Keratoconjunctivitis associated with sialodacryoadenitis in rats. YIN-LOK LAI, ROBERT O. JACOBY, PRAVIN N. BHATT, AND ALBERT M. JONAS.

A high incidence of keratoconjunctivitis was observed in a closed colony of inbred Lewis/Wistar rats. Clinical signs including blinking, ocular discharge, circumcorneal flush, corneal opacity, ulceration, pannus, hypopyon, and hyphema were observed at about three weeks of age. Acute disease subsided by six weeks of age, but some lesions progressed to low-grade chronic keratitis. Six per cent of affected rats developed megaloglobus, which usually appeared by three weeks of age. Lesions included focal or diffuse interstitial keratitis, corneal ulceration, anterior synechia, and inflammatory exudate in the anterior chamber. A high incidence of keratoconjunctivitis was observed in a closed colony of Wistar/Lewis rats. Histopathological serological and virological evidence indicates there is an association between eye lesions and sialodacryoadenitis (SDA), a corona-viral disease of rats.1-8

Materials and methods.
Rats. Twenty to forty per cent of Wistar/Lewis weanlings in an isolated colony room had a “blinking” syndrome. Signs usually lasted less than two weeks and most rats recovered. Five to 10 per cent of affected weanlings developed lesions of keratoconjunctivitis which resolved in two to three weeks. About six per cent of weanlings with keratoconjunctivitis developed megaloglobus.

Two groups of rats were obtained from the breeding colony. Group 1 consisted of weanlings that were “blinking” or had other signs including: circumcorneal flush, corneal opacities, ulcers, hyphema, hypopyon, and synechia. Group 2 consisted of seven- to ten-week-old rats with megaloglobus. Rats were housed in plastic boxes with filter lids and were fed standard rations and water ad libitum.

Clinical examination. Group 1 was randomly divided into several subgroups for clinical evaluation and euthanasia. Eyes were examined by direct ophthalmoscopy once each week and drawings were made of each eye to record the characteristics and progression of lesions. Group 2 was examined in a similar way on arrival.

Tissue collection and processing. Group 1 randomly selected rats were euthanatized with ether at ages 3, 5, 10, and 18 weeks. Group 2 rats were euthanatized within one week after arrival. Eyes were fixed in 2 per cent phosphate-buffered gluteraldehyde, pH 7.4, embedded in paraffin, sectioned at 5 µm through the optic nerve and stained with hematoxylin and eosin. Other tissues were fixed in 10 per cent neutral buffered formalin and processed as described above.

Virological and serological tests. Eyes and nasopharyngeal washings from selected rats were assayed for SDA virus as previously described. Serums from selected rats were tested for antibodies to a panel of murine viruses by a commercial laboratory (Microbiological Associates, Bethesda, Md.).

Results.
Clinical signs. Lesions were usually bilateral in Group 1 rats, although the severity of lesions often varied between eyes of individual rats. Ocular discharge, circumcorneal flush, diffuse corneal opacity, corneal ulcers, pannus, hypopyon, and hyphema appeared by three weeks. Lesions were self-limiting and gradually subsided between three and five weeks. Megaloglobus occurred in six per cent of eyes by three weeks, but only one new case developed thereafter.

Megaloglobus in both Group 1 and Group 2 rats was accompanied by severe chronic keratitis. Histopathology. The most prominent ocular lesion was keratoconjunctivitis which was usually associated with dacryoadenitis of the homolateral harderian gland. Acute keratitis was characterized by necrosis of corneal epithelium with infiltration of epithelium and subjacent stroma by neutrophils (Fig. 1). This lesion was usually most severe in the center of the cornea. Ulcerative keratitis usually resulted in extensive destruction of the cornea, hypopyon, hyphema, and anterior synechia. Milder lesions gradually subsided, but chronic active keratitis persisted and stromal fibrosis and pannus developed. Anterior and/or posterior synechia was common in the iris and ciliary body, but primary iritis or iridocyclitis was not observed.

Organization of inflammatory exudates was accompanied by deposition of fibrinogranular, eosinophilic material in the interstices of Zinn zonules.
Ulcercative keratitis, hypopyon, and hyphema were present in a high proportion of three-week-old rats, but the incidence dropped sharply by five weeks. The incidence of other lesions also gradually decreased (Table I, A).

Lesions associated with megaloglobus were identical in Group 1 and Group 2 rats. They were characterized by synechia, hemorrhage, and lenticular and retinal changes (Table I, B).

Lenticular changes were prominent and included necrosis and focal hyperplasia of the lens epithelium (Fig. 2, A).

Retinal lesions were either focal or diffuse and commonly included a substantial loss of ganglion cells which was often accompanied by generalized thinning of retinal layers and a severe reduction in the number of photoreceptors. Occasionally, there was also thinning of choroid and sclera underlying affected areas of retina (Fig. 2, B).

