An immunohistochemical study of uveodermatologic syndrome in two Japanese Akita dogs

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Abstract

Materials Ocular and cutaneous tissues from two Japanese Akita dogs with uveodermatologic syndrome (UVD) were subjected to immunohistochemical analysis.

Results Light microscopic examination of the globes confirmed the presence of panuveitis of different severity in each case. The infiltrate was primarily granulomatous with prominent perivascular lymphoid aggregates. Melanophages were present throughout the affected areas, and there were scattered plasma cells. Immunohistochemistry using CD79a, CD3, MAC387 and MHC class II markers indicated that there were relatively few T lymphocytes and that most lymphocytes were of the B-cell lineage. The two skin biopsies examined also appeared to represent different stages of cutaneous pathology. The biopsy from one case was consistent with the reported features of skin lesions of canine UVD syndrome, including granulomatous dermatitis with extensive T-cell infiltration extending into the epidermis. In contrast, the skin lesion from the second case showed less inflammation, more pigmentary incontinence and evidence of dermal fibrosis. There was no immunoglobulin or complement deposition at any level within the cutaneous or ocular lesions.

Conclusions The findings of these two cases suggest that the skin lesions of these two dogs with UVD syndrome were mediated by T cells and macrophages (Th1 immunity), whereas the ocular lesions were more consistent with a B cell and macrophage response (Th2 immunity). This is, however, a preliminary investigation and these features may not be the same for all cases of UVD syndrome.

Key Words: autoimmunity, dog, immunohistochemistry, Japanese Akita, uveodermatologic syndrome, Vogt–Koyanagi–Harada

INTRODUCTION

Uveodermatologic syndrome (UVD) is a chronic panuveitis with cutaneous involvement resulting in vitiligo and poliosis, which was first described in two Japanese Akitas in 1977.1 Since then, other case reports have been published documenting a similar syndrome in other breeds such as the Siberian Husky and Golden Retriever, although the majority of cases have been in the Japanese Akita.1–13

Canine UVD has been likened to human Vogt–Koyanagi–Harada (VKH) syndrome, which is a chronic multiorgan granulomatous inflammatory condition of unknown etiology, which principally affects the eyes, auditory system, meninges and skin.14 The common link between the ocular, cutaneous and central nervous system signs is a similar embryologic development of pigment containing cells, which are the target of the granulomatous inflammation.14 The mechanism that triggers the autoimmune reaction is unknown, but sensitization to melanocytic antigens by means of cutaneous injury or possible viral infection has been postulated.15,16 Experimental evidence suggests that VKH involves a T-lymphocyte mediated autoimmune process directed against an unknown antigen associated with melanocytes. Exposure of lymphocytes from human patients with VKH disease to peptides derived from the tyrosinase family of proteins led to significant proliferation of lymphocytes. The tyrosinase family of proteins are enzymes involved in melanin formation and are expressed specifically by melanocytes. Immunization of these peptides into pigmented rats induced ocular and extraocular changes that highly resembled human VKH disease.17

Canine UVD is similarly believed to be due to an immune-mediated reaction against melanocytes, but there is little evidence that demonstrates an underlying trigger factor, although
both bacterial and fungal causes have been suggested.\textsuperscript{2,3,6,18} In one study, histopathologic examination of a young male Japanese Akita revealed sequestration of uveal melanocytes surrounded by large accumulations of inflammatory cells, in a very similar pattern to that presented in humans. However, the immunologic basis for this inflammation has not been clearly identified in the veterinary literature.\textsuperscript{9}

Studies conducted in humans suffering from VKH indicated that CD3\(^+\) \(T\) cells constituted the majority of lymphocytes present in aqueous humor and cerebrospinal fluid (CSF).\textsuperscript{19} The percentage of CD4\(^+\) lymphocytes in uveitic aqueous humor and CSF was significantly higher than was present in the peripheral blood \((P < 0.001)\), and activated CD4\(^+\) and CD8\(^+\) cells were significantly more frequent in aqueous humor than in CSF and the peripheral circulation.\textsuperscript{19} Fas antigen was also highly expressed by CD4\(^+\) cells in aqueous humor and there was a significantly higher proportion of Fas\(^+\) and memory cells in the aqueous humor than in the CSF or peripheral blood.\textsuperscript{19}

