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Disciplines
Medicine and Health Sciences | Ophthalmology | Veterinary Medicine

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VIRAL-ANTIBODY COMPLEXES IN CANINE ADENOVIRUS TYPE 1 (CAV-1)
OCULAR LESIONS:
LEUKOCYTE CHEMOTAXIS
AND ENZYME RELEASE

L. E. CARMICHAEL1, B. L. S. MEDICI, S. I. BISTNER2, and G. D. AGUIRRE1

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Accepted October 24, 1974

ABSTRACT Canine adenovirus-type 1 (CAV-1)-antibody complexes caused severe anterior uveitis with corneal edema ("blue eye") when injected into the anterior chamber of normal dogs. The response of the anterior uvea to such immune complexes (IC) was similar to the spontaneously occurring disease. In the presence of complement (C3), IC caused release of neutrophile chemotactic factors. Following phagocytosis of IC-C3, leukocytes released lysosomal enzymes, as indicated by the presence of acid phosphatase in the surrounding medium. Membrane bound viral aggregates, presumably IC, were common in neutrophiles and in macrophages that had infiltrated the anterior chamber of opaque eyes that occurred after intravenous (IV) inoculation with attenuated CAV-1. These data were incorporated into a postulated scheme for the pathogenesis of CAV-1 uveitis with corneal edema.

KEY WORDS: DOG, CANINE ADENOVIRUS-1, ANTERIOR UVEITIS, CORNEAL EDEMA, IMMUNE COMPLEX, LEUKOCYTE CHEMOTAXIS, PATHOGENESIS.

(Cornell Vet., 1975, 65:331-351)
INFECTION by canine adenovirus type-1 (CAV-1) causes a generalized, frequently fatal disease in young dogs; however the disease in older animals usually is mild, often being recognized only by an increase in specific antibody or by the appearance in recovered dogs of severe inflammation of the anterior uvea and corneal edema (“blue eye”). Ocular manifestations also are seen following vaccination with attenuated CAV-1 strains (4,9). Previous reports from this laboratory (1,5,6) have described clinical and pathological features of the ocular disease in relation to changes associated with viral growth in the eye and the associated systemic and local immune responses. It was hypothesized that the ocular lesions were mainly a consequence of Type III (immune complex-mediated) hypersensitivity, for a feature of the disease was the demonstration of viral antigen and specific antibody in the anterior uveal tracts of virtually all affected eyes. Also, severe uveitis occurred after the intraocular injection of canine adenoviral-antibody complexes (immune complexes). Inflammatory responses were more severe if complement (C') had been added to immune complex (IC) preparations before injections were made. Extensive and severe panuveitis also occurred within 4 hours following intravitreal injection of non-living CAV-1 soluble antigens into eyes of immunized dogs with high levels of complement-fixing and neutralizing CAV-1 antibodies. In the latter instances, histological examination of affected eyes indicated that recovery would not have occurred.

In a recent study (1), ultrastructural changes were described in the corneal endothelium of dogs at various times during the course of experimental CAV-1 infection. During the early stage of inapparent or mild uveitis, virus could be isolated from the aqueous fluid and clusters of viral particles were observed in the nuclei of occasional corneal endothelial cells. Later, during the stage of severe anterior uveitis (usually 7 to 8 days after inoculation by the intravenous route), virus was not isolated from the aqueous fluid, but viral antigen and immunoglobulins could be demonstrated by immunofluorescence within inflammatory cells which had infiltrated into the anterior chamber and trabecular spaces. The inflammatory cells, consisting of mononuclear and somewhat fewer numbers of polymorphonuclear leukocytes, occurred as clusters within a fibrin meshwork (keratic precipitates) and commonly were found closely adherent to the corneal endothelium. Electron microscopic study revealed phagocytized aggregates of membrane-bound CAV-1 particles within the cytoplasm of apparently normal and degenerating macrophages and neutrophiles. Such aggregates were interpreted as IC within phagolysosomes.

