Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis

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Objective—To determine the distribution of cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) in skin (including hair follicles and sweat and sebaceous glands) of clinically normal dogs and dogs with atopic dermatitis (AD) and to compare results with those for positive control samples for CB1 (hippocampus) and CB2 (lymph nodes).

Sample—Skin samples from 5 healthy dogs and 5 dogs with AD and popliteal lymph node and hippocampus samples from 5 cadavers of dogs.

Procedures—CB1 and CB2 were immunohistochemically localized in formalin-fixed, paraffin-embedded sections of tissue samples.

Results—In skin samples of healthy dogs, CB1 and CB2 immunoreactivity was detected in various types of cells in the epidermis and in cells in the dermis, including perivascular cells with mast cell morphology, fibroblasts, and endothelial cells. In skin samples of dogs with AD, CB1 and CB2 immunoreactivity was stronger than it was in skin samples of healthy dogs. In positive control tissue samples, CB1 immunoreactivity was detected in all areas of the hippocampus, and CB2 immunoreactivity was detected in B-cell zones of lymphoid follicles.

Conclusions and Clinical Relevance—The endocannabinoid system and cannabimimetic compounds protect against effects of allergic inflammatory disorders in various species of mammals. Results of the present study contributed to knowledge of the endocannabinoid system and indicated this system may be a target for treatment of immune-mediated and inflammatory disorders such as allergic skin diseases in dogs. (*Am J Vet Res* 2012;73:988–995)

The term cannabinoid indicates any of the various chemical constituents of marijuana, of which Δ^9-tetrahydrocannabinol (commonly known as THC) is the best known for its psychotropic and potentially beneficial physiologic effects.[1] During the past decade, there has been growing interest in the biological effects of cannabinoids. Two main subtypes of cannabinoid receptors (CB1 and CB2) have been identified in mammalian tissues; agonists and antagonists selective for these receptors have been identified.[2] Nucleotide sequences of CB1 and CB2 are strongly conserved in mammals, and these receptors belong to the G-protein–coupled receptor superfamily.[3]

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Abbreviations

AD	Atopic dermatitis
CA	Cornu ammonis
CB	Cannabinoid receptor type
PEA	N-palmitoylethanolamide

All endogenous agonists of cannabinoid receptors (ie, endocannabinoids) identified to date are long-chain polyunsaturated fatty acids that are derivatives of arachidonic acid and have various degrees of selectivity for 1 or both subtypes of cannabinoid receptors.[4] Cannabinoid receptors, their endogenous agonists, and the enzymes responsible for production and degradation of these agonists constitute the endocannabinoid system. Moreover, N-acylethanolamines such as PEA that are structurally related to other endocannabinoids are widely distributed in CNS and peripheral tissues, and although their lack of affinity for CB1 and CB2 calls into question their classification as endocannabinoids, they are considered cannabimimetic compounds.[5]

The endocannabinoid PEA has anti-inflammatory, analgesic, and antipruritic effects.[6–10] These effects are mediated, at least in part, by the so-called entourage

effect (ie, activation of CB1, CB2, and transient receptor potential vanilloid type 1 ion channels[6] by PEA enhances effects of the endocannabinoid anandamide).

Cannabinoid receptor type 1 is strongly expressed in brain cells (primarily cells of the hippocampus[11]) of humans and laboratory animals, whereas CB2 is strongly expressed in cells of the immune system.[12–14] Cannabinoid receptor type 2 is upregulated during early phases of inflammation in cells of CNS and peripheral tissues.[14] Although the distribution of cells expressing CB1 and CB2 in tissues of laboratory animals[11,15,16] and humans[17,18] has been determined, CB1 has been identified only in cells of salivary glands[19] and hair follicles[20] in dogs (via immunohistochemical localization).

Other authors[21] have reported that mice lacking CB1 and CB2 have greater allergic inflammation than mice that have those receptors, and contact dermatitis is attenuated in mice that have high expression of endocannabinoids. Authors of that study also reported that cannabinoid receptor antagonists exacerbate allergic inflammation, whereas cannabinoid receptor agonists attenuate inflammation. These findings suggest there is a protective role of the endocannabinoid system in contact allergies of skin, and components of that system may be potential targets for treatment.

Considering that AD is a major medical problem for dogs, a better understanding of the endocannabinoid system in this species might be useful for development of treatments. To this end, the objective of the study reported here was to investigate the distribution of CB1 and CB2 in skin, lymph nodes, and the hippocampus of clinically normal dogs and skin of dogs with AD.