Histopathologic analysis of cutaneous lesions in humans with VKH revealed mononuclear cell infiltration of the slightly edematous dermis, especially surrounding hair follicles and sweat glands.\textsuperscript{20} Melanin-laden cells in the epidermis were partially lost, and the infiltrating mononuclear cells consisted primarily of \(T\) lymphocytes and a small number of \(B\) lymphocytes.\textsuperscript{20} Most cells expressed HLA-DR (human leukocyte antigen-DR), and CD4\(^+\) lymphocytes were dominant over CD8\(^+\) in a ratio of 3 : 1. The results suggest that cell-mediated immunity plays an important role in the pathogenesis of cutaneous lesions of humans with VKH.\textsuperscript{20}

The central nervous signs of human VKH are rarely seen in dogs; however, a single Japanese Akita presenting with clinical central nervous system signs not explained by another disease process has been reported.\textsuperscript{5} One study described a single Siberian Husky with a subacute meningitis at necropsy that was similar to that found in humans with VKH.\textsuperscript{15} Therefore, although canine patients cannot unequivocally be shown to have VKH syndrome, they may have a VKH-like syndrome. This syndrome in the dog has been named uveodermatologic syndrome (UVD).

In this paper the clinicopathologic findings of uveodermatologic syndrome in two Japanese Akita dogs are reviewed. Histopathologic assessment of both ocular and cutaneous lesions were performed in both cases and tissues from these dogs were subjected to immunohistochemical studies to define further the nature of the cellular immune response in this disease.

MATERIALS AND METHODS

Case material

Medical records from the Small Animal Hospital of the School of Clinical Veterinary Science, University of Bristol were searched for cases of UVD in Japanese Akita dogs.

The criteria for case selection were: (i) breed; (ii) no previous history of ocular trauma or surgical intervention; (iii) no clinical or laboratory evidence suggestive of other ocular disease; (iv) bilateral ocular involvement; (v) evidence of chorioretinitis with or without anterior uveitis, vitreous inflammatory reaction, focal areas of subretinal fluid or bullous retinal detachment, and (vi) alopecia, poliosis or vitiligo.

Four cases satisfied the inclusion criterion of a confirmed diagnosis of this condition and in two cases tissue suitable for immunohistochemistry was collected. Case 1 was diagnosed solely on clinical and postmortem examinations, while case 2 had been previously diagnosed via clinical examination, serologic evaluation and histopathologic sampling of the skin.

Histopathology

Entire globes and skin biopsies were taken from both cases and placed into 10% neutral buffered formalin. Tissues were paraffin wax embedded, and 4-micron sections were stained with hematoxylin and eosin for routine histopathology. Serial sections were cut from selected blocks for immunohistochemical studies, and these were mounted onto organosilane-coated microscope slides.

Immunohistochemistry

Sections from each sample were subjected to two categories of immunohistochemical evaluation. Category one included labeling to detect the presence of \(T\) lymphocytes (expressing the CD3 marker), \(B\) lymphocytes and plasma cells (expressing surface membrane or cytoplasmic CD79a, respectively), macrophages or dendritic cells (expressing class II molecules of the major histocompatibility complex, MHC), or monocyte-macrophages (expressing the antigen MAC 387, calprotectin).

Unstained sections were hydrated through graded alcohols and treated with 0.5% hydrogen peroxide in methanol for 30 min to eliminate endogenous peroxidase activity. After washing in distilled water, the sections were subjected to antigen retrieval by boiling for 6 min in citrate buffer (pH 6, 0.01 \(\text{M}\)) at half power in a 750 watt microwave oven. Following washing in phosphate buffered saline (PBS, pH 7.4, 0.01 \(\text{M}\)) sections were incubated with normal goat or normal mouse serum (each diluted at 1/20; Dako), mouse antihuman MAC 387 (diluted 1/20; Dako) and mouse antihuman CD79a (diluted 1/20; Dako). Two negative control sections were incubated with either normal rabbit serum or normal mouse serum (each diluted at 1/800) at this stage.