To further investigate the pathogenic functions of IC in CAV-1 ocular disease, the generation of leukocytic chemotactic factors (CF) and release of lysosomal hydrolases as possible mediators of corneal endothelial injury were examined. In this paper, additional evidence is presented that indicates a pathogenetic role for viral-antibody complexes in the anterior uvea of dogs infected with CAV-1. In addition to accounting for the sudden and extensive accumulations of inflammatory cells in the anterior chamber and trabecular spaces of affected eyes, *in vivo* and *in vitro* studies were made to attempt to relate lysosomal enzyme release to corneal endothelial damage.

**MATERIALS AND METHODS**

**Immune complex preparations.** Cornell-1 strain CAV-1 (infectious canine hepatitis virus) was used. Methods of virus isolation, growth in dog kidney cell (DKC) cultures, and the preparation of viral antigens have been described (5,6). Immune complex preparations were made as reported previously (6), however in some experiments dissociated viral antigens were prepared by a methanol precipitation method (16) followed by dialysis against pH 10.3 buffer (0.1 M glycine-NaOH). An approximate 40-fold concentration of viral antigen, as evaluated by complement-fixation tests, was obtained. Mixtures of viral antigen and varying amounts of anti-CAV-1 immunoglobulin solution, prepared by ammonium sulfate precipitation (final saturation of 33%) were made in a series of dilution tubes and incubated for about 2 hours at 37C, at which time a floccular precipitate appeared in some tubes. Mixtures
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then were allowed to react at 4C for an additional period of 24 hours, and the contents of the tube with the greatest amount of precipitate were combined with those of tubes with two- and four-fold greater amounts of antigen. The pooled precipitates then were centrifuged at 2,500 rev/min (PR-2 Centrifuge, International Eqpt. Co., Needham Heights, MA.) for 20 minutes at 4C and washed twice in 0.15 M phosphate-buffered saline, pH 7.4 (PBS). After two additional washes, precipitates were suspended in PBS to one-fifth the original volume. Preparations of IC were stored at -15C until used for dog inoculations or for in vitro experiments. Such IC preparations, after reaction with C', consistently produced intense uveitis, commencing within 2-6 hours after injection into the anterior chamber of CAV-1 susceptible dogs' eyes. Arthus-type skin reactions also occurred in dogs within 4-6 hours after intradermal inoculation of 0.2 ml amounts of IC into the abdominal skin.

After tests in dogs had demonstrated the capability of an IC preparation to engender anterior uveitis, the preparation was considered satisfactory for in vitro experiments.

Dog studies. A total of 66 specific-pathogen-free Beagle dogs from the Veterinary Virus Research Institute's colony were used. Their ages ranged from 9 to 17 weeks. Inoculation of IC or lysed leukocyte suspensions into the anterior chamber of dogs' eyes and collection of aqueous fluid samples was done as described elsewhere in detail (6). Selected eyes from dogs which had developed typical CAV-1 "blue eyes" following intravenous inoculation with attenuated CAV-1 were enucleated shortly after the onset of corneal opacification and stored in sealed plastic vials at -70C until cryostat sections (4 nm) were made for immunofluorescent study. Eyes for electron microscopic examinations were fixed in situ, as described below.

Leukocyte preparations. Samples (16 ml) of peripheral blood were obtained from the jugular vein and placed in silicone-treated 50 ml glass centrifuge tubes containing 4 ml of sterilized 5% dextran (MW 250,000) made up in Hank's saline solution, pH 7.4. The blood-dextran mixtures were allowed to stand for 1 hour at 35C and the leukocyte-rich plasma portions, which always contained a small number of erythrocytes, were harvested and centrifuged for 5 min. at 800 rev/min. in an International PR-2 centrifuge. Cell pellets were then suspended to one-half the original plasma volume in RPMI 1640 medium containing 4% heated lamb serum. Leukocyte counts indicated 0.8 to 1.2 X 10^7 cells/ml.

Electron microscopy. Ultrathin sections of portions of the anterior segment (cornea and angle) of eyes with early uveitis and corneal edema were fixed in situ and stained as described previously (1). For in vitro studies, leukocyte preparations were fixed with 2.5% glutaraldehyde and osmic acid and embedded for sectioning according to a described method (12). Examinations were done with a Philips EM-300 electron microscope.

