1	D
11	grade 1	P	1	normal	0	1	D
12	grade 1	L	0	focal loss	1	0	D
13	grade 1	none	0	normal	0	0	D
15	grade 1	none	0	normal	0	0	D

[a] grade 1 = 0-5% inflammatory cells; grade 2 = 5-25% inflammatory cells; grade 3 > 25% inflammatory cells

[b] P = plasmacytic; L = lymphocytic; M = macrophage, E = eosinophil; N = neutrophil; mixed = pyogranulomatous

[c] 0 = normal architecture

1 = normal architecture but heavy multifocal to diffuse inflammation

2 = normal architecture replaced by inflammatory cells

3 = normal architecture replaced by fibrous connective tissue

[d] 1 = mild; 2 = moderate; 3 = severe; 4 = obliterative

[e] D = dilated; SM = squamous metaplasia; A = atrophic

f HK = hyperkeratosis

[g] second anal sac not retrievable

DISCUSSION

Clinical Observations

The clinical results of this study were consistent with the results of previous reports for the treatment of perianal fistulas using cyclosporin,[2,5] with full healing of sinus tracts in all dogs completing the treatment course. Previously reported frequency of recurrence (between 30%[2] and 41.2%[5]) following termination of immunosuppressive therapy were likewise similar, with one third of cases recurring in the current study.

Two-thirds of the dogs in the present study were male (2:1 male-to-female ratio), and of these 10 dogs, nine were sexually intact. A similar distribution was seen by Killingsworth et al.[12] Only one of the 22 male dogs in that study was castrated, prompting the hypothesis that hormonal abnormalities may possibly account for the overrepresentation of intact male dogs with perianal fistula. A similar gender distribution is seen with tumors of the hepatoid circumanal glands (perianal adenomas and adenocarcinomas). Hepatoid circumanal glands are present in both males and females; however, they are more developed and more numerous in intact male dogs, and indeed are the most numerous gland in the zona cutanea of the male dog. Perianal adenomas are the third most common tumor in dogs.[18] They occur 12 times more frequently in intact male dogs, and are more common in ovariohysterectomized females than in intact females.[18] As well, perianal adenomas are more prevalent in German Shepherd dogs; however, this prevalence is not as remarkable as the distinct breed predisposition seen with respect to perianal fistula.[19]

With regards to perianal fistulas in people, Lunniss et al commented on the increased incidence of idiopathic anal fistulas in men compared with women and

investigated the levels of circulating sex hormones to account for gender differences.[20] They found no evidence of increased circulating androgen levels in male or female patients, compared with healthy controls, and suggested the possibility of increased local androgen conversion in the glands to account for the gender differences in predilection.[20] A similar alteration in hormone metabolism may be suggested for canine perianal fistula.

Characterization of the Inflammatory Infiltrate

In the present study, the primary pattern of inflammation associated with perianal fistula was diffuse plasmacytic/lymphocytic inflammation, with frequent lymphoid nodules associated with glandular components of the subcutis. This is consistent with the previous reports on perianal fistula.[4,9] Sections directly adjacent to actual sinus tracts showed a mixed inflammatory pattern, most likely due to associated opportunistic bacterial infection secondary to the ulcerated epidermis.

Evaluation of the Anal Sacs/Ducts

Extension of primary anal sac disease was first proposed as a mechanism in the pathogenesis of perianal fistulas based on the findings from a series of cases in which sinus tracts were identified involving the anal sacs.[21] In support of this theory it has been noted that early gross lesions of perianal fistulas often appear centered over the ducts of the anal sacs.[22] In the present study, one-third of cases had sinus tract formation around the region of the anal sac ducts, but no direct communication between sinus tracts and anal sacs was noted in any of the cases. Clinical evaluation of the anal sacs and ducts revealed the most consistent abnormality, which was fibrosis, associated with the tissues

surrounding the anal sac ducts, although occlusive scarring (preventing canulation) of the duct was present in only one case. Microscopically, the most consistent changes seen in association with the anal sacs consisted of diffuse mononuclear inflammation in the subepithelium, in association with ducts of the apocrine glands, and dilation of the apocrine glands, with or without concurrent mononuclear apocrine adenitis. When apocrine gland adentitis was present it was never associated with the same severe, obliterative pattern seen in the zona cutanea. The anal sacculitis was much less intense following treatment with cyclosporin, however, anal sac inflammation persisted to a greater degree than did the inflammation in the zona cutanea. The microscopic lesions seen were most consistent with apocrine gland obstruction secondary to adjacent perianal inflammation and did not support the presence of primary anal sac disease.