After primary incubation, sections were washed in PBS before application of the appropriate secondary antibody for a 30-min period at room temperature. These secondary antisera were either biotin-conjugated goat antimouse immunoglobulin (diluted 1/20; Sigma Chemical Company, Poole, Dorset, UK) or biotin-conjugated goat antirabbit IgG (diluted 1/20; Sigma). Following a further PBS wash, the
final incubation for all sections was with avidin-conjugated horseradish peroxidase (diluted 1/20; Sigma). Finally, immunolabeling was visualized by applying 0.05% 3, 3'-diaminobenzidine tetrahydrochloride with 0.01% hydrogen peroxide for 2 min. Slides were washed with distilled water and counterstained with Mayer's hematoxylin before being dehydrated through graded alcohols prior to mounting under DPX.

The immunohistochemical procedure applied to sections in category two was designed to demonstrate the presence of immunoglobulins (IgG, IgM or IgA) or the third component of complement (C3) within the tissue samples. Pretreatment of these sections was as described above; however, the antigen retrieval was by incubation for 30 min at 37 °C in a solution of 0.1% trypsin in calcium chloride. The initial 30-min incubation of the sections was with either a 1/5 dilution of normal goat serum in PBS (for IgG, IgM and IgA) or with a 1/5 dilution of normal rabbit serum in PBS (for complement C3). Following washing in PBS, the primary antisera were applied for a 30-min period at room temperature with the slides incubated in a humid chamber. These antisera included: rabbit antidog IgG (Fc specific), rabbit antidog IgM (Fc specific), rabbit antidog IgA (Fc specific) and goat antigoat IgG conjugated to horseradish peroxidase (diluted 1/20; Sigma). Finally, the secondary reagents were goat antirabbit IgG conjugated to horseradish peroxidase (diluted 1/200 in PBS) at this stage.

After incubation with primary antisera, the sections were again washed in PBS before incubation with the appropriate secondary antisera for a 30-min period at room temperature. The secondary reagents were goat antirabbit IgG conjugated to horseradish peroxidase (diluted 1/100 in PBS) and rabbit antigoat IgG conjugated to horseradish peroxidase (diluted 1/200 in PBS). Following further PBS washing, the final stages of bound antibody visualization, counterstaining and mounting were as described for the first procedure above.

RESULTS

Case history

Case 1 An 11-year-old, neutered female Japanese Akita was presented with a 2-day history of watery, ‘red’ eyes. The owners had observed that the dog was in pain and blind before seeking veterinary advice. On clinical examination there was no menace or dazzle response, or pupillary light reflex from either eye, and conjunctival and episcleral hyperemia were present bilaterally. Slit-lamp biomicroscopy revealed mild corneal edema and peripheral corneal neovascularization. Intraocular examination revealed grade three flare, iridal hyperemia with increased prominence of vasculature, and iris bombe present bilaterally. Retinal examination was difficult due to vitreal haze and opacification, but revealed large areas of bullous retinal detachment and degeneration. The intraocular pressure was 14 mmHg in the right eye and 10 mmHg in the left eye. At the time of examination there were no obvious skin lesions present, although a dermatologist did not examine the case.

A diagnosis of panuveitis was made and, in view of the poor prognosis, the owners opted for euthanasia. Both globes and skin biopsies from normal appearing facial skin and nasal planum were submitted for histopathologic examination. Nasal planum was selected for biopsy due to dense pigmentation and a previously published predilection for cutaneous clinical signs. The referral pathologist in private practice had elucidated no other cause for the ocular lesions and histopathology of the skin showed granulomatous dermatitis consistent with UVD. The condition had been treated with topical prednisolone acetate 1% (Predforte, Allergan Ltd, High Wycombe, Buckinghamshire, UK) four times daily and systemic prednisolone at up to 1 mg/kg twice daily over the previous 4 years by the referring veterinarian. Initial treatment with azathioprine (Imuran; Glaxo Wellcome, Stockley Park, Middlesex, UK) at 50 mg once daily for 3 weeks and then 25 mg every 2 days for 3 months had initially controlled the disease process. The referring veterinary surgeon had discussed the case with the ophthalmology and dermatology units at the University of Bristol after which the owners requested euthanasia. Necropsy samples of depigmented skin from the face and nasal planum, and one globe were taken. The cutaneous sites were selected on the basis of clinical evidence of depigmentation and on a predilection for this disease already documented.