Chemotactic factor (CF) assay. An assembly that contained two chambers separated by a membrane filter (AHLCO Mfg. Co., New Britain, Conn.) was used. Micropore filters (Millipore Corp., Bedford, Mass.), 0.65 nm pore size, were employed for the assay of CF elaborated by peripheral blood leukocytes. The method used has been described in detail (18). Immune complex-C' (IC-C') preparations to be tested for CF activity, together with appropriate controls, were injected into the lower compartment of a chemotaxis assembly; peripheral blood leukocyte suspensions were prepared as described above and 1 ml amounts then were injected into the upper chamber. After incubation for 3 hours at 37C in 5% CO2 and air, fluids were aspirated from the chambers and the filters were fixed and stained according to a described method (17). Quantitation of the leukotactic responses was done by counting (375 X magnification) the number of leukocytes that had migrated in at least 5 randomly selected fields. A chemotactic index (CI) was recorded (number of migrating cells reacted with test preparations divided by the number of leukocytes that had migrated when the lower chambers contained medium only). Only those cells which clearly had migrated into the plane of focus of the lower portion of the filter were counted. The 0.65 nm filter permitted evaluation only of neutrophile responses, since macrophages are excluded by the pore size of the filters used (18). Phago-
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**Acid phosphatase assays.** Acid phosphatase is a common marker enzyme, indicating liberation of lysosomal hydrolases from damaged leukocytes, or their release following phagocytosis of bacteria, zymosan particles, or immune complexes (20). In this study, acid phosphatase activity of aqueous fluid and serum samples from normal dogs and from dogs with CAV-1 induced corneal opacities were determined colorimetrically, using commercially prepared reagents (Sigma, St. Louis, Mo., Techn. Bull. No. 104, 1973). Similar tests were done using leukocyte suspensions (1 ml amounts) reacted in silicone-treated glass tubes with viral antigen, immunoglobulin, or IC preparations (0.3 ml amounts), to which 0.1 ml of C′ had been added. In some instances, reactions were carried out on nonphagocytosable surfaces (Micropore filters, 0.45 nm pore size) according to described procedures (12,13). Enzyme activities were expressed as Sigma Units (Sigma, St. Louis, Mo., Techn. Bull. No. 104, 1973), or as per cent release of enzyme activity released into supernatant fluids obtained after centrifugation of leukocyte reaction mixtures for 10 min. at 2,000 rev/min. at 4°C. The total enzyme activity of leukocyte suspensions was liberated by treatment with 0.2% Triton X-100 (Rohm and Hass Co., Philadelphia, Pa.).

**RESULTS**

**Neutrophile chemotaxis by IC.** In the 6 dogs used for these experiments, uveitis commenced within 3 to 8 hours following intraocular inoculation with IC-C′, reaching maximal intensity approximately postinoculation (PI) hours 24-36 (Fig. 1). Reactions varied somewhat, but there was chemosis, iridal edema and marked aqueous flare in all injected eyes. Microscopic lesions of the anterior uveal tract shortly after onset of corneal edema consisted of heavy infiltrations by neutrophiles, with somewhat lesser numbers of mononuclear cells (Fig. 2). The cells appeared to originate from the root.
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Frozen sections of such eyes stained with fluorescein-labelled anti-dog C3 revealed bright membrane-associated fluorescence of cells adherent to the corneal endothelium and within the trabecular spaces (Fig. 4).

Examination of frozen sections of eyes obtained from two dogs soon after the onset of corneal edema (PI days 7 and 8 following intravenous inoculation with attenuated CAV-1) also revealed viral antigen within cytoplasmic cellular compartments but, in contrast with dogs given intraocular injections with IC-C', intranuclear virus also was observed in small groups of corneal endothelial cells located approximately 1 to 3 mm from the corneal-iridal angle (Fig. 5).