Materials and Methods

Animals—Five healthy dogs (1 male and 4 females; age range, 4 to 13 years) and 5 dogs with AD (2 males and 3 females; age range, 1 to 11 years) were included in the study. Healthy dogs were client-owned dogs admitted for neutering. Dogs with AD were client-owned dogs referred for dermatologic problems; at least 3 major and 3 minor diagnostic criteria[22] for AD were fulfilled for each of these dogs. Dogs with AD had clinical signs of erythema, alopecia, pruritus, and mild crusting of skin and had positive results for intradermal allergen testing, which supported the clinical diagnosis of AD. To avoid bias attributable to inclusion of skin samples with AD lesions from various anatomic sites, only samples of skin lesions on the ventral aspects of cervical and axillary regions of dogs were obtained. All AD dogs underwent flea control measures and were fed a hypoallergenic restriction diet for 8 weeks prior to collection of skin samples to exclude allergies to fleas or food as possible causes of AD. When necessary, dogs were treated for secondary yeast or bacterial infections of skin. Immediately before collection of skin samples for immunolocalization of CB1 and CB2, cytologic examination of skin at planned collection sites was performed for each dog with AD; bacteria and fungi were not detected. Treatments for secondary bacterial or yeast infections were discontinued at least 2 to 3 weeks prior to collection of skin samples in these dogs.

Hippocampus and popliteal lymph node samples were obtained from 5 cadavers of dogs during necropsy at the Department of Animal Pathology of the University of Pisa. These tissue samples served as positive control samples for detection of CB1 (hippocampus) and CB2 (popliteal lymph node).

Institutional ethical committee approval was not required for the study because AD skin samples were obtained and histologically examined for diagnostic purposes and hippocampi and lymph nodes were collected from cadavers during necropsy. Written informed consent was obtained from owners of dogs with AD for inclusion of those dogs in the study. Written informed consent was obtained from owners of healthy dogs for collection of skin samples from those dogs during neutering.

Collection of tissue samples—One skin biopsy sample was obtained from the abdominal region of each of the 5 healthy dogs during inhalation anesthesia (isoflurane) for surgery (ie, neutering). One sample each of grossly normal hippocampus and popliteal lymph node was obtained during necropsy from each of the 5 cadavers of dogs; these dogs did not have clinical or gross evidence of neurologic or infectious diseases. In humans and laboratory animals, CB1 and CB2 are strongly expressed in hippocampus and lymph nodes, respectively.[11,12,23] Therefore, samples of hippocampi and lymph nodes were used as positive control samples for CB1 and CB2, respectively. Skin samples were obtained from each of the 5 client-owned dogs with AD. These dogs were sedated with butorphanol tartrate[a] and medetomidine hydrochloride[b] for intradermal allergen testing, and skin samples were collected by use of local anesthesia with lidocaine (2%). A 6-mm punch biopsy[c] instrument was used to obtain samples from skin lesions; 2 biopsy samples were obtained from each of 2 lesions per dog.

Samples of skin, hippocampus, and lymph nodes were placed in neutral-buffered 4% formalin immediately after collection and subsequently embedded in paraffin. Tissue samples were sectioned (thickness, 5 µm), stained with H&E, and examined via light microscopy to detect histologic lesions.

Immunolocalization of CB1 and CB2—Because the canine CB1 protein sequence is homologous to the human CB1 protein sequence,[3] tissue samples were labeled with a commercially available polyclonal rabbit anti–human-CB1[d] antibody. Similarly, the CB2 protein sequence is conserved among species of mammals; therefore, tissue samples were labeled with polyclonal rabbit anti–human-CB2[e] antibody. Paraffin-embedded tissue samples were sectioned (thickness, 5 µm), mounted on glass slides,[f] deparaffinized, and rehydrated. Quenching of endogenous peroxidases was performed with 3% H_2O_2 in distilled water (30 minutes at room temperature [approx 22°C]). Nonspecific binding of antibody was prevented by incubation of slides in 25% normal horse serum (diluted in 0.1M PBS solution) for 20 minutes at room temperature. Slides were washed and incubated with anti-CB1 or anti-CB2 primary antibody (diluted 1:100 in PBS solution) overnight (approx 16 hours) at 4°C. Slides were washed and incubated for 30 minutes at room temperature with a peroxidase-polymer–conjugated secondary antibody.[g] Slides were developed by incubation with diaminoben-

zidine[h] for 5 minutes at room temperature. Tissue sections were counterstained with hematoxylin, dehydrated, and mounted to glass slides with coverslips and a permanent mounting medium. Negative control tissue samples were prepared by replacement of the primary antibody with an unrelated peptide[i] and preabsorption of the unrelated peptide for 20 minutes at room temperature with CB1 or CB2 blocking peptide[j,k] (diluted 1:500 in the PBS solution containing the primary antibody that corresponded to the blocking peptide); this method was used to prepare negative control samples of skin, hippocampus, and lymph nodes.