In the present study, bilateral anal sacculectomy was performed as part of the biopsy protocol on 11 of the 12 dogs that completed the study. The fact that bilateral anal sacculectomy did not appear to alter the frequency of perianal fistula recurrence, combined with a very low presenting history of previous anal sac disease, would further support my conclusion that primary anal sac disease is not a component in the pathogenesis of perianal fistulas. The one dog that did not have both anal sacs removed did not experience any recurrence following cessation of immunosuppressive therapy.

Evaluation of the Cutaneous Zone

One limitation in this study was the absence of biopsy tissues from the intermediate and columnar zones and thus, particular comment on the pathology in these regions is lacking. Killingsworth et al,[4] however, evaluated histologic section from all

three regions of the anal canal in dogs with perianal fistulas and found that the most severely inflamed lesions were located in the zona cutanea.

The results of this study support the theory of "abscessation" of glandular structures of the zona cutanea, although, in fact, no suppurative inflammation was seen primarily affecting the glands of the zona cutanea, so the term abscessation is probably inaccurate. Killingsworth et al[4] found hidradenitis (apocrine gland adenitis) to be the most consistent inflammatory pattern, present in 50% of cases of perianal fistula in that study. This close association of inflammation with apocrine glands was not consistently identified in the present study. Although apocrine gland adenitis was a common feature in conjunction with sebaceous adenitis and hepatoid circumanal gland adenitis, hepatoid circumanal gland adenitis was the most frequent and most severe inflammatory pattern identified in all the histologic sections of zona cutanea (Figures 29-32). Following treatment with oral cyclosporin and full clinical resolution of perianal fistulas, very mild paratrichial sebaceous adenitis was the only consistent residual inflammatory pattern, present in all except two cases evaluated. In this regard, with reference to the term anal furunculosis used in publications from the United Kingdom, only one instance of folliculitis or furunculosis was identified in all the sections examined, including pre- and post-treatment sections.

The observations from this study did not provide conclusive evidence to support the primary involvement of any one gland type in the pathogenesis of canine perianal fistulation. Obliterative inflammation within the deep dermis and subcutis precluded any identification of most structures of the subcutis and dermis, a pattern also quite typically seen in advanced cases of hidradenitis suppurativa in people. However, the pattern of

Figure 29. Zona cutanea of a spayed female German Shepherd with perianal fistula (case 9) showing primary mononuclear inflammation affecting only the hepatoid circumanal glands (arrow). Masson's trichrome, X 100

Figure 30. Zona cutanea of a spayed female German Shepherd with perianal fistula (case 9) showing primary mononuclear inflammation affecting only the hepatoid circumanal glands (arrow). Masson's trichrome, X 1000