Histopathology and immunohistochemistry

Case 1 In the first case there was some preservation of basic ocular microarchitecture; however, there was a severe granulomatous inflammatory infiltration of the iris, ciliary body, choroid and retina extending to sclera (Fig. 1). The infiltrate was dominated by macrophages, with a prominent neutrophilic component. At the margins of the ciliary body the lesions comprised perivasculare aggregates of small lymphocytes, and there were striking small nodular perivasculare aggregates of small lymphocytes at regular intervals within the choroid and retina. Melanophages were present throughout the affected areas and there were scattered plasma cells. The optic nerve was unaffected, but there were dense granulomatous infiltrates surrounding the nerve caudal to the globe. Additional isolated granulomatous foci were noted within the scleral tissue. Immunohistochemistry revealed that the small lymphoid aggregates within this lesion comprised B lymphocytes, which on separate sections were noted to express CD79a+ and MHC class II, with low numbers of individual T cells present throughout (Fig. 2). By contrast,
the majority of the small lymphocytic aggregates at the periphery of the ciliary body lesion were T cells. The infiltrating macrophages had weak cytoplasmic expression of MHC class II, but class II labeled cells with dendritic morphology were not prominent. There were, however, numerous MAC387+ cells within the infiltrates. Plasma cells scattered throughout the lesion expressed CD79a and predominantly IgG. There was no evidence of complement C3 deposition.
A biopsy from nasal planum of clinically normal appearance in case 1 was examined. The biopsy was taken from this region due to the typical presentation of lesions at this site, but the lack of visible alteration in pigmentation may suggest that the histopathologic findings were indicative of preclinical disease. There was a diffuse granulomatous infiltration of the superficial dermis, extending focally in places to involve the superficial adnexal structures. Although the dominant cell type in the infiltrate was the macrophage, neutrophils, small lymphocytes and plasma cells were also present (Fig. 3). The infiltrate was principally below the level of the epidermal basement membrane zone, but occasionally there was a mild infiltration into the basal epidermis with accompanying local spongiosis. There was no evidence of basal keratinocyte vacuolation, or of keratinocyte apoptosis. Although the epidermis was pigmented, there was minimal evidence of pigmentary incontinence. Immunohistochemical studies revealed strong expression of MHC class II by the dermal macrophages and dermal dendritic cells. Additionally, there were very prominent class II+ epidermal Langerhans cells (Fig. 4), and there was focal cytoplasmic and membrane expression of MHC class II by keratinocytes (Fig. 5). MAC387+ cells were prominent within the dermal infiltrates. The lesions were heavily infiltrated by CD3+ T lymphocytes, and these cells were seen to migrate into the epidermis (Fig. 6).

Figure 6. Section of skin from case 1 labeled with antibody specific for the T lymphocyte marker CD3. There are numerous T cells within the superficial dermis and scattered T cells are seen to infiltrate into the overlying epidermis. Avidin-biotin immunohistochemistry, bar = 50 µm.

Figure 7. Section of ciliary body from case 2. There is obliteration of normal structure by extensive granulomatous infiltration and necrosis with prominent melanophages. A perivascular lymphoid aggregate is present to the right side of the photomicrograph. Hematoxylin and eosin, bar = 200 µm.

Figure 8. Section of ciliary body from case 2 labeled with antibody specific for the T lymphocyte marker CD3. The majority of the lymphocytes do not express this molecule, suggesting that they are of the B cell lineage. Avidin-biotin immunohistochemistry, bar = 50 µm.