Electron microscopic examination of eyes fixed shortly after the onset of corneal clouding revealed large numbers of cells (macrophages and neutrophiles) containing membrane-bound aggregates of adenoviral particles (Figs. 6a and 6b); some of the phagocytosed viral clusters appeared partially digested. Intranuclear (replicating) virus was not observed at this stage by electron microscopy. However, as noted above, immunofluorescence microscopy revealed occasional small clusters of cells, usually near the corneal-iridal angle, that contained intranuclear viral antigen. Attempts to isolate virus from such eyes were unsuccessful, probably because of...
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Fig. 3. CAV-1 antigen in the cytoplasm of leukocytes within the aqueous fluid of dog's eye 8 hours following injection with viral-antibody complexes, plus complement. Fluorescent antibody stain applied to smear preparation. X400.

Fig. 4. Leukocytes adherent to the corneal endothelium (CE). Cell-bound complement (C') revealed by anti-dog C' fluorescent antibody stain applied to frozen section of anterior segment of eye. Twenty-four hours following injection of the anterior chamber with immune complex suspension. X400.
neutralizing antibody which always is found in the aqueous fluid in opaque eyes (5).

*Fig. 5.* Frozen section of the anterior segment of eye with corneal edema obtained 7 days after intravenous inoculation with CAV-1. Brightstaining intranuclear viral antigen (arrow) in corneal endothelial cells. Aggregates of viral antigen also were seen in cytoplasm of clumped leukocytes within anterior chamber. Fluorescent antibody stain, ×400.

**In vitro** studies to determine the chemotactic activity of IC are summarized in Table 1. From these results, it was clear that chemotaxis by CAV-1/antibody complexes was dependent on the presence of fresh serum, i.e., a C" component(s). Figs. 7a, 7b and 7c illustrate the chemotactic responsiveness of peripheral blood leukocytes (neutrophiles) to IC-C". Because results were essentially the same using IC preparations made from methanol-precipitated partially purified virus (Fig. 8) or viral subunit antigens prepared by dissociation at pH 10.3, data in Table 1 does not distinguish the tests done with these different antigens.

*Figs. 6a and b.* Double membrane-bound aggregates of viral particles within the cytoplasm of macrophage (M) juxtaposed against Descemet's membrane (D). Corneal endothelial cells were absent in this area (Fig. 6a). Electron photomicrograph, ×25,000. Similar cytoplasmic structures (arrows) within macrophage comprising portion of keratic precipitate. Note fibrin (F). Electron photomicrograph, ×13,000.
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![Fig. 5](image)

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Table 1. Neutrophile chemotaxis by canine adenovirus-1 (CAV-1)-antibody complexes (immune complexes)

<table>
<thead>
<tr>
<th>Test mixture*</th>
<th>Test no.</th>
<th>Mean number cells/field**</th>
<th>Chemotactic index (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC + fresh dog serum (C')</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46</td>
<td>15.3</td>
<td></td>
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<tr>
<td>2</td>
<td>54</td>
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</tr>
<tr>
<td>3</td>
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<td>&gt;15</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&gt;100</td>
<td>&gt;21.7</td>
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<tr>
<td>IC + RPMI 1640 medium</td>
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</tr>
<tr>
<td>1</td>
<td>4</td>
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<td>2</td>
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<tr>
<td>4</td>
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<td>&gt;21.7</td>
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<tr>
<td>CAV-1 antigen + fresh dog serum (C')</td>
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<tr>
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<td>4</td>
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<td>Anti-CAV-1 globulin + fresh dog serum (C')</td>
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<tr>
<td>RPMI 1640 medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
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<td>3</td>
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<tr>
<td>3</td>
<td>11</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
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<td></td>
</tr>
</tbody>
</table>

*Test mixture indicates reactants in lower well of chemotaxis assembly, made up to final volumes of 1 ml with RPMI 1640 medium. Upper wells contained about 10^7 leukocytes, as described in text.

**At least 5 fields (40X objective magnification) counted per stained filter.

IC = immune complex.