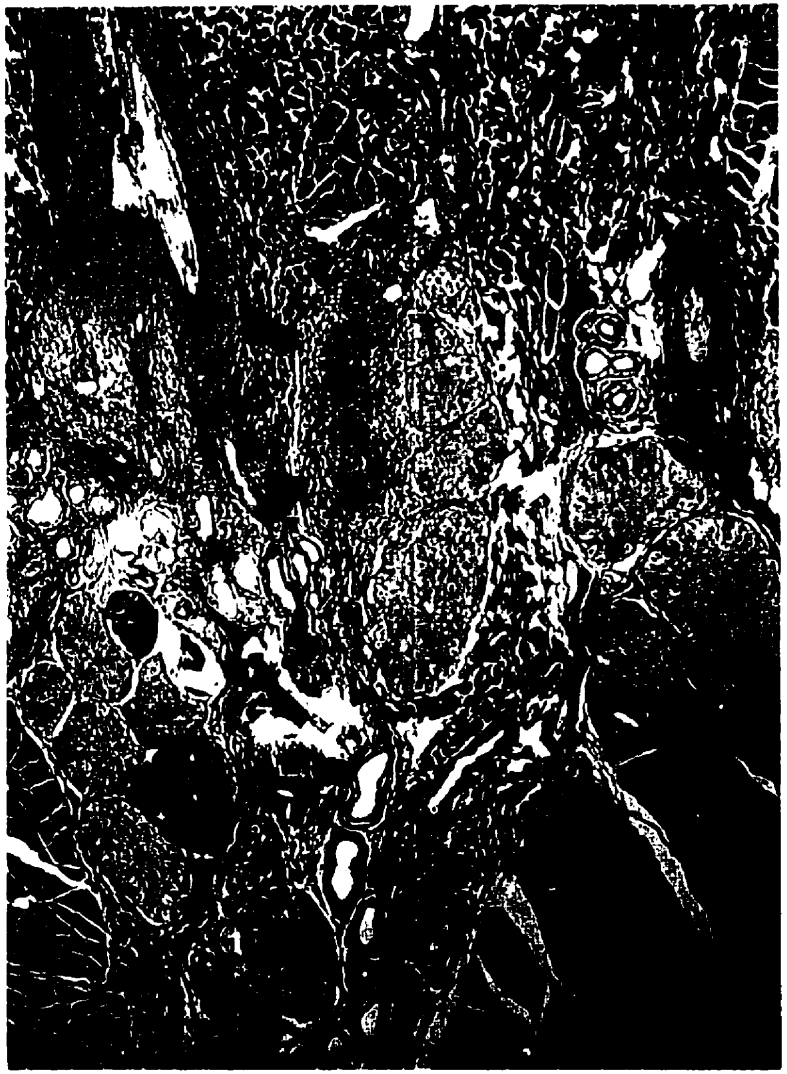

Figure 29.

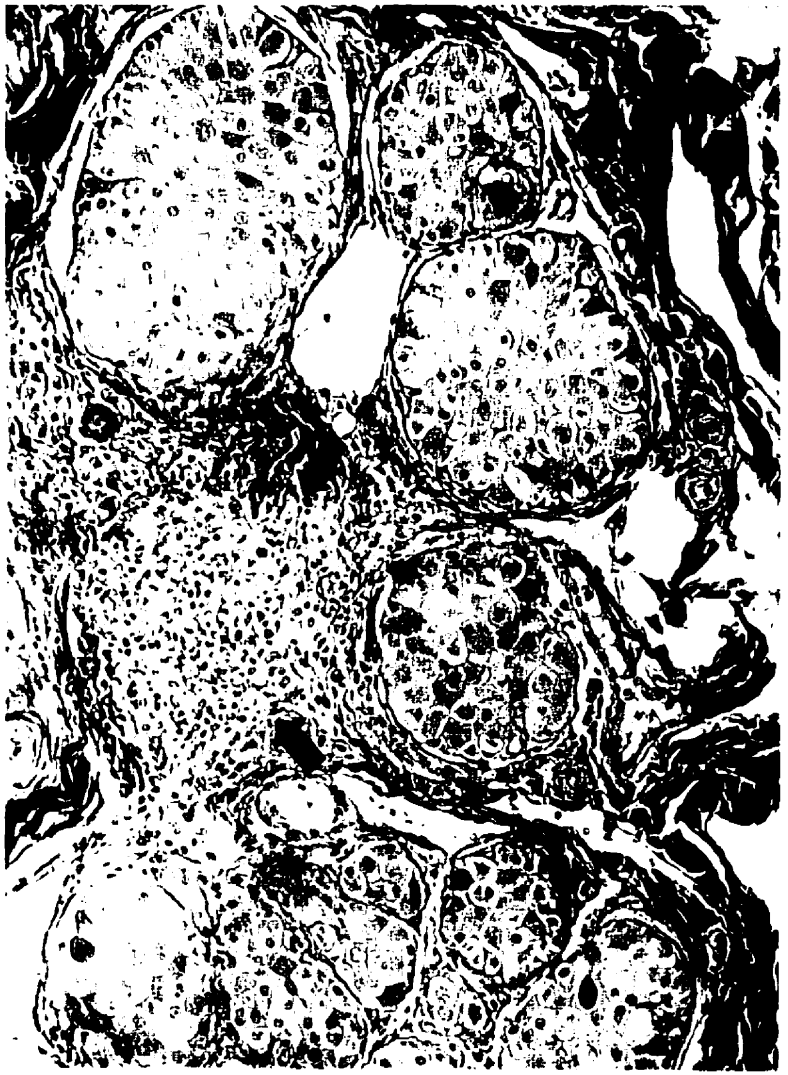

Figure 30

Figure 31. Zona cutanea of a spayed female German Shepherd with perianal fistula (case 13) showing primary mononuclear inflammation affecting only the hepatoid circumanal glands (arrows). Masson's trichrome, X 100

