Experimental allergic uveitis

III. Manifestations produced in the guinea pig by immunization with homologous retina

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Following one injection of guinea pigs with 10 mg. homologous retina in adjuvant emulsion, the large majority developed uveitis within 3 weeks. The clinical signs and histological manifestations observed at onset and for several weeks thereafter, during which the disease progressed from an iridocyclitis to cyclitis and choroiditis, are described. The development of cytologic events in the uveal tract resembled those of delayed hypersensitivity, in which lymphocytes predominated in the early infiltrate and plasma cells in the subsequent infiltrate. Although morphologic alteration of the retina was observed in some animals, it did not occur until after there was extensive cellular infiltration of the choroid. The possible relationship of this model to that produced previously with uveal tissue is discussed.

Key words: allergic uveitis, retina, immune reaction, delayed hypersensitivity, guinea pigs, histopathology, cyclitis, choroiditis, iritis, retinal atrophy, lymphocytes, plasma cells.

A relationship between uveitis and autoimmunity has been discussed for many years, during which time many attempts were made to produce an experimental model of autoimmune uveitis by immunization with uveal tissues. These attempts resulted in uveitis when a lengthy schedule of repeated immunizations was tried. The role of retinal antigens in production of a similar phenomenon was first suggested when it was found that repeated immunization of guinea pigs with homologous retina also led to allergic uveitis. The latter finding presented the seemingly anomalous situation of elicitation of uveal pathology by antigens derived from non-uveal tissue, and it contrasted with the generally accepted relationship between uveal antigenicity and autoimmune uveitis.

Results of subsequent investigations supporting the role of retinal specific antigens in production of uveitis were recently reported. Comparative studies of the antigenicity of various homologous ocular tissues demonstrated that one injection of either guinea pigs or rabbits with homol-
ogous retina led to a high incidence of allergic uveitis. These findings were contrasted with ineffectiveness of one injection of other homologous ocular tissues, including the uvea, in production of this phenomenon. Heterologous ocular tissues were also ineffective, with the exception of heterologous retina in some instances. In addition, unlike the simultaneous production of uveitis and encephalomyelitis in some species of animals immunized with central nervous tissues, production of uveitis by injection of homologous retina was not associated with either clinical signs or histological manifestations of allergic encephalomyelitis.

Retinal specificity was also demonstrated by analysis of immune responses. Guinea pigs, immunized with homologous retina, produced both humoral antibody and delayed hypersensitivity to antigens shown to be retinal specific. Furthermore, immune responses to 2 such antigens were observed, which could be crudely separated by ultracentrifugation of retinal homogenate. One of the antigens was present in the sedimented particulate fraction which, when used as an antigenic stimulus, elicited humoral antibody formation but not uveitis. The other antigen present in the clear supernatant saline extract of retina led to production of humoral antibody, delayed hypersensitivity, and uveitis.

In the present communication the nature of the clinical and histopathological ocular manifestations produced in the guinea pig by sensitization to homologous retina is presented. The data show primary inflammation of the uveal tract and subsequent involvement of the retina in some animals with relatively severe uveal pathology.

Materials and methods

Experimental animals were female Hartley albino guinea pigs weighing 400 to 500 grams each, which were given a single injection in the hindfoot pad of 0.1 ml tissue adjuvant emulsion, containing either 10 mg whole homologous retina or the equivalent in retinal extract. The adjuvant preparation used was Freund's complete adjuvant obtained from Difco Laboratories, Detroit, Michigan, and marketed as Bacto-Adjuvant, Complete H37 Ra. When 20 per cent (w/v) retinal suspension was emulsified in an equal volume of this adjuvant, the emulsion contained 100 mg of retina and 0.5 mg of killed and dried tubercle bacilli per milliliter. This ratio of retinal antigen to bacterial component was found to be optimal for production of uveitis.

The dissection and preparation of tissue for immunization, the preconditioning and handling of animals, and the methods of clinical and histological examination were previously described.

Of 208 guinea pigs injected in the above manner with homologous retina, 10 were killed for histological examination before onset, 33 during early development of clinical signs, 125 at 21 days after immunization, and 40 at approximately 3 weeks after onset. Control animals were killed at 21 days after immunization and did not show either clinical or histological manifestations of uveitis after a similar single injection of the following emulsions: 16 animals with guinea pig uvea; 6 each with guinea pig lens, ciliary zonula, vitreous, or optic nerve; and 58 with saline adjuvant emulsion.

Results

Clinical findings. Fig. 1 shows the time of onset and incidence of uveitis in guinea pigs after one injection of homologous retinal emulsion. The eyes appeared nor-

![Fig. 1. Time of onset and cumulative incidence of uveitis in guinea pigs immunized with homologous retina.](https://i.imgur.com/8y5Z5.png)
nal during the first 9 days of observation with the ophthalmoscope. Examination with the slit-lamp microscope during this period showed many animals that developed a suggestion of iris vessel dilation and minimal aqueous flare by Day 7. These minimal signs were considered prodromal and were in contrast to the marked vascular and exudative changes described below which appeared abruptly in the eyes and characterized the day of onset. More than half of the animals developed clinical signs of uveitis by Day 14 and about 90 per cent (17/2108) by 3 weeks' postimmunization.