Sections of tissue samples were independently examined via light microscopy by 3 of the authors (LC, VM, FA). Representative images were acquired by use of photodocumentation software.[l]

Results

Immunolocalization of CB1 in hippocampus samples—Cannabinoid receptor type 1 immunoreactivity was detected in hippocampus samples of healthy dogs (**Figure 1**). Strong CB1 immunoreactivity was detected in all areas of hippocampus. Immunoreactivity was detected in each of the 4 regions of the CA; CB1 immunoreactivity decreased progressively from CA1 to CA4. Cannabinoid receptor type 1 immunoreactivity was detected in the neuropil of the CA and in the dentate gyrus. Dot-like structures with CB1 immunoreactivity lined the external surface of neuronal cell bodies, particularly those of pyramidal and granular neurons, whereas cytoplasm of neurons did not have CB1 immunoreactivity.

Immunolocalization of CB1 in skin samples of healthy dogs—Cannabinoid receptor type 1 immunoreactivity was detected in skin samples of healthy dogs. In the epidermis, CB1 immunoreactivity was detected in cytoplasm of basal and suprabasal layer cells (Figure 1). Hair follicles had CB1 immunoreactivity in cytoplasm of inner epithelial root sheath cells (**Figure 2**). In contrast, cells of the outer root sheaths of hair follicles and arrector pili muscles did not have CB1 immunoreactivity. Strong CB1 immunoreactivity was