Figure 32. Zona cutanea of a spayed female German Shepherd with perianal fistula (case 7) showing primary mononuclear inflammation affecting only the hepatoid circumanal glands. Note the intraepithelial inflammatory cells (arrow). Masson's trichrome, X 1000

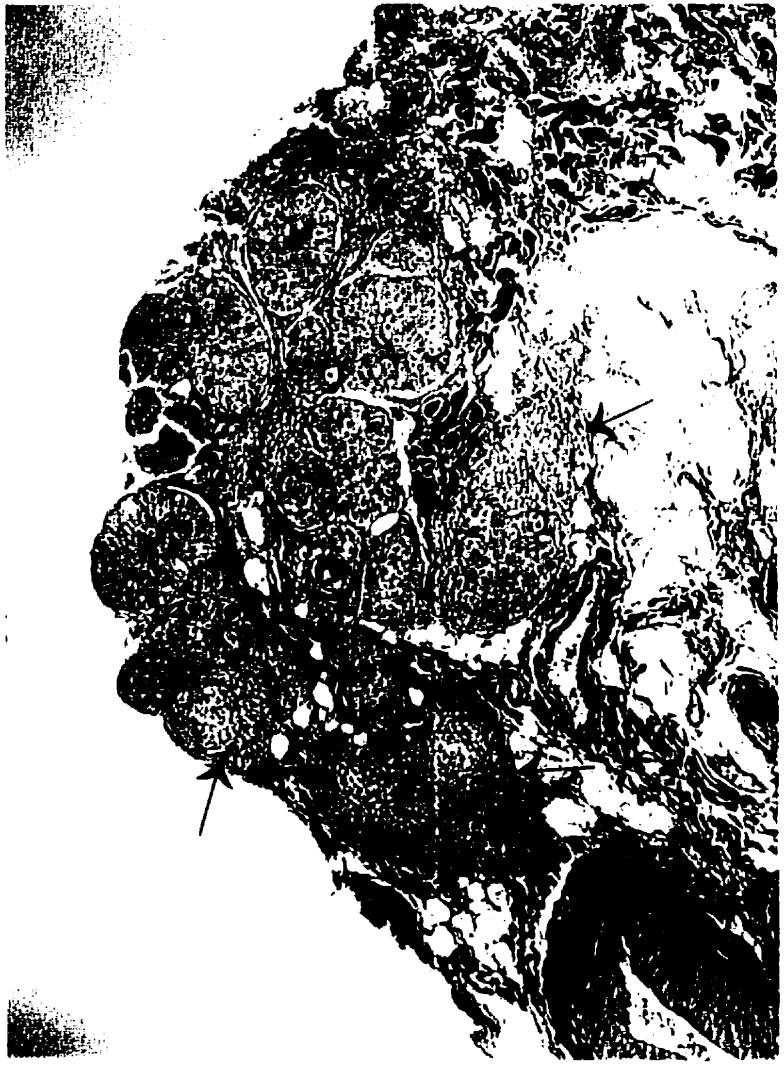

Figure 31.