Figs. 7a, b and c. Chemotactic response of canine peripheral leukocytes (neutrophiles) to CAV-1-antibody complexes, plus complement (C'). Neutrophiles on top of filter membrane (7a) have migrated through filter pores to lower side (7b) in response to immune complex-C'-generated chemotactic factors. Absence of chemotactic factors (control) indicated by reduced leukocytic migration (7c). Arrow indicates single migrating cell within filter pore. Focus on top of filter, giving distorted image of cells on bottom of filter. H & E. ×800.
Table 1. Neutrophile chemotaxis by canine adenovirus-1 (CAV-1)-antibody complexes (immune complexes)

<table>
<thead>
<tr>
<th>Test mixture*</th>
<th>Test no.</th>
<th>Mean number cells/field**</th>
<th>Chemotactic index (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC† + fresh dog serum (C')</td>
<td>1</td>
<td>54</td>
<td>15.3</td>
</tr>
<tr>
<td>2</td>
<td>&gt;150</td>
<td>&gt;15</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;100</td>
<td>&gt;21.7</td>
<td></td>
</tr>
<tr>
<td>IC + RPMI 1640 medium</td>
<td>1</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>CAV-1 antigen + fresh dog serum (C')</td>
<td>1</td>
<td>5</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Anti-CAV-1 globulin + fresh dog serum (C')</td>
<td>1</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
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<td>---</td>
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<td>2</td>
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<td>3</td>
<td>11</td>
<td>---</td>
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</tr>
</tbody>
</table>

*Test mixture indicates reactants in lower well of chemotaxis assembly, made up to final volumes of 1 ml with RPMI 1640 medium. Upper wells contained about 10⁷ leukocytes, as described in text.

**At least 5 fields (40× objective magnification) counted per stained filter.

†IC = immune complex.

Figs. 7a, b and c. Chemotactic response of canine peripheral leukocytes (neutrophiles) to CAV-1-antibody complexes, plus complement (C'). Neutrophiles on top of filter membrane (7a) have migrated through filter pores to lower side (7b) in response to immune complex-C'-generated chemotactic factors. Absence of chemotactic factors (control) indicated by reduced leukocytic migration (7c). Arrow indicates single migrating cell within filter pore. Focus on top of filter, giving distorted image of cells on bottom of filter. H & E. ×800.
Lysosomal enzyme studies. There was no measurable acid phosphatase activity in aqueous fluid samples from normal eyes, however elevated enzyme levels (0.1 to 1.0 Sigma units) were found in virtually all samples from opaque eyes (Table 2). Enzyme levels of serum samples obtained from 14 normal dogs were 0.19 ± 0.09 Sigma units (range 0.07 to 0.41). Similar serum enzyme levels also were found in dogs with corneal edema. In 15 of 29 samples obtained from opaque eyes, enzyme levels of aqueous fluid samples were not significantly different from those of the serum; however 14 samples had acid phosphatase activities greater than found in serum, suggesting some local release.

In vitro studies of the release of acid phosphatase from leukocytes by IC-C′ preparations are summarized in Table 3.

The enzyme release from leukocytes reacted with IC-C′ was 7.7 ± 0.75%; in contrast, release from leukocytes incubated with CAV-1 antigen, C′, or antiseraum amounted to only 1.1 ± 0.01%. Reaction mixtures were monitored by immunofluorescence and, occasionally, by electron microscopy for phagocytic capabilities of the cell preparations. Active phagocytosis was revealed by the appearance of bright green cytoplasmic fluorescence in leukocytes reacted with IC-C′ (Fig. 9). Also, cells that had ingested IC-C′ occurred in clumps. Cells exposed only to viral antigen suspensions could not be distinguished from controls.

Electron microscopy of leukocytes exposed to IC-C′ revealed heavily vacuolated cells, with some double membrane-bound vacuoles containing clumps of viral particles and amorphous material. However, in the in vitro preparations,
Lysosomal enzyme studies. There was no measurable acid phosphatase activity in aqueous fluid samples from normal eyes, however elevated enzyme levels (0.1 to 1.0 Sigma units) were found in virtually all samples from opaque eyes (Table 2). Enzyme levels of serum samples obtained from 14 normal Ig.

Table 2. Acid phosphatase levels in aqueous fluid samples from normal eyes and eyes with corneal edema.