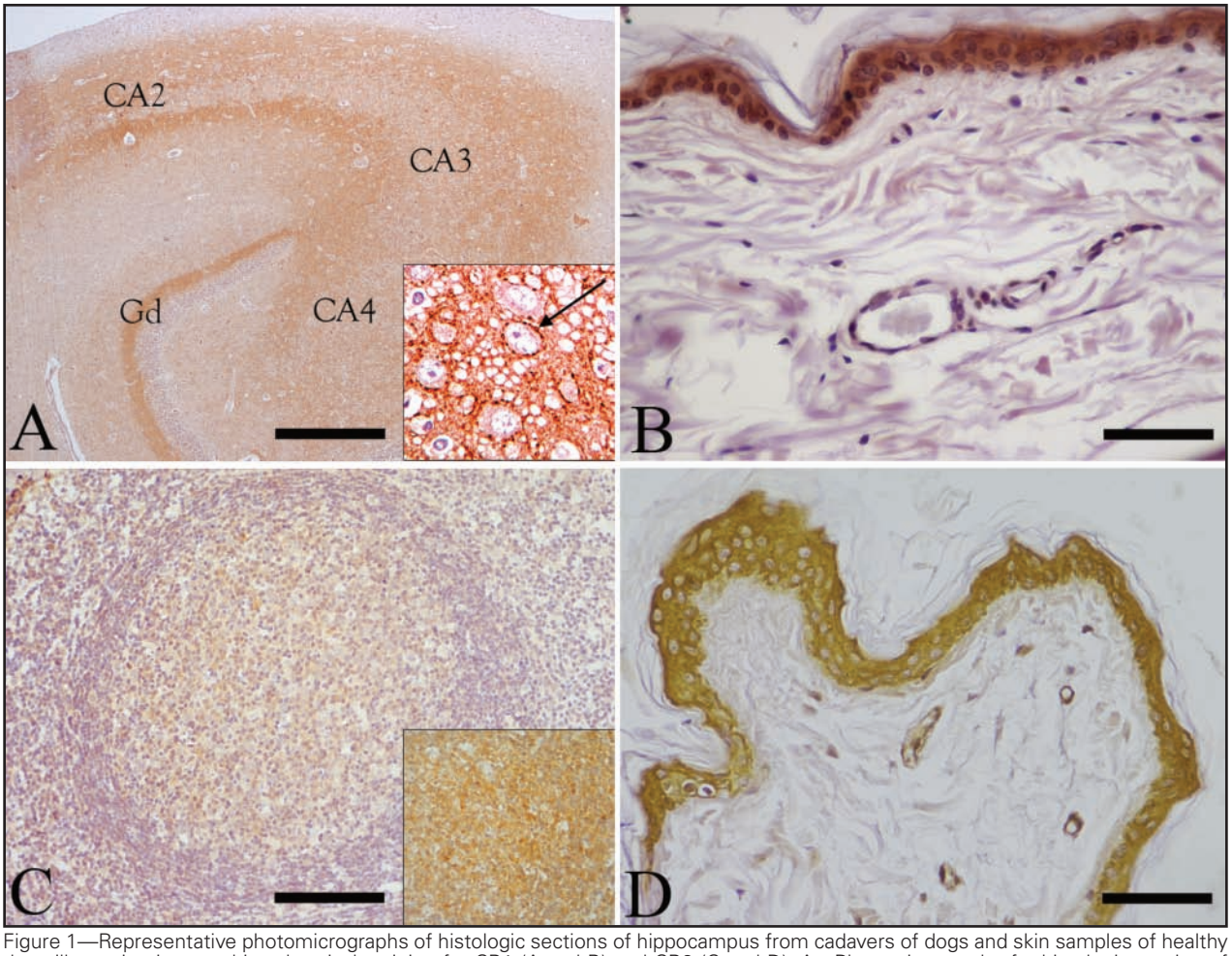

Figure 1—Representative photomicrographs of histologic sections of hippocampus from cadavers of dogs and skin samples of healthy dogs illustrating immunohistochemical staining for CB1 (A and B) and CB2 (C and D). A—Photomicrograph of a histologic section of hippocampus. Notice strong CB1 immunoreactivity in various regions of the hippocampus. Bar = 500 μm. Inset—Neuronal cell bodies in the neuropil of the pyramidal layer are surrounded by dot-like structures with CB1 immunoreactivity (arrow). B—Photomicrograph of a histologic section of skin. Notice CB1 immunoreactivity in keratinocytes in the basal and suprabasal epidermal layers. Bar = 50 μm. C—Photomicrograph of a histologic section of skin. Notice CB2 immunoreactivity in lymphocytes in the follicular B-cell regions. Bar = 100 μm. Inset—Lymphocytes in the follicular germinal center have CB2 immunoreactivity. D— Photomicrograph of a histologic section of skin. Notice CB2 immunoreactivity in basal and suprabasal epidermal keratinocytes. Bar = 50 μm. Gd = Dentate gyrus.

detected in reserve cells (ie, proliferative undifferentiated sebocytes) at the periphery of sebaceous glands. Cannabinoid receptor type 1 immunoreactivity was also detected in the cytoplasm of secretory and ductal cells of sweat glands. In the dermis, diffuse cytoplasmic staining was observed in perivascular cells with typical mast cell morphology. Cannabinoid receptor type 1 immunoreactivity was also detected in the cytoplasm of scattered fibroblasts and, rarely, in endothelial cells.

Immunolocalization of CB2 in lymph node samples—Cannabinoid receptor type 2 immunoreactivity