Association of eye lesions with SDA. More than 90 per cent of rats had lesions compatible with acute or convalescent stages of SDA. Virological and serological tests indicate that SDA virus infection exists in the colony and that more than 80 per cent of rats have antibodies to SDA by eight weeks of age. SDA virus was recovered from the nasopharynx of weanling rats in the nucleus colony, but attempts to isolate virus from affected eyes were unsuccessful.

In a separate experiment ten 3-week-old rats and ten 8-week-old rats from the same colony room, all with eye lesions were euthanatized. Nasal washes were cultured for virus, serum was assayed for antibody to common murine viral pathogens, and tissues were examined histologically. Results are summarized in Table II. SDA virus was isolated from four of ten 3-week-old rats and sera from all rats had anti-SDA antibody.

Nine rats in the eight-week-old group had keratitis and/or keratoconjunctivitis. All 10 rats had either focal nonsuppurative Harderian dacrooadenitis and/or focal squamous metaplasia of the Harderian gland.
A. Eye lesions in Group 1 rats:  

However, none of the rats had lesions indicating pharynx of several rats and most weanling rats. The affected colony is characterized lesions of SDA. In addition, rats with eye lesions have not been determined by serological testing and by recovery of SDA virus from the nasopharynx of weanlings with eye lesions. Therefore, a close temporal association was found between active SDA infection and keratoconjunctivitis and serological studies were not done.

Table I. Histopathological classification

<table>
<thead>
<tr>
<th>Age in weeks</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of eyes</td>
<td>46</td>
<td>44</td>
<td>112</td>
<td>70</td>
</tr>
</tbody>
</table>

**Cornea:**
- Acute keratitis 87% 27% 4%
- Ulcers 54% 9%
- Keratoprecipitation 21%
- Hypopyon or/and hyphema 34% 7%
- Megaloglobus 7% 5% 6%

B. Eye lesions in Group 2 rats (7 to 10 weeks old):

<table>
<thead>
<tr>
<th>No. of eyes</th>
<th>20</th>
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<tbody>
<tr>
<td>Chronic ulcerative keratitis</td>
<td>20%</td>
</tr>
<tr>
<td>Synchia</td>
<td>100%</td>
</tr>
<tr>
<td>Intraocular hemorrhage</td>
<td>65%</td>
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</table>

**Lens:**
- Epithelial alterations 70%
- Subcapsular cataract 20%
- Capsular alteration 10%
- Retinal degeneration 75%

Sendai virus was recovered from the nasopharynx of several rats and most weanling rats had anti-Sendai hemagglutination inhibiting (HAI) antibody. In addition, most rats had serum antibody to pneumonia virus of mice (PVM), Kilham rat virus (KRV), and Toolan H-1 virus. However, none of the rats had lesions indicating active infection with any of these agents.

**Discussion.** Our observations indicate there is good correlation in weanlings between the occurrence of acute keratoconjunctivitis and characteristic lesions of SDA. In addition, rats with chronic interstitial keratitis and synchia had residual lesions of SDA. The affected colony is infected with SDA virus as determined by serological testing and by recovery of SDA virus from the nasopharynx of weanlings with eye lesions. Therefore, a close temporal association was found between active SDA infection and keratoconjunctivitis.

**Enzootic keratoconjunctivitis:** Associated with sialoadenitis in Sprague-Dawley rats has been reported by Heywood. Fifty per cent of animals were affected, but histological, virological, and serological studies were not done.

Evidence of exposure to pneumonia virus of mice (PVM), Kilham rat virus (KRV), H-1, rat coronavirus (RCV), and Sendai viruses was found in weanling and adult rats, but there was no morphological evidence of infection with these agents. Moreover, eye lesions have not been described in natural outbreaks due to these viruses.

**SDA virus-induced eye lesions** are conceptually attractive since virus replicates readily in nasopharynx and hardier gland. Thus corneal contamination with infected tears or nasal secretions is likely. Nevertheless, SDA virus was not recovered from affected eyes. Since eye lesions are associated with hardier dacyroutenis, impedance to lacrymal flow and proptosis could result in keratitis sicca. Ostensibly, normal conjunctival bacterial flora could proliferate rapidly under these conditions and increase the severity of lesions.

**Preliminary findings** using immunofluorescence labeling indicate that viral antigen is widespread to lachrymal flow and proptosis could result in keratitis sicca. Ostensibly, normal conjunctival bacterial flora could proliferate rapidly under these conditions and increase the severity of lesions.