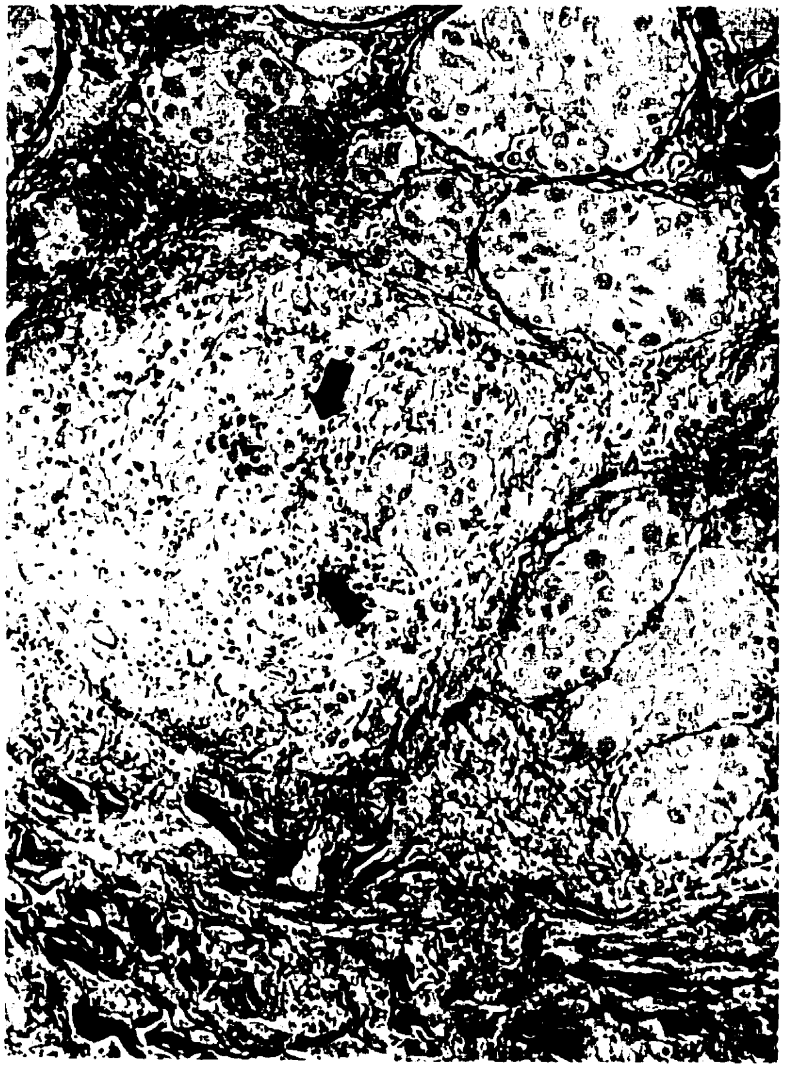

Figure 32.

inflammatory aggregation in the subcutis was suggestive of the distribution typical for hepatoid circumanal glands in normal perianal skin. Evaluation of paralesional histologic sections showed most of the inflammation associated with hepatoid circumanal gland lobules and evidence of acinar destruction, with an impression of only secondary involvement of apocrine glands. With respect to the apocrine glands, an intraluminal inflammatory infiltrate (necessary in order to classify a lesion as a primary apocrine adenitis or hidradenitis) was only identified in one case.[23]

There are many similarities between hidradenitis suppurativa in people and canine perianal fistulas, and indeed it has been stated that the clinical appearance of canine perianal fistulas most closely resemble the appearance of hidradenitis suppurativa in people.[4] In spite of the clinical similarities though, the primary histopathologic lesions differ with respect to these two diseases. The most consistent histologic features seen with hidradenitis suppurativa include follicular hyperkeratosis and marked follicular occlusion, with apocrine gland inflammation occurring only as a bystander effect.[23-27] In contrast, follicular occlusion or primary folliculitis was not a feature of perianal fistula in the present study. This is consistent with the results of Killingsworth et[4] al in which follicular plugging with keratin was not a consistent feature. Killingsworth et[4] al concluded that the lesions of perianal fistula originated from periadnexal inflammation within the zona cutanea.[4] In the present study, a distinct attempt was made to identify the originating source of the periadnexal inflammation. Differentiation of primary versus secondary inflammation was made based on the presence or absence of intraepithelial or intraluminal inflammatory infiltrate associated with the various glandular structures of the zona cutanea.[23] With respect to these defining limitations, only the hepatoid circumanal

glands showed an associated inflammatory pattern consistent with primary inflammation. In addition, inflammation was consistently more severe around hepatoid circumanal gland lobules compared with paratrichial sebaceous glands or apocrine sweat glands. Additionally, when hepatoid inflammation was seen in non-sinus-associated skin, it was typically perifollicular and superficial.