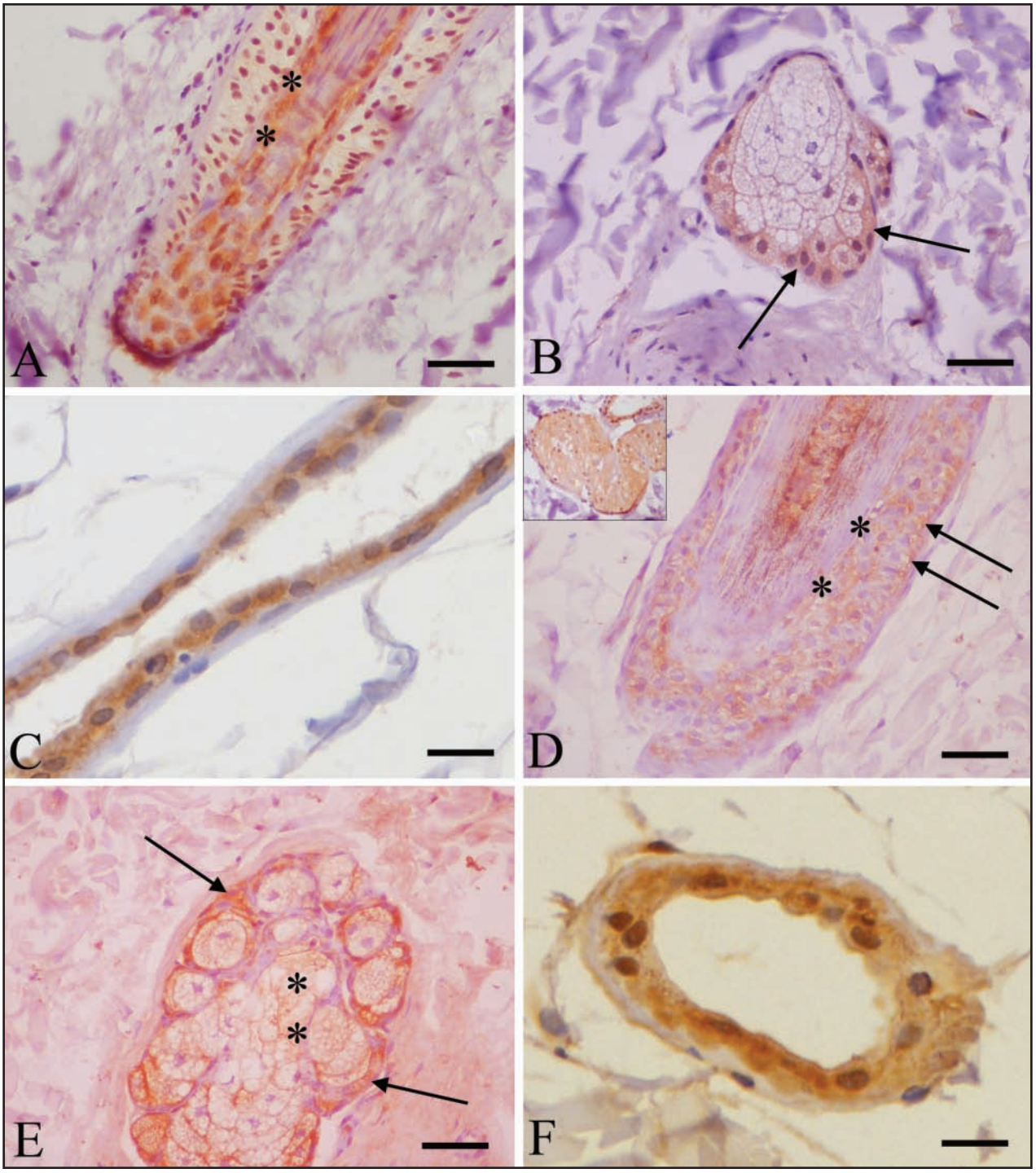

Figure 2—Representative photomicrographs of histologic sections of skin samples from healthy dogs illustrating immunohistochemical staining for CB1 (A, B, and C) and CB2 (D, E, and F) in hair follicles and sebaceous and sweat glands. A—Notice CB1 immunoreactivity in the inner root sheath (asterisks) of the follicle. Bar = 50 μm. B—Notice CB1 immunoreactivity in peripheral sebaceous reserve cells (arrows). Bar = 50 μm. C—Notice CB1 immunoreactivity in ductal epithelium of apocrine sweat glands. Bar = 125 μm. D—Notice CB2 immunoreactivity in the inner (asterisks) and outer (arrows) root sheaths of a hair follicle. Bar = 50 μm. Inset—Notice CB2 immunoreactivity in the arrector pili muscles. E—Notice CB2 immunoreactivity in peripheral sebaceous reserve cells (arrows) and mature sebocytes (asterisks). Bar = 50 μm. F—Notice CB2 immunoreactivity in ductal epithelium of apocrine sweat glands. Bar = 125 μm.

was detected in lymph node samples of healthy dogs (Figure 1). Strong CB2 immunoreactivity was detected in B-cell zones of lymphoid follicles, mainly in the germinal centers of secondary follicles, whereas weak CB2 immunoreactivity was detected in the outer mantle and marginal zones of lymph nodes. Lymphocytes in the paracortex of lymph nodes did not have CB2 immunoreactivity. Cannabinoid receptor type 2 immunoreactivity was detected on membranes and in cytoplasm of B cells.

