Immunopathology of Pineal Glands from Horses with Uveitis

Carolyn M. Kalsow,1 Richard R. Dubielzig,2 and Ann E. Dwyer1,3

**PURPOSE.** Pinealitis accompanying uveitis is well established in laboratory models of autoimmune uveoretinitis. In naturally occurring uveitis, pinealitis has been demonstrated in the pineal gland from a mare with active uveitis and is suspected in some human uveitides. We have evaluated pineal glands from horses with various stages of uveitis for signs of immunopathology accompanying spontaneous uveitis.

**METHODS.** Pineal glands from 10 horses with uveitis and from 13 horses without uveitis were evaluated for histochemical (H&E, collagen) and immunohistochemical (MHC class II antigen expression, infiltration of T and B lymphocytes, and glial fibrillary acidic protein (GFAP) and vimentin upregulation) evidence of inflammation.

**RESULTS.** Septal areas of pineal glands from horses with uveitis had clusters of MHC class II antigen-expressing cells, T lymphocytes, and enhanced collagen deposition. These changes were not as readily observed in pineal glands from horses without uveitis. B lymphocytes were detected only in the pineal gland from the one mare with active uveitis in which T and B lymphocytes were organized into follicles. No differences in GFAP or vimentin immunoreactivity were noted in pineal glands from horses with or without uveitis.

**Conclusions.** These pineal gland changes suggest that the pinealitis associated with equine uveitis is transient just as the uveitis of these horses is recurrent. Study of pineal glands from horses with clinically documented uveitis allows demonstration of subtle pineal changes associated with natural uveitis. Similar changes would be difficult to document in human patient populations.

**Lymphocytic infiltration of the pineal gland is regularly observed in laboratory models of experimental autoimmune uveoretinitis (EAU). This experimental autoimmune pinealitis (EAP) is a generalized phenomenon that has been induced in a variety of species by various photoreception-associated proteins that are also present in pineal gland.**

In humans, lower serum levels of the pineal neurohormone melatonin in patients with uveitis suggests pineal gland abnormalities in these individuals. However, there is no direct histopathologic evidence of pineal gland changes in human patients with uveitis. Aged pineal glands are usually not available for direct observation, especially at the time of active inflammation, and noninvasive techniques have yet to be developed to detect pineal inflammation in situ.

Equine recurrent uveitis is a natural disorder that can serve as a model of human uveitis. This is a spontaneous inflammation in which there is no artificial perturbation of the systemic immune response, and tissues from horses euthanatized because of blindness or other reasons are more readily obtainable for study than are tissues from human patients. Documented pinealitis in a mare with active uveitis prompted this investigation of immunohistoopathologic changes of pineal glands from several horses with and without uveitis.

**MATERIALS AND METHODS**

**Pineal Glands**

Pineal glands from 10 horses with a diagnosis of uveitis and from 13 horses without uveitis were provided by Ann Dwyer of the Genesee Valley Equine Clinic, William Rebhun, DVM, of Cornell University, and Richard Dubielzig, DVM, of the University of Wisconsin. The tissue was recovered from horses at the time of euthanasia or natural death, fixed in formalin, 95% ethanol or Bouin’s solution and embedded in paraffin.

Clinical information was available for 6 of the 10 horses with uveitis. All 6 had had a recurrence in one eye within one year and had a duration of uveitis for greater than a year at the time of euthanasia. Only one horse was considered to have active uveitis at the time of euthanasia, i.e., recurrence within 2 weeks rather than 2 months or greater. These horses had been treated expeditiously. The other 4 horses with uveitis...
presented to a tertiary center for euthanasia. The had clinical and histopathologic evidence of uveitis, but detailed clinical information was not available.

**Histochemistry and Immunohistochemistry**

Histopathologic changes were evaluated on sections stained with hematoxylin and eosin (H&E), and collagen was visualized with Verhoef’s stain for elastin and collagen (Department of Surgical Pathology, University of Rochester).

Major histocompatibility complex (MHC) class II antigen expression, T lymphocytes, B lymphocytes, vimentin, and glial fibrillary acid protein (GFAP) were visualized by a peroxidase-antiperoxidase immunohistochemical procedure. After clearing, sections were treated with 3% H$_2$O$_2$ for 5 minutes to remove endogenous peroxide and were blocked with Superblock blocking buffer (Pierce, Rockford, IL) for 20 minutes at room temperature. Peroxidase was visualized with DAB Enhanced Substrate Kit (Pierce, Rockford, IL) for 20 minutes at room temperature. Sections were dehydrated for mounting in Refrax (Anatech, Ltd., Battle Creek, MI), viewed by brightfield microscopy and photographed with TMAX 400 film (Eastman Kodak Co., Rochester, NY). Although primary antibodies HLA-DRα and B cell were derived from mice sensitized to human antigens, their equine reactivity was confirmed on sections of equine lymph node.