<table>
<thead>
<tr>
<th>Aqueous fluid samples</th>
<th>Acid phosphatase activity (Sigma units)</th>
<th>Per cent enzyme release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal eyes (non-infected)</td>
<td>&lt;0.1 0.1-0.3 0.4-1.0</td>
<td>1 2 3</td>
</tr>
<tr>
<td>Normal eyes (CAV-1 infected)**</td>
<td>18/18* 0/18 0/18</td>
<td>2 3 4</td>
</tr>
<tr>
<td>Corneal edema (CAV-1 infected)†</td>
<td>20/29 18/29 11/29</td>
<td>2 3 4</td>
</tr>
</tbody>
</table>

*The numerator is the number of samples with acid phosphatase activity within the range listed; the denominator is the total number of samples tested in each class.

**Of the 39 samples in this category, 26 were from the non-affected eye of dogs with unilateral corneal opacities. The remainder were obtained from normal eyes of dogs at the time of onset of anterior uveitis with corneal edema in one or more littermates.

†Samples obtained within 12 hours after onset of corneal edema.

In vitro studies of the release of acid phosphatase from leukocytes by IC-C' preparations are summarized in Table 3.

Table 3. Acid phosphatase release from canine leukocytes by canine adenovirus-1 (CAV-1)-antibody complexes (immune complexes).

<table>
<thead>
<tr>
<th>Test mixtures</th>
<th>Experiment</th>
<th>Acid-phosphatase (Units)*</th>
<th>Per cent enzyme release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes + IC-C'**</td>
<td>1</td>
<td>3.6</td>
<td>6.9</td>
</tr>
<tr>
<td>2</td>
<td>6.5</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Leukocytes + antigen, C', or immunoglobulin</td>
<td>1</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Leukocytes + 0.2% Triton X-100</td>
<td>1</td>
<td>52 ± 5</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>52 ± 2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*Enzyme activity expressed as Sigma Units x 100. Values represent mean of at least three replicate tests.

**IC-C' indicates immune complex suspensions reacted with complement prior to addition of leukocytes.

The enzyme release from leukocytes reacted with IC-C' was 7.7 ± 0.75%; in contrast, release from leukocytes incubated with CAV-1 antigen, C', or antisera at only 1.1 ± 0.01%. Reaction mixtures were monitored by immunofluorescence and, occasionally, by electron microscopy for phagocytic capabilities of the cell preparations. Active phagocytosis was revealed by the appearance of bright green cytoplasmic fluorescence in leukocytes reacted with IC-C' (Fig. 9). Also, cells that had ingested IC-C' occurred in clumps. Cells exposed only to viral antigen suspensions could not be distinguished from controls.

Electron microscopy of leukocytes exposed to IC-C' revealed heavily vaculated cells, with some double membrane-bound vacuoles containing clumps of viral particles and amorphous material. However, in the in vitro preparations,
phagocytosed IC never were as compact as those observed in
leukocytes within the anterior chamber of dogs with acute
anterior uveitis and corneal edema.

**Fig. 9.** Canine peripheral leukocytes reacted with CAV-1-antibody complexes, plus complement. Cells are clumped. Bright areas represent cytoplasmic fluorescence of phagocytized CAV-1 antigen. Fluorescent antibody stain. X250.

**DISCUSSION**

When the present studies were initiated, it had been
established that two distinct types of ocular reactions may
occur in the dog following infection with CAV-1: (I) An in­
tense uveitis with corneal edema and systemic signs occurs
within 48 to 72 hours following injection of virus directly
into the anterior chamber (10); (II) A delayed reaction may
occur 1 to 3 weeks following the natural disease or after vac­
cination with attenuated virus consisting of mild to severe
anterior uveitis, often with corneal edema—"blue eye"—
(4,5,9).

The pathogenesis of the first, unnatural, condition is
readily understood. Viral growth occurs rapidly in cells of the
corneal endothelium and trabecular meshwork with extension
of the inflammation to the posterior uvea. As a result, there

is total loss of the corneal endothelium, cataractous changes in
the lens, and severe uveitis that invariably terminates in total
loss of vision in the inoculated eye. The second, spontaneous,
type of ocular response to CAV-1 is more complex, for an­
terior uveitis without corneal edema is more common follow­
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In a recent ultrastructural study (1) of the changes in
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viral particles were observed in corneal endothelial cells dur­
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edema, augmenting previous virological studies (5). Later,
at the time of onset of severe anterior segment inflammation
with corneal edema, there was sequential infiltration of the
anterior chamber and trabecular spaces, initially by neu­
trophiles and, some hours later, by macrophages. These cells
were found to contain viral aggregates within phagolysosomes
in the cytoplasm. The aggregates were regarded as viral-anti­
body complexes.