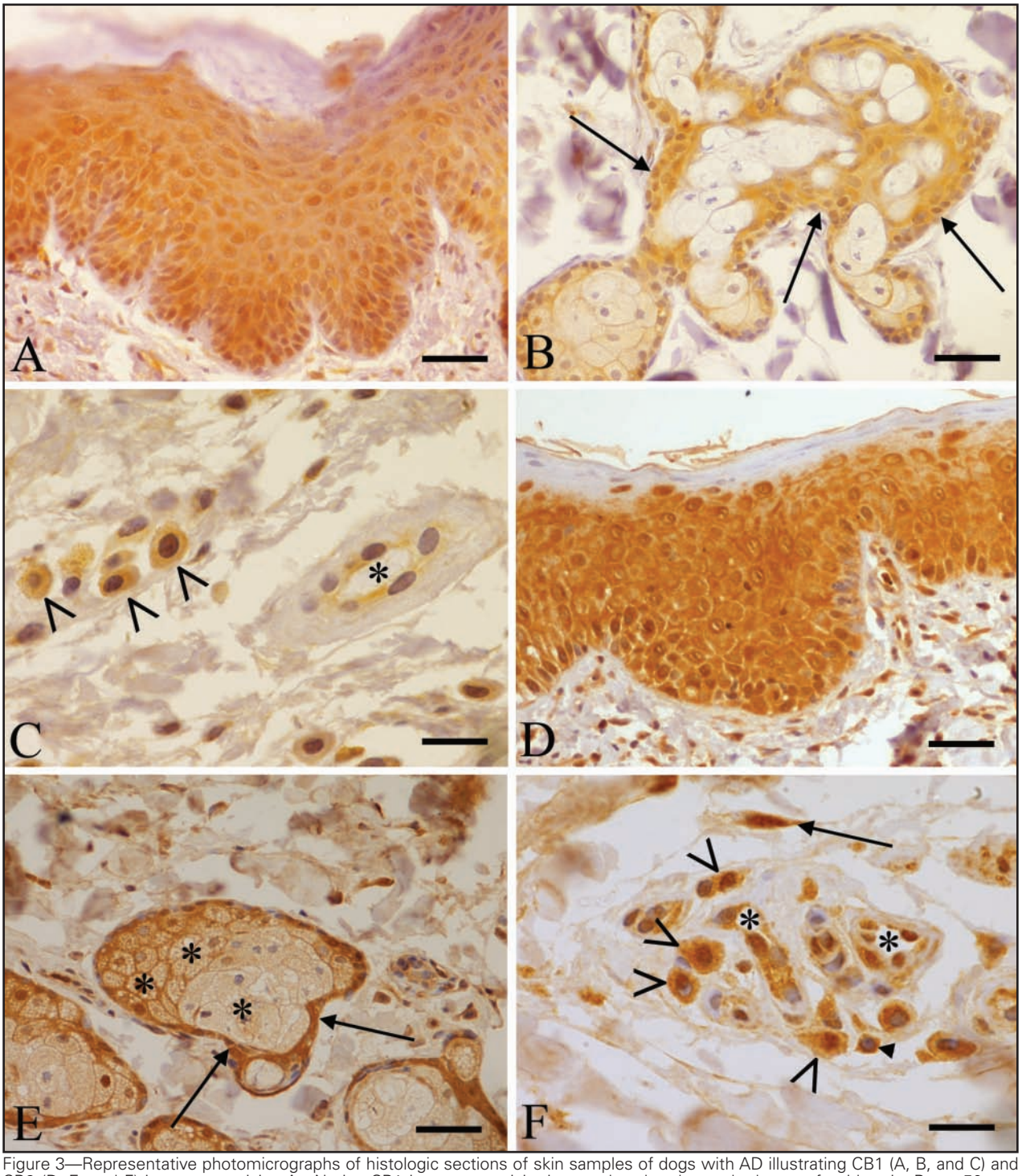

Figure 3—Representative photomicrographs of histologic sections of skin samples of dogs with AD illustrating CB1 (A, B, and C) and CB2 (D, E, and F) immunoreactivity. A—Notice CB1 immunoreactivity in suprabasal and granular layers of epidermis. Bar = 50 μm. B—Notice CB1 immunoreactivity in reserve cells of hypertrophic sebaceous glands (arrows). Bar = 50 μm. C—Notice CB1 immunoreactivity in perivascular cells with mast cell morphology (open arrowheads) and endothelial cells lining the lumen of a small blood vessel (asterisk). Bar = 125 μm. D—Notice CB2 immunoreactivity in basal, suprabasal, and granular layers of epidermis. Bar = 50 μm. E—Notice CB2 immunoreactivity in peripheral sebaceous reserve cells (arrows) and centrally located mature sebocytes (asterisks). Bar = 50 μm. F—Notice CB2 immunoreactivity in perivascular cells with mast cell morphology (open arrowheads), endothelial cells lining lumina of small blood vessels (asterisks), a fibroblast-like dermal cell (arrow), and a lymphocyte (small solid arrowhead). Bar = 125 μm.

Immunolocalization of CB2 in skin samples of healthy dogs—Cannabinoid receptor type 2 immunoreactivity was detected in skin samples of healthy dogs. In the epidermis, CB2 immunoreactivity was detected in cytoplasm of cells of the basal and suprabasal layers (Figure 1). Hair follicles had CB2 immunoreactivity in the basal and suprabasal cells of the outer and inner epithelial root sheaths (Figure 2). Mild cytoplasmic CB2 immunoreactivity was detected in cells of arrector pili muscles and secretory and ductal cells of sweat glands. Sebaceous glands had CB2 immunoreactivity in the cytoplasm of peripheral reserve cells and, to a lesser extent, in the differentiated-cell compartment. In the dermis, diffuse cytoplasmic staining was observed in perivascular cells with typical mast cell morphology. Cannabinoid receptor type 2 immunoreactivity was also detected in diffusely located fibroblasts and endothelial cells.

Immunolocalization of CB1 and CB2 in skin samples of dogs with AD—The epidermis in skin samples of the 5 dogs with AD was hyperplastic and focally hyperkeratotic. Strong cytoplasmic CB1 immunoreactivity was observed in keratinocytes of the suprabasal spinous layers of the epidermis. Conversely, epidermal basal keratinocytes had only weak focal CB1 immunoreactivity, and cells of the stratum corneum did not have CB1 immunoreactivity (**Figure 3**). Keratinocytes of the basal and suprabasal layers of the epidermis had marked cytoplasmic CB2 immunoreactivity, whereas corneocytes did not have CB2 immunoreactivity.

Sweat and sebaceous glands were hyperplastic. In sebaceous glands, CB1 and CB2 immunoreactivity was observed in cytoplasm of reserve cells and CB2 immunoreactivity was observed in cytoplasm of undifferentiated reserve cells and mature sebocytes; nuclei of some cells had CB1 and CB2 immunoreactivity. Cytoplasmic CB1 and CB2 immunoreactivity was detected in perivascular cells with mast cell morphology, endothelial cells lining postcapillary venules of the dermis, and diffusely located elongated fibroblastic cells.