Signs of uveitis at onset were vascular dilation and edema in the perilimbal area, marked dilation of iris vessels, increased flare in the anterior chamber, and cellular exudation in the anterior vitreous and aqueous fluids. In a number of instances, the vascular dilatory changes were noted one day prior to the exudation changes. The perilimbal congestion subsided after several days in the majority of animals, but in some with severe changes the reaction progressed to edema and clouding of the cornea.

The intraocular changes usually increased in intensity for a few days after onset and then appeared to stabilize. The degree of ocular involvement varied from mild to severe, as shown in Table I. Whether albino, pigmented, or mixed preparations of homologous retina were used for immunization, the findings were similar. Some animals showed a liberal sprinkling of cells in the anterior vitreous, which occurred either with or without significant aqueous cellular exudation or prominent vascular dilation in the iris and perilimbal area. This type of reaction did not progress significantly after onset and was graded as mild. In other animals many cells appeared in the vitreous and usually the aqueous, accompanied by dilation of iris and limbal vessels. This was graded as a moderate reaction. Animals with moderately severe uveitis showed marked vitreous and aqueous cellular exudation, small vitreous opacities, usually keratic precipitates, and ocular congestion. Some animals developed, in addition to these changes, large white opacities and fibrous changes in the vitreous. These severely affected eyes were usually difficult to examine, due to the extent of intraocular cellular exudation and clouding of the cornea.

The progress of signs was followed in 40 animals with uveitis by twice weekly slit lamp and ophthalmoscopic examinations (Fig. 2). Although each animal demonstrated cellular exudation in the anterior vitreous, some with mild uveitis failed to show exudation in the aqueous fluid. Furthermore, the reaction in the anterior chamber, having reached maximal intensity within a few days after onset, began to subside. By one week after onset, aqueous cells were no longer detected in a few of the animals, and by 3 to 4 weeks

<table>
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<tr>
<th>Preparation injected*</th>
<th>No. of animals</th>
<th>Negative</th>
<th>Mild</th>
<th>Moderate</th>
<th>Moderately severe</th>
<th>Severe</th>
<th>Total occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albino retina (Hartley)</td>
<td>123</td>
<td>14</td>
<td>31</td>
<td>38</td>
<td>52</td>
<td>28</td>
<td>109/123</td>
</tr>
<tr>
<td>Pigmented retina (English short hair)</td>
<td>47</td>
<td>7</td>
<td>12</td>
<td>9</td>
<td>15</td>
<td>4</td>
<td>40/47</td>
</tr>
<tr>
<td>Mixed albino and pigmented retina</td>
<td>28</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>23/28</td>
</tr>
</tbody>
</table>

*Injection was 0.1 ml. emulsion containing 10 mg. of retina. Animals were Hartley albino guinea pigs.

†Degree of manifestations are described in text.
later the anterior chamber was clear of cells in the majority. In contrast, cells persisted in the vitreous throughout the period of examination. The persistence of cells in the vitreous does not in itself indicate continued inflammatory activity. Nevertheless, in agreement with these clinical findings, histological evidence cited further in this article shows continued marked inflammation of the choroid, but not of the iris, in animals examined at this time.

**Histological findings.** Cellular infiltration of the uveal tract was found in all 172 animals that had clinical signs. In addition, minimal cellular infiltration of the ciliary body was found in 4 of the remaining 26 immunized animals, whose eyes had appeared clinically normal. No inflammatory changes were noted in the eyes of 10 additional immunized animals, which were killed for examination at the first indication of prodromal signs noted above.

The infiltrate was present in the ciliary body of each animal with uveitis and infrequently was the only histological manifestation. More often, cyclitis occurred in combination either with iritis or choroiditis, and in many animals, all 3 uveal segments were affected. Representative findings in Table II from animals examined in relation to the time of onset indicated that iritis was typically an early manifestation, which subsequently diminished, whereas choroiditis was commonly a later manifestation.