In the present series of experiments, observations were
extended to account for the very rapid onset of the corneal
lesions and the keratic precipitate formation. The role of IC
and complement in experimental tissue injury has been re­
viewed by several authors (7,8,19). As regards the eye, a
aturally occurring immunologically mediated disease is less
clearly documented; however, convincing evidence has been
compiled (2,3) for a pathological role for viral-antibody com­
plexes in diseases of other organs, especially the kidney and
vascular system. A notable difference between Type III re­
tions as described for experimental immunologic uveitis
(15) and ocular lesions of CAV-1 is the localization of virus
principally within the avascular corneal endothelium and
trabecular meshwork cells, rather than within arterioles of
the uveal tract. It was shown previously (1,5) that the
presence of occasional viral-infected cells in the corneal endo­
thelium does not generally lead to sufficient functional change
to cause osmotic flow of aqueous fluid into the corneal stroma
and result in edema. Rather, other mechanisms also must
operate. The data presented here have demonstrated the
phagocytosed IC never were as compact as those observed in leukocytes within the anterior chamber of dogs with acute anterior uveitis and corneal edema.

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In the present series of experiments, observations were extended to account for the very rapid onset of the corneal lesions and the keratic precipitate formation. The role of IC and complement in experimental tissue injury has been reviewed by several authors (7,8,19). As regards the eye, naturally occurring immunologically mediated disease is less clearly documented; however, convincing evidence has been compiled (2,3) for a pathological role for viral-antibody complexes in diseases of other organs, especially the kidney and vascular system. A notable difference between Type III reactions as described for experimental immunologic uveitis (15) and ocular lesions of CAV-1 is the localization of virus principally within the avascular corneal endothelium and trabecular meshwork cells, rather than within arterioles of the uveal tract. It was shown previously (1,5) that the presence of occasional viral-infected cells in the corneal endothelium does not generally lead to sufficient functional change to cause osmotic flow of aqueous fluid into the corneal stroma and result in edema. Rather, other mechanisms also must operate. The data presented here have demonstrated the
in vitro release of a neutrophilic chemotactic factor by virus-antibody complexes plus C'. Also, the finding of cellbound C' by immunofluorescence in lesions, along with viral-antibody aggregates, provided further evidence for the Type III hypersensitivity reaction. The actual cause of functional corneal endothelial damage was not established by these studies; however, the finding, in some instances, of elevations in aqueous fluid acid phosphatase levels, sometimes above serum enzyme levels, suggests release, and a possible role, of lysosomal enzymes in tissue damage. Neutrophilic lysosomal extracts have been reported to cause reversible changes in cultures of corneal epithelial cells (14), however in recent experiments done in our laboratory, we were unable to observe severe damage to cultured corneal endothelial cells using similar methods. The failure to cause corneal endothelial damage may have been due to the rapid washing out of the small molecular weight acid proteases, since the trabeculae were not occluded by infiltrated cells, as occurs in spontaneous CAV-1 eye lesions. Also, adherence of large numbers of leukocytes and fibrin to the corneal endothelium as keratic precipitates could focus a sustained release of injurious constituents such as acid proteases, collagenase, and permeability factors (7,11,13). Such a mechanism is likely, since lesions are relatively focal and corneal edema generally resolves within a few days, sometimes weeks. The clumping of neutrophiles and monocytes within the plasmoid aqueous fluid may represent an immune adherence phenomenon, since in vitro observations indicated clumping of cells only in the presence of C'. Removal of the stimulus (IC-leukocytes) then allows repair of tissue damage if corneal endothelial destruction were not extensive.

In vitro release of acid phosphatase from leukocyte suspensions by IC-C' amounted to approximately 7.7%, as compared with the total enzyme activity released by a detergent (Triton X-100). The quantitative significance could not be assessed; however, similar release of acid phosphatase from human neutrophiles exposed to immune complexes has been reported (20,21) as indicative of liberation of lysosomal hydrolases during phagocytosis.