Results were consistent among tissue samples of each type (skin samples from healthy dogs, skin samples from dogs with AD, hippocampus samples, and lymph node samples). Negative control tissue samples for which primary antibodies were replaced with an unrelated antibody that was preabsorbed to the corresponding CB1 or CB2 blocking peptide did not have immunoreactivity (data not shown), thus confirming specificity of the anti-CB1 and anti-CB2 antibodies.

Discussion

Results of the present study indicated CB1 and CB2 were present in the skin of healthy dogs and dogs with AD. It is worth mentioning that the endocannabinoid system supports homeostasis (ie, regulation of cell proliferation, survival, and differentiation) in skin of healthy animals and has protective functions for cells against effects of many acute and chronic inflammatory skin diseases.[24]

In skin samples of clinically normal dogs, we detected a homogeneous distribution of CB1 and CB2 in all epidermal layers, whereas in humans, CB1 is mainly detected in epidermal spinosum and granulosum layers, and CB2 is mainly detected in basal keratinocytes.[18] The epidermis of dogs is thinner than that of humans (2 to 3 vs 6 to 7 nucleated cell layers, respectively), and this difference might account for the lack of a difference in distribution between CB1 and CB2 in epidermal layers of skin samples of normal dogs in the present study. Alternately, there may be species differences in the epidermal distribution of CB1 and CB2. Other authors[25] investigating distribution of CB1 and CB2 in normal and neoplastic skin of humans found that the 2 receptors were almost equally distributed in suprabasal layers of epidermis; those authors also reported that staining of basal keratinocytes was only sporadically detected, although they did not specify to which of the 2 receptors this finding applied. In skin samples of dogs with AD in the present study, hyperplastic epidermal changes were detected, strong CB1 and CB2 immunoreactivity was detected in suprabasal keratinocytes, and weak CB1 and strong CB2 immunoreactivity was detected in basal keratinocytes. These findings indicated these 2 receptors may be upregulated during inflammation.

In hair follicle epithelium of dogs in the present study, we found CB1 immunoreactivity in keratinocytes of inner root sheaths and CB2 immunoreactivity in keratinocytes of inner and outer root sheaths. This finding supported the hypothesis of other authors[26] that function of hair follicles is sensitive to the effects of psychoemotional stress and findings of another study[27] that Δ^9-tetrahydrocannabinol is incorporated into hair of humans.[27] Immunoreactivity specific for CB1 has been detected in keratinocytes of inner[18] and outer[28] root sheaths of hair follicles of humans. Cannabinoid receptor type 2 immunoreactivity has been detected in outer root sheaths of hair follicles of humans.[18] Furthermore, authors of another report[20] detected CB1 in cells of the inner root sheaths of cycling hair follicles of dogs. Findings of 2 recent studies[20,24] indicated CB1 is expressed in a hair-cycle–dependent manner in hair follicle epithelium, suggesting that hair follicles have a CB1-mediated endocannabinoid signaling system that might act as a negative regulator of hair growth via autocrine and paracrine signaling. To the authors' knowledge, distribution of CB2 in hair follicle epithelium of dogs has not been previously described; the pattern of distribution we found in cells of inner root sheaths and basal and suprabasal keratinocytes of outer root sheaths of dogs in the present study was similar to findings for cycling hair follicles of humans.[18]

In glandular skin structures of dogs in the present study, CB1 and CB2 immunoreactivity was typically detected in secretory and ductal cells of sweat glands. These findings were similar to those of another study[18] in which CB1 and CB2 were detected in myoepithelial cells of eccrine glands and epithelium of sweat gland ducts of humans.[18] Receptors CB1 and CB2 each have specific localizations in ductal and acinar structures; authors of another study[29] suggested the differing localizations of CB1 and CB2 in glandular structures in tissues other than skin of rats may be related to the 2 fundamental steps of saliva production.[29] Similarly, CB1 is present in ductal rather than acinar cells in salivary glands of dogs.[19]

Cannabinoid receptor type 1 was detected in undifferentiated reserve sebaceous cells, and CB2 was detected in mature and undifferentiated sebocytes in skin samples of dogs in the present study. These findings do not correlate with localization of CB1 and CB2 in humans; undifferentiated sebaceous cells have strong immunoreactivity for CB2, whereas differentiated sebocytes express CB1 in skin of humans.[18] Results of the present study regarding expression of CB1 and CB2 in sweat and sebaceous glands of dogs, even though they were somewhat different from results for humans, indicated a possible functional role of the cannabinoid system in sweat and sebaceous glands.[18]

Glandular structures are hyperplastic in skin of dogs with AD.[30] In skin samples of dogs with AD in the present study, CB1 and CB2 immunoreactivities were strong in cytoplasm of cells of sweat and sebaceous glands. The pattern of distribution of CB1 and CB2 in skin samples from dogs with AD was similar to that in glandular structures of skin samples of healthy dogs in the present study.