It is hypothesized that early perianal fistula lesions are associated with inflammation of the superficial circumanal glands. As the severity of inflammation progresses, the deeper hepatoid circumanal glands become involved along with adjacent glandular structures, resulting in the obliterative inflammation associated with advanced cases of perianal fistula. Baker stated that cords of hepatoid circumanal gland cells can extend deeply into the tissues around the anal canal[28] and this may account for the extreme depth of sinus tracts identified in some advanced cases of perianal fistula. Alternatively, the depth of sinus tracts may be due to secondary involvement of the anal glands of the intermediate and columnar zones and account for the rare observance of rectal fistulas in severe, late-stage lesions of perianal fistula.[4]

Additional support for this hypothesis of primary hepatoid cell targeting exists with clinical evaluation of the anal sacs. As previously mentioned, in the present study one-third of cases had sinus tract formation around the region of the anal sac ducts (Figures 33-35), without any evidence of direct communication between sinus tracts and the anal sacs. This is consistent with the work of Robins and Lane where they observed that "early cases of perianal fistula are frequently centered around the ducts of the anal sacs".[22] In addition, clinical evaluation of the anal sacs in this present study revealed the

Figure 33. Perianal region of an eight-year-old, intact male German Shepherd illustrating the frequent development of perianal fistula over the regions of the anal sac ducts.

Figure 34. Perianal region of a six-year-old, spayed female German Shepherd illustrating the frequent development of perianal fistula over the regions of the anal sac ducts.

Figure 33

Figure 34

Figure 35. Perianal region of a four-year-old, intact male German Shepherd illustrating the frequent development of perianal fistula over the regions of the anal sac ducts.

majority of fibrosis associated with the tissues surrounding the anal sac ducts rather than the anal sacs themselves, and the histopathology of the anal sacs was most consistent with apocrine gland obstruction rather than that of primary anal sac disease. Parks identified hepatoid circumanal glands elements in the walls of the anal sac ducts as well as the subcutis of the zona cutanea.[29] This observation makes inherent sense since it is readily acknowledged that the anal sacs are diverticula of the cutaneous zone.[30]

The Efficacy of Cyclosporin

Cyclosporin an immunosuppressive drug that is not myelosuppressive or cytotoxic.[31] The primary effect of cyclosporin is the inhibition of T lymphocytes activation and function, particularily T-helper cells, by blocking the production and action of interleukin-2.[31,32] The absence of these inhibitory effects on T-suppressor cell function serves to augment this effect.

The primary pattern of inflammation associated with perianal fistula is diffuse lymphocytic/plasmacytic inflammation, with frequent lymphoid nodules associated with glandular components. It is reasonable to theorize that cyclosporin causes resolution of perianal fistula by inhibiting the activation and function of the lymphocytes involved in the pathogenesis of the lesions, allowing second intention healing to occur. If this is accurate, other drugs that affect T cell function, such as corticosteroids, cyclophosphamide, and azothiaprine should also have an effect on perianal fistula, and indeed this is the case.[33,34]

Cyclosporin may, however, exert other effects that could result in improvement of the clinical signs and appearance of perianal fistula. Cyclosporin does inhibit

phospholipase A_2,[35] and in this way it may decrease the inflammation associated with perianal fistula. However, it is difficult to believe that this anti-inflammatory effect of cyclosporin is substantially responsible for the resolution of perianal fistula since other anti-inflammatory medications do not alter the progression of perianal fistula unless immunosuppression is achieved.

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CHAPTER III

GENERAL DISCUSSION AND CONCLUSIONS

Summary of Findings

Evaluation of the anal sacs revealed diffuse mononuclear inflammation in the subepithelium associated with the ducts of the apocrine glands, and dilation of the apocrine glands of the anal sacs, with or without concurrent mononuclear apocrine adenitis. These changes were most consistent with apocrine gland obstruction secondary to adjacent perianal inflammation and did not support the presence of primary anal sac disease.

With regards to the cutaneous zone, the hepatoid circumanal glands were by far the predominant structures occupying the normal subcutis. Evaluation of paralesional histologic sections from the cutaneous zone showed directed (intraepithelial) mononuclear inflammation targeting the hepatoid circumanal gland lobules, with evidence of lobular destruction, and an impression of only secondary involvement of apocrine glands. Obliterative inflammation associated with lesional histologic sections precluded identification of most structures of the subcutis and dermis; however, a pattern of inflammatory aggregation in the subcutis was suggestive of the distribution typical for hepatoid circumanal glands in normal perianal skin.