**RESULTS**

T-lymphocyte-reactive cells were observed in 9 of 10 pineal glands from horses with uveitis and in 8 of 13 pineal glands from horses without uveitis (Table 2, Fig. 1). More strikingly, MHC class II antigen reactivity of septal infiltrating cells was seen in 8 of 10 (80%) pineal glands from horses with uveitis but in only 4 of 13 (31%) pineal glands from horses without uveitis (Table 2, $\chi^2 = 5.49, P = 0.019$). MHC class II antigen-reactive cells were only found in those pineal glands that also had T-lymphocyte-reactive cells.

MHC class II antigen immunoreactivity was also observed on the vascular endothelium of the pineal glands in which MHC class II antigen-expressing cells were observed in the septa, and in some additional pineal glands (Fig. 1C). MHC class II antigen immunoreactivity of vascular endothelium was observed in 9 of 10 pineal glands from horses with uveitis and 6 of 13 pineal glands from horses without uveitis.

B-lymphocyte immunoreactivity was observed only in the pineal gland from the one horse euthanatized at the time (within 2 weeks) of active uveitis. Some of the extensive accumulation of both T and B lymphocytes in the septa was organized as distinct follicles with B-lymphocyte MAb-immunoreactive cells in the follicle surrounded by T-lymphocyte MAb-immunoreactive cells. MHC class II antigen-immunoreactive cells were observed in both the follicular and the extrafollicular areas (Fig. 2).

Verhoeef’s stain for elastin and collagen demonstrated strong reactivity for collagen in the septa of pineal glands from each of the 10 horses with uveitis. There was no such reactivity in 6 of 13 pineal glands from horses without uveitis (Fig. 3). These measurements were confirmed morphometrically (data not shown). GFAP immunoreactivity of stellate cells was observed in each of the pineal glands, and GFAP immunoreactivity of fiber bundles was present in most but not all pineal glands, i.e., immunoreactive fiber bundles were observed in 7 of 10 pineal glands from horses with uveitis and in 10 of 13 pineal glands from horses without uveitis. Hence, presence or absence of such reactivity did not correlate with a diagnosis of uveitis. Highly variable vimentin reactivity of vessels, cells, and fibers did not correlate with a diagnosis of uveitis.

**DISCUSSION**

Detection of lymphocytes in pineal gland is not unique. Clusters of lymphocytes have been described in specific mouse and

### Table 1. Primary Antibodies for Immunohistochemistry

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Source</th>
<th>Clone</th>
<th>Specificity</th>
</tr>
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<tbody>
<tr>
<td>HLA-DRα</td>
<td>DAKO Corp.*</td>
<td>TAL 1B5</td>
<td>MHC class II antigen</td>
</tr>
<tr>
<td>WS6657</td>
<td>D. P. Lunn, DVM†</td>
<td>CVS 6</td>
<td>T lymphocytes, PMN</td>
</tr>
<tr>
<td>B cell, CD20</td>
<td>DAKO Corp.</td>
<td>L26</td>
<td>B lymphocyte</td>
</tr>
<tr>
<td>GFAP</td>
<td>DAKO Corp.</td>
<td>GF-2</td>
<td>Astrocytes</td>
</tr>
<tr>
<td>Vimentin</td>
<td>DAKO Corp.</td>
<td>V9</td>
<td>Intermediate filaments of cells of mesenchymal origin</td>
</tr>
</tbody>
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* Santa Barbara, CA.  
† University of Wisconsin.

### Table 2. T lymphocyte and MHC Class II Antigen Immunoreactive Cells in Pineal Glands from Horses with and without Uveitis

<table>
<thead>
<tr>
<th>Immunoreactivity</th>
<th>Ocular Status (n = 23)</th>
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<tbody>
<tr>
<td></td>
<td>With Uveitis (n = 10)</td>
</tr>
<tr>
<td><strong>MHC class II</strong></td>
<td></td>
</tr>
<tr>
<td>T cell + (n = 12)</td>
<td>8</td>
</tr>
<tr>
<td>T cell - (n = 0)</td>
<td>0</td>
</tr>
<tr>
<td><strong>MHC Class II</strong></td>
<td></td>
</tr>
<tr>
<td>T cell + (n = 5 )</td>
<td>1</td>
</tr>
<tr>
<td>T cell - (n = 6)</td>
<td>1</td>
</tr>
</tbody>
</table>
FIGURE 1. Immunohistopathology of pineal gland from a horse with uveitis. Lymphocytes (arrows) in the septa of the pineal gland (A, H&E) include cells immunoreactive for T lymphocytes (arrows) (B) and for MHC class II antigen (arrows) (C). Original magnification, ×400.

rat species, and in an occasional human pineal gland. This is not totally unexpected in light of trafficking of T lymphocytes through tissues, including neural tissues, and absence of a full blood-brain barrier in the pineal gland. However, normal presence of lymphocytes in the pineal gland must be differentiated from pathologic changes associated with uveitis or with other disease states.