Mast cells have an essential role in the pathogenesis of cutaneous inflammatory diseases in humans and dogs.[31–33] In skin samples of healthy dogs and dogs with AD in the present study, perivascular dermal cells with typical mast cell morphology and distribution had immunoreactivity specific for CB1 and CB2. We considered these to be mast cells because of their location and morphology and the finding of other authors[34] that skin of dogs typically has mast cells but not macrophages. That finding of the present study was also supported by results of other studies that cells of the mast cell line RBL2H3 express CB1 and CB2[35,36] and that agonists of CB1 and CB2 decrease mast cell degranulation.[37,38] Notably, the cannabimimetic compound PEA inhibits release of inflammatory mediators by immunologically challenged mast cells freshly isolated from skin biopsies of dogs.[39]

Cannabinoid receptor type 1 and CB2 were immunolocalized to the cytoplasm of epidermal and follicular keratinocytes, sweat and sebaceous gland epithelial cells, and mesenchymal dermal cells of skin samples in the present study. It is believed that CB1 permanently and constitutively cycles between the plasma membrane and endosomes of cells, causing intracellular localization of this receptor,[40] which would support findings of the present study and results of another study[18] that intense immunolabeling for CB1 and CB2 is detected in the cytoplasm of epithelial cells.

Receptors CB1 and CB2 and their agonists mitigate several effects of disease, including inflammation and increased nociception in humans and laboratory animals.[8,9,41,42] Administration of endocannabinoids and cannabimimetic compounds (eg, PEA) decreases skin inflammation in rats[43] and sensory phenomena in skin such as signs of pain and pruritis in mice,[44] suggesting the endocannabinoid system might be a promising target for treatment of skin disorders.[24,45] Notably, expression of PEA in skin of mice is markedly elevated during experimentally induced allergic dermatitis, and PEA protects against the effects of inflammation in keratinocytes (in vitro) and effects of allergic inflammation in skin (in vivo).[46] Dermatitis is a major medical issue in the field of veterinary medicine, and AD, a common dermatologic condition in humans and dogs,[30,31,47] poses a substantial diagnostic and therapeutic challenge during a patient's lifetime because no single treatment is universally effective.

Because they have immunosuppressive properties, cannabinoids could potentially be considered anti-inflammatory agents.[45] Other authors[21,44,46] have proposed that the cannabinoid system may be involved in attenuation of allergic responses and pruritus. Administration of cannabimimetic compounds to reduce clinical signs during hypersensitivity dermatitis in cats has been investigated.[48] Oral administration of the cannabimimetic compound PEA delays development of clinical signs in dogs with experimentally induced AD.[m]

Since the present study was conducted, results of another study[10] have been reported in which the benefits of administration of PEA to dogs with allergic skin disease were investigated. That study[10] included hypersensitive Beagles; the Beagles were immunologically challenged before a single dose of PEA (3, 10, and 30 mg/kg) was administered PO. Results of that study[10] indicate administration of PEA significantly reduces allergic responses (ie, skin wheal area) in dogs. Results of the present study suggested those effects of PEA may have been mediated, at least in part, by CB1 and CB2. Results of the present study indicated the endocannabinoid system is present in skin of healthy dogs and dogs with AD. Further studies are warranted to investigate the structure and function of the endocannabinoid system in dogs, which may potentially be a target for treatment of AD and other inflammatory diseases in companion animals.

a. Dolorex, Merck Animal Health, Boxmeer, The Netherlands.
b. Domitor, Orion Pharma, Espoo, Finland.
c. Stiefel Laboratories, Redecesio di Segrate, Italy.
d. Rabbit polyclonal anti-CB1, catalog No. ab23703, Abcam, Cambridge, England.
e. Rabbit polyclonal anti-CB2, catalog No. ab45942, Abcam, Cambridge, England.
f. SuperfrostPlus slides, Fisher Scientific, Pittsburgh, Pa.
g. ImmPRESS Universal, Vector Laboratories, Burlingame, Calif.
h. ImmPACT DAB, Vector Laboratories, Burlingame, Calif.
i. NCL-CALp rabbit polyclonal anti-calcitonin, Novocastra, Newcastle, Northumberland, England.
j. Cannabinoid receptor 1 peptide, catalog No. ab50542, Abcam, Cambridge, England.
k. Cannabinoid receptor 2 peptide, catalog No. ab45941, Abcam, Cambridge, England.
l. NIS-Elements Basic Research, Nikon Instruments Inc, Melville, NY.
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