Figure 9. Serial sections of the same area of ciliary body from case 2 shown in Fig. 8. This section is labeled with antibody specific for class II molecules of the major histocompatibility complex. The majority of lymphocytes express this molecule, providing further evidence for a B lymphoid lineage. Avidin-biotin immunohistochemistry, bar = 50 µm.
CD79a+ plasma cells were scattered throughout the lesion, and most of these expressed cytoplasmic IgG. There was no evidence of immunoglobulin or complement deposition associated with the keratinocytes, basement membrane zone or blood vessels.

Case 2 A complete sagittal hemisection of the globe from case 2, including eyelids, optic nerve and adjacent musculature was prepared. The microscopic appearance was of a severe endophthalmitis. The entire structure of the eye had been obliterated, with the exception of the lens, and there was replacement of the iris, ciliary body, choroid and retina by a dense circumferential band of granulation tissue. Within the inner zone of this tissue there were foci of necrosis, areas of granulomatous inflammation, and a prominent infiltration of small lymphocytes and plasma cells that often aggregated around blood vessels. Large clusters of melanophages were also prominent focally (Fig. 7). The optic nerve was degenerate, but other pericellular structures were unaffected. The nature of the infiltrate was characterized by immunohistochemistry. There were relatively few T lymphocytes within the infiltrates, and these comprised a small proportion of the perivascular aggregates (Fig. 8). By contrast, most of the small lymphocytes expressed membrane MHC class II (Fig. 9). Although the same cells did not appear to express CD79a, their most likely identity was of the B lymphoid lineage. The macrophages infiltrating the lesion did not express MHC class II; however, this marker did delineate a population of cells with dendritic morphology scattered throughout. MAC387+ cells were not a feature of the infiltrate. The plasma cells expressed cytoplasmic CD79a and IgG. There was no evidence of complement deposition associated with vessels or epithelium in the section.

A skin biopsy was also taken from case 2. In this biopsy there was partial ulceration of the epidermis, with formation of a fibrinocellular crust in the areas of epidermal loss. The superficial dermal infiltration was milder than that described above, and was predominantly mononuclear in nature. There was prominent pigmentary incontinence, with melanophages clustered within the superficial dermis. Additionally, there was evidence of early fibrosis in the superficial dermal region. On immunohistochemistry, the dominant labeling was for MHC class II+ dermal macrophages and dendritic cells. Epidermal Langerhans cells were less prominent in this skin lesion, and there was no keratinocyte expression of class II MHC. Lymphocytes and plasma cells were sparse, and there was no deposition of immunoglobulin or complement at any site.

DISCUSSION

Although the four cases identified within the hospital database were well defined clinically, appropriate tissue biopsies were only available from two dogs for evaluation, and these became the focus of the present study. The low number of tissues examined means that it is not possible to make definitive statements about the pathology or local tissue immune response in these dogs. However, there are a number of useful observations that can be highlighted.

The two ocular lesions examined may represent different stages of the disease process. It is likely that the globe from case 1 presented earlier pathology than the severe endophthalmitis noted in the sample from case 2. There were similarities and differences in the nature of the tissue immune response in these two samples. Both were primarily a granulomatous inflammation. However, the nature of the macrophage population differed in each lesion. In case 2 the macrophages did not express MHC class II, although a population of class II+ dendritic-like cells were present. By contrast, the macrophages in the eye of case 1 were class II+ but dendritic cells were not abundant. Moreover, the population of recent blood emigrants represented by MAC387+ cells was only present in the lesions from case 1. These observations may be a reflection of the relative chronicity of the lesions in case 2. The chronic nature of the latter lesion might also explain the lack of T cells in that sample, in contrast to the T cell aggregates present at the margin of the ciliary body in case 1 (Table 1).