In this study, horses with and without uveitis had T-lymphocyte clusters in the septa of their pineal glands, but MHC class II antigen reactivity of infiltrating lymphocytes was seen more frequently in pineal glands from horses with uveitis. Because activated and memory T lymphocytes of horses express MHC class II antigen but naive T lymphocytes do not, it is probable that pineal lymphocytes of horses with uveitis included activated or memory T lymphocytes. Conversely, the T lymphocytes without MHC class II antigen expression, from horses without uveitis, were probably naive lymphocytes.

MHC class II antigen expression of infiltrating cells was linked to infiltration of T lymphocytes. Although macrophage-monocyte lineage cells may have been part of this MHC class II antigen-reactive infiltrate, they could not be demonstrated as such by present techniques (unpublished data). Furthermore, because MHC class II-expressing cells were not seen in the absence of T lymphocytes, macrophage-monocyte lineage cells are probably not constitutively present in the equine pineal gland, as has been demonstrated in the rat pineal gland. Observation of MHC class II antigen in vascular endothelium of almost half of the pineal glands from horses without uveitis and in almost all the horses with uveitis suggests that activation of the endothelium is a more general response not restricted to uveitis.

Pineal glands from two horses with uveitis did not show the characteristic finding of T lymphocytes and MHC class II antigen-expressing cells in the septa. Because cells of other tissues from both horses were reactive with these antibodies, absence of reactivity indicates absence of expression rather than inability of cells of these individual horses to react with the MAb. Absence of findings in these two horses with uveitis could indicate end stage disease or a transient nature of the pineal gland involvement.

Conversely, observation of T lymphocytes and MHC class II-expressing cells in four horses without a diagnosis of uveitis suggests that there was an undiagnosed uveitis or that infiltration of MHC class II antigen-expressing T lymphocytes is not uveitis specific. Although eyes were examined at the time of euthanasia, a complete history of all horses, especially those without uveitis was not always available.

Detection of B lymphocytes only in the pineal gland from the one horse with active uveitis suggests that their presence is transient and restricted to active inflammation. It is significant that these B and T lymphocytes were organized into follicles as observed in the eyes from this horse and from other horses with uveitis and from humans with uveitis, but not in eyes from mice or rats with EAU. Because we have studied only one pineal gland obtained within 2 weeks of an episode of uveitis, additional pineal glands from horses with active uveitis will be required to confirm whether these findings are peculiar to this horse or truly associated with active uveitis.

EAP associated with EAU in laboratory animals has similarities to the changes of the pineal glands from these horses with uveitis. The infiltrate in both is predominantly mononuclear, including T lymphocytes and MHC class II antigen-expressing cells. Also, a few eosinophiles have been observed in EAP, just as they were in equine uveitis-associated pinealitis. Although not as prominent as T lymphocytes, B lymphocytes have been observed in EAP in rats and primates, as they were seen here in equine pinealitis. Accumulation of lymphocytes in the pineal gland does not appear to be overtly cytotoxic to the pinealocytes of either laboratory animals or horse, and activation of the pineal vascular endothelium also has been detected in EAP. Furthermore, even as the inflammation of labora-
FIGURE 2. Lymphocytic nodules in pineal gland from a horse with active uveitis. The septa had heavy infiltration of lymphocytes some of which were organized into distinct follicles (A, H&E) with B-lymphocyte MAb-reactive cells in the follicle (B) surrounded by T lymphocyte MAb-reactive cells (C). MHC class II antigen immunoreactive cells were observed in both the follicular and the extrafollicular areas (D). Original magnification, ×160.

FIGURE 3. Collagen histochemistry in equine pineal gland. The septa (arrows) of the pineal gland from a horse without uveitis had minimal collagen reactivity (A, B) compared to the significant collagen reactivity in the pineal gland from a horse with uveitis (C, D). Verhoef's stain for elastin and collagen. Original magnification, (A, C) ×160; (B, D) ×400.
specific for uveitis. Even so, this fibrosis may well be indicative of previous pineal infiltrate associated with uveitis.

In summary, activated T lymphocytes were observed in fibrotic septa of pineal glands from horses with uveitis. B lymphocytes, organized into follicles, were only present during active uveitis. These results suggest that pinealitis is associated with uveitis and that the pinealitis may be transient, just as the uveitis is recurrent. Furthermore, accessibility of equine pineal glands at appropriate times permits documentation of the subtle and/or transient pineal changes associated with spontaneous uveitis. Similar changes would be more difficult to document in human patient populations.

Acknowledgments

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References