Comparative evaluation of the degree of inflammatory infiltrate found in sinus, and previously sinus, associated skin during treatment with cyclosporin showed overall reduction in the mononuclear inflammatory response in conjunction with increased fibrosis of the subcutis. Compared with entirely unaffected regions of the cutaneous zone

prior to treatment, after four weeks of therapy all previous sinus associated regions evaluated showed either atrophy of hepatoid circumanal glands, with evidence of regenerating nests, and/or increased dissecting fibrosis among hepatoid circumanal gland lobules.

Conclusions

1) The histopathologic lesions of perianal fistula reveal a pattern consistent with primary inflammation involving the hepatoid circumanal glands. An overrepresentation of perianal fistulas in intact male dogs, both in this study and previous studies, would also be consistent with an abnormality of the hepatoid circumanal glands.
2) Early perianal fistula lesions are associated with mononuclear inflammation of the superficial circumanal glands. As the severity of inflammation progresses, the deeper hepatoid circumanal glands become involved along with adjacent glandular structures, resulting in the obliterative inflammation associated with advanced cases of perianal fistula.
3) The inflammation associated with lesions of perianal fistula are not mediated by an obvious bacterial infectious source, as indicated by the absence of any substantial neutrophilic component, the absence of observed bacteria in the histologic sections, and that this inflammation resolves with systemic immunosuppression using oral cyclosporin.
4) The anal sacs are not primarily involved in the pathogenesis of perianal fistula; however, other compelling evidence for primary involvement of the hepatoid circumanal glands is found with documentation of hepatoid cell elements within the

walls of the ducts of the anal sacs[1] in conjunction with documentation of pronounced clinical fibrosis around the anal sac ducts and frequent documentation of sinus tracts surrounding the anal sac ducts in the current study.

In summary, both the clinical and histologic assessments from the current study give supportive evidence to the primary involvement of the hepatoid circumanal glands in the pathogenesis of perianal fistulas.

Future Areas of Research

It must be stressed that canine perianal fistula has not been proved to be an immune-mediated disease. The only evidence to suggest that it may develop as a result of immunoregulatory dysfunction or immune-mediated disease is the clinical responsiveness of the disease to immunosuppressive therapy, particularly with the use of cyclosporin. Additional investigation is necessary to determine what primary role the immune system plays, if any, in the pathogenesis of perianal fistula.

Clinically similar lesions to, and histologically indistinguishable from, hidradenitis suppurativa occur in people with a cutaneous manifestation associated with Crohn's disease and ulcerative colitis conditions.[2] Like canine perianal fistula, the etiology of Crohn's fistulas is unknown, but immunoregulatory dysfunction has been implicated, regardless of whether it is a primary factor responsible for the onset of disease or whether it is secondary factor responsible for persistence of the inflammation.[3] Evidence supporting an autoimmune response in the pathogenesis of inflammatory bowel disease and ulcerative colitis has been demonstrated by quantitative measurement of circulating soluble interleukin-2 receptors (IL-2R - see figure 8) in the serum of patients

affected with these diseases compared with healthy patients.[4,5] Levels of soluble interleukin-2 receptor are significantly elevated in the sera of Crohn's disease and ulcerative colitis patients and the mononuclear cells of these inflammatory bowel disease patients release large amounts of IL-2R.[4] In addition, the number of circulating T lymphocytes bearing activation markers is increased in people with Crohn's disease, all of which suggest an autoimmune response.[4] Similar investigation in canine patients with perianal fistulas could help clarify the role of the immune system in the pathogenesis of this poorly understood disease and prompt further investigation into additional treatment options, including alternative immunosuppressive drugs or alternative routes of administration (topical vs. systemic).

Other indirect evidence that immune-mediated disease is involved in the etiology and/or pathogenesis of perianal fistula can be found by considering the population of dogs most at risk for developing the disease. The marked over-representation of German Shepherd dogs automatically makes one suspicious that genetics plays a critical role in the development of perianal fistula. Genetic predisposition is also a primary underlying factor in the development of immune-mediated disease, and this probably involves abnormalities with the major histocompatibility complex (MHC) molecules or MHC genes.[6] With this in mind, it would be interesting to investigate alterations in MHC expression as a possible reason for the marked predisposition of perianal fistula in German Shepherd dogs. Work in this area would also be helpful in reinforcing or dismissing the idea of an immune-mediated basis for canine perianal fistula.

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