The most unexpected finding, that was consistent in both biopsies, was the dominance of B lymphocytes over T lymphocytes within the lesions. This was particularly so in the first case, where there were striking nodular aggregates of B cells within the choroid and retina. Taken together, the finding of granulomatous inflammation associated with a B, rather than T, cell infiltration would be consistent with the form of granulomatous inflammation driven by a type 2 (Th2)

<table>
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<th>Table 1. Antigen presenting cell populations present in ocular and cutaneous lesions</th>
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| Case 1 | 1. Macrophages express MHC class II in eye  
2. No dendritic cell population in eye  
3. MAC387+ cells present in eye  
4. Granulomatous infiltrate of MHC class II+ macrophages and dermal dendritic cells, more prominent vs. case 2  
5. Class II expression by epidermal keratinocytes and prominent Langerhans cells  
6. Larger number of MAC387 cells within the skin vs. case 2  
7. Heavy infiltrate of T cells in dermis and focally in epidermis |
| Case 2 | 1. Macrophages do not express MHC class II within the eye  
2. Class II+ dendritic cells present within the eye  
3. No MAC387+ cells present within the eye  
4. Granulomatous infiltrate of MHC class II+ macrophages and dermal dendritic cells |
lymphoid population. Recognition that all granulomatous inflammation may not be associated with type 1 (Th1) immunity is relatively recent\textsuperscript{21} and the ocular inflammation in canine UVD syndrome might be an example of a type 2 granulomatous process. There was, however, no evidence for immunoglobulin or complement deposition within these lesions, although local plasma cells were present.

The two skin biopsies examined again appeared to represent different stages of cutaneous pathology. The biopsy from case 1 was consistent with the reported features of the skin lesions of canine UVD syndrome, including granulomatous dermatitis with minimal involvement of the overlying epidermis. Although heavily infiltrated, there was minimal pigmentary incontinence in this biopsy. In contrast, there was less inflammation, more pigmentary incontinence and evidence of dermal fibrosis in the skin lesion from case 2. These features might suggest that the lesions in case 2 were more chronic in nature than those sampled from case 1. However, it is important to remember that the lack of visible pathology at the time of biopsy may indicate that the pathologic evaluation was of a preclinical stage, or that the results were due to another different disease pathology not solely related to UVD. These histopathologic findings support the clinical picture seen in cases 1 and 2, with case 2 having required systemic prednisolone at doses of up to 1 mg/kg twice daily over the proceeding 5 years for both ocular and cutaneous manifestation of suspected UVD. Medical management had restricted progression of the cutaneous clinical profile of these cases. Because of this, it is possible that the cutaneous and ocular lesions initially both start with a type 2 reaction and that, during development of the disease, the cutaneous lesions transform into a type 1 reaction.

In the medical literature an attempt has been made to determine the immunologic factors associated with VKH. Experimental observations have demonstrated that tyrosinase peptides are immunogenic, and may be a candidate autoantigen in VKH.\textsuperscript{22} Later studies have reported that lymphocytes from patients with VKH made strong proliferative responses when cultured with peptides derived from the tyrosinase family proteins.\textsuperscript{17} Injection of these peptides into pigmented rats induced ocular and extraocular changes that resembled human VKH. These studies support the hypothesis that VKH involves an autoimmune response to the tyrosinase family proteins.

Studies in humans have shown that the presence of the major histocompatibility complex allele HLA-DRB-1 was associated with VKH syndrome.\textsuperscript{23} This was further investigated in the Han Chinese using molecular genetic techniques and these studies found that HLA-DRB1*0405 and DRB1*15 were closely associated with the susceptibility to VKH syndrome. DRB1*0405 was probably the major susceptibility gene, with DRB1*15 having a minor association. There was also a negative association between DRB1*14, DRB1*08 and the susceptibility to VKH syndrome, suggesting that there may be resistance genes.\textsuperscript{24} In Korean patients with DRB1*0405, a reduction in visual acuity and increase in ocular complications were common, suggesting that HLA-DRB1*0405 itself, or a haplotype
including this allele, greatly increased the risk for developing VKH syndrome.\(^5\) Although the canine MHC class II genes are now well defined,\(^6\) there have been no studies of genetic associations with UVD in the Japanese Akita.

The recent research in humans may lead to some new areas for investigation in the susceptible canine population, and may help to localize the immunologic factors that are involved in this devastating canine disease.

REFERENCES