**All eyes with megaloglobus had synchia and 65 per cent also had hyphema. These lesions undoubtedly blocked or impeded outflow of aqueous humor and caused the secondary glaucoma.** Furthermore, chronic ulcerative keratitis was detected primarily in megaloglobic eyes whereas corneal lesions subsided by five weeks in eyes that did not develop megaloglobus. Thus megaloglobus probably delayed or prevented healing of corneal lesions. Similarly, lenticular and retinal degeneration were probably secondary to elevated intraocular pressure in megaloglobic eyes, since these changes were not observed in eyes with only inflammatory lesions.

**Although the ocular lesions described are largely self-limiting, rats with this disease are unsuitable for ocular research. Studies on the pathogenesis of the disease are continuing.**

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Table II. Incidence of lesions, virus isolation, and serum antibody in rats with keratoconjunctivitis

<table>
<thead>
<tr>
<th>Lesions:</th>
<th>3-week-old</th>
<th>8-week-old</th>
</tr>
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<tbody>
<tr>
<td>Keratoconjunctivitis</td>
<td>9/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Dacryoadenitis</td>
<td>9/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Sialoadenitis</td>
<td>9/10</td>
<td>1/10</td>
</tr>
</tbody>
</table>

**Virus isolation:**
- SDA | 4/10 | 0/10 |
- Sendai | 1/10 | 0/10 |

**Serum antibody:**
- NT | 8/10 | 6/10 |
- CF | 8/10 | 1/10 |
- RCV | 4/10 | 1/10 |
- HAI | 10/10 | 8/10 |
- PVM | 0/10 | 0/10 |
- Reo 3 | 5/10 | 7/10 |
- H-1 | 8/10 | 7/10 |

HAI = Hemagglutination inhibiting; CF = Complement fixing; NT = Neutralizing.
Conclusions. This work was supported in part by Public Health Service Grants USPHS 5 PO6 RR 00393 and USPHS 1 RO1 RR 00700, and 1 RO1 EY01769-01. Submitted for publication Dec. 23, 1975. Reprint requests: Dr. Yin-Lok Lai, Section of Comparative Medicine, Yale University School of Medicine, 375 Congress Ave., New Haven, Conn. 06510.

Key words: keratoconjunctivitis, sialodacryoadenitis, virus, rat, enzootic megaloglobulus, cataract, retinal degeneration.

REFERENCES

Table I. Rejection rates of penetrating corneal transplants in rabbits followed for 10 weeks

<table>
<thead>
<tr>
<th>No Splenectomy</th>
<th>Splenectomy</th>
</tr>
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<tbody>
<tr>
<td>Number rejected</td>
<td>6/14 (43%)</td>
</tr>
<tr>
<td>Mean survival time (days)</td>
<td>30.5</td>
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</table>


Recent studies have suggested that the spleen may be essential for the "immunologic privilege" enjoyed by corneal grafts. In one of these studies, enhancement of subsequent skin allograft survival was elicited in rats by preinjecting donor lymphoid cells into the anterior chamber of the eye or the vein of the animal. This prolongation of skin graft survival did not occur when splenectomized recipient rats were used, so that the spleen is somehow necessary for this enhancement-like response. The low incidence of corneal graft rejection may be due in part to the intravascular presentation of antigen via the alymphatic anterior chamber and the subsequent spleen-dependent response. Antigen from grafts in many other organs presents first in the draining lymph nodes and therefore may be handled differently. Since sensitizing antigen from penetrating corneal transplants probably first enters the recipient via the anterior chamber, it may lead to enhancement just as did the lymphoid cells and, therefore, may play a role in prolonging corneal graft survival. Prior splenectomy might then be expected to lead to a greater percentage of rejected corneal grafts and/or earlier graft rejection, just as it eliminated the prolonged skin graft survival described above. This possibility seemed to deserve further investigation. Furthermore, it would provide a convenient means of attaining higher rejection rates of clear penetrating corneal grafts in experimental animals. We examined this experimental model in rabbits.

Materials and methods. Central 7 mm. penetrating keratoplasties were exchanged between the right eyes of 19 normal adult outbred albino rabbits and 19 similar rabbits that had undergone splenectomy 2 weeks previously. The entire spleen plus any accessory splenic tissue was removed. The animals were anesthetized with sodium pentobarbital (30 mg. per kilogram) for both the splenectomy and keratoplasty procedures. The operating microscope was used for the keratoplasty. Heparin solution (10,000 U. per milliliter) was applied topically after the anterior chamber had been entered. Interrupted 10-0 nylon sutures were used and removed on the tenth postoperative day. One per cent atropine ointment was applied to each eye daily for 1 week following keratoplasty. Technical failures, grafts not clear on the seventh postoperative day, and rabbits that died during the 10 week period of study were all eliminated from the project. No steroids were administered. The rabbits were examined daily for the first month and then every few days until