Based on these data, a postulated scheme for the pathogenesis of “blue eye” is presented (Fig. 10).

CAV-1 ANTERIOR UVEITIS

VIREMIA:

Viral growth in RE tissue, kidney, liver

+ VIRAL GROWTH IN ANTERIOR UVEA/CORNEAL ENDOTHELIUM (CE)

No growth--no uveitis
Growth--CE, vascular endothelium, trabeculae

LOCAL IMMUNE RESPONSE

Lymphocytes and plasma cells in anterior uvea
Restricted viral growth

VIRAL GROWTH ARRESTED OR VIRAL PERSISTENCE IN RESTRICTED SITES

No persistence--no clinical signs
Persistence--CE cells, trabecular meshwork cells
Viral antigen release into aqueous fluid

IMMUNE COMPLEX FORMATION (CAV-1/antibody)

Virus/antibody excess: slight or no pathogenicity
Virus/antibody balance: complement (C') binding; pathogenic

C'-MEDIATED RELEASE OF BIOLOGICALLY ACTIVE COMPONENTS

Leukocyte chemotactic factors
Immune adherence factor
Enhanced phagocytosis of immune complexes

LEUKOCYTE INFILTRATION INTO ANTERIOR CHAMBER

PHAGOCYTOSIS OF IMMUNE COMPLEXES-C' BY NEUTROPHILES AND MACROPHAGES

LYSOSOMAL ENZYME RELEASE

Injury (focal) to CE at points of contact with leukocytes?

INFILTRATION OF LEUKOCYTES BETWEEN CE AND DESCemet's MEMBRANE

Aqueous fluid permeates into corneal stroma
Corneal edema

DIGESTION OF IMMUNE COMPLEXES WITHIN PHAGOCYTIC VACUOLES & REMOVAL

If lesions in CE focal: resolution in 1-3 weeks
If lesions extensive: no CE regeneration, persistent or permanent corneal damage. Bullous keratopathy, hydrophthalmos, or extensive corneal pigmentation with complete loss of vision.

Fig. 10. Postulated scheme of pathogenesis of acute anterior uveitis following CAV-1 infection. The pathogenic role of lysosomal enzyme release from leukocytes is not fully established.
The in vitro release of a neutrophil chemotactic factor by virus-antibody complexes plus C'. Also, the finding of cellbound C' by immunofluorescence in lesions, along with viral-antibody aggregates, provided further evidence for the Type III hypersensitivity reaction. The actual cause of functional corneal endothelial damage was not established by these studies; however, the finding, in some instances, of elevations in aqueous fluid acid phosphatase levels, sometimes above serum enzyme levels, suggests release, and a possible role, of lysosomal enzymes in tissue damage. Neutrophilic lysosomal extracts have been reported to cause reversible changes in cultures of corneal epithelial cells (14), however in recent experiments done in our laboratory, we were unable to observe severe damage to cultured corneal endothelial cells using similar methods. The failure to cause corneal endothelial damage may have been due to the rapid washing out of the small molecular weight acid proteases, since the trabeculae were not occluded by infiltrated cells, as occurs in spontaneous CAV-1 eye lesions. Also, adherence of large numbers of leukocytes and fibrin to the corneal endothelium as keratic precipitates could focus a sustained release of injurious constituents such as acid proteases, collagenase, and permeability factors (7,11,13). Such a mechanism is likely, since lesions are relatively focal and corneal edema generally resolves within a few days, sometimes weeks. The clumping of neutrophiles and monocytes within the plasmoid aqueous fluid may represent an immune adherence phenomenon, since in vitro observations indicated clumping of cells only in the presence of C'. Removal of the stimulus (IC-leukocytes) then allows repair of tissue damage if corneal endothelial destruction were not extensive.

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- Corneal edema

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- If lesions extensive: no CE regeneration, persistent or permanent corneal damage. Bullous keratopathy, hydrophthalmos, or extensive corneal pigmentation with complete loss of vision.

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