Eyes examined on the day of onset of uveitis showed diffuse infiltration of the ciliary body and iris, with exudation of cells into the anterior vitreous and aqueous fluids (Fig. 3, A). Cellular infiltration extended into the pars plana, Fig. 3, B), but seldom into the choroid at this time. Infiltration of the limbus and peripheral cornea was usually observed (Fig. 3, C). Cells in the vitreous and limbus were of mixed types, a significant number of which were neutrophilic granulocytes. Those in the uvea were predominantly mononuclear and consisted of lymphocytes with some plasma cells and a sprinkling of granulocytes (Fig. 4, A). The uveal infiltrate retained its mononuclear character in eyes examined 1 and 2 days after onset. Plasmacytosis was evident at 3 days, and at

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**Table II. Distribution and severity of lesions in the uveal tract after onset**

<table>
<thead>
<tr>
<th>Time of examination</th>
<th>Guinea pig no.</th>
<th>Clinical severity*</th>
<th>Histopathology†</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Iris</td>
<td>Ciliary body</td>
</tr>
<tr>
<td>Onset</td>
<td>775</td>
<td>±</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>793</td>
<td>1+</td>
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</tr>
<tr>
<td></td>
<td>795</td>
<td>±</td>
<td>1+</td>
</tr>
<tr>
<td>Onset + 3 days</td>
<td>781</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>801</td>
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<td>798</td>
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<tr>
<td>Onset + 7 days</td>
<td>740</td>
<td>2+</td>
<td>3+</td>
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<tr>
<td></td>
<td>742</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>344</td>
<td>1+</td>
<td>2+</td>
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<tr>
<td></td>
<td>391</td>
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<td>3+</td>
</tr>
<tr>
<td></td>
<td>393</td>
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<tr>
<td>Onset + 3 weeks</td>
<td>744</td>
<td>3+</td>
<td>0</td>
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<tr>
<td></td>
<td>745</td>
<td>3+</td>
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<td></td>
<td>749</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>749</td>
<td>2+</td>
<td>1+</td>
</tr>
</tbody>
</table>

*Degree of uveitis as shown in Table I.
10, ±, 1+, 2+, 3+, and 4+ indicated degrees of cellular infiltration corresponding to none, slight, mild, moderate, moderately severe, and severe.

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Fig. 3. Ocular inflammatory changes at onset of uveitis: (A) iridocyclitis with cellular exudation into anterior vitreous and aqueous fluids (x72); (B) infiltration of pars plana and exudation into anterior vitreous (x200); and (C) infiltration of limbus and cornea (x120).
subsequent intervals of examination plasma cells were the predominant cell type in the anterior uvea (Fig. 4, B). Extensive infiltration of such cells was commonly found in the corona of the ciliary body (Fig. 5, A) and adjacent to blood vessels in the iris (Fig. 5, B).

Extension of inflammation to the choroid was observed in the majority of animals killed 6 or more days after onset. In mild choroidal inflammation, cellular infiltration appeared especially in the proximity of major choroidal vessels located in the anterior section near the pars plana (Fig. 6, A). Small perivascular inflammatory foci were also observed in the peripheral and posterior choroid in some animals (Fig. 6, B). More frequently, however, cellular infiltration was diffuse, affected large areas, and increased thickness of the choroid several times the normal size (Fig. 6, C). The infiltrate consisted mainly of plasma cells and lymphocytes, with a few macrophages and granulocytes. In severely affected eyes, the choroid in cross section was inflamed the entire length from the pars plana to the optic papilla. In such eyes there was marked cellular exudation...
Fig. 5. Plasma cell infiltration of anterior uvea at onset + 5 days: (A) corona of ciliary body (×240); (B) iris (×240).

and usually fibrinous changes in the vitreous (Fig. 7).

The retina was morphologically unaffected in animals that had iridocyclitis only, or those with mild to moderate infiltration of the choroid. Although the retina was often artfactitiously detached, it also appeared to be normal in many animals with extensive choroidal pathology (Fig. 6, C). In other animals, the layer of rods and cones and portions of the outer nuclear layer of the retina adjacent to areas of marked choroidal infiltration were frequently missing (Fig. 8, A). Despite loss or atrophy of outer retinal layers, cellular infiltration of the retina was slight and consisted of a few plasma cells in the ganglion layer. In more advanced lesions, however, prominent cellular infiltration and disorganization of the retina were observed (Fig. 8, B). This reaction progressed almost to complete destruction of the retina in some animals (Fig. 8, C). Unlike inflammatory cells present in the uveal tract, which were predominantly mononuclear, cells which invaded the retina were mainly neutrophilic granulocytes. Phagocytosis of retinal debris was observed in association with these changes.
Fig. 6. Inflammatory changes in the choroid: (A) mild infiltration of the anterior choroid extending from the pars plana (×72); (B) mild perivascular cellular infiltration in the peripheral choroid (×240); (C) moderately severe infiltration and thickening of the peripheral choroid (×160).
Fig. 7. Cellular exudation and fibrinous changes in the vitreous associated with severe cyclitis and choroiditis (×72).

Discussion

Significant features of the present model of experimental uveitis are the small amount of retinal antigen used, the efficacy of a single immunization, the short induction period, and the development of signs in the large majority of animals within a 10 day period. The data show that the reaction began in the ciliary body and iris with cellular exudation into the intraocular fluids and subsequently was extended to the choroid. Although the manifestations persisted in the choroid and ciliary body throughout the period of observation, those in the iris diminished and/or disappeared. Whether some or all of the uveal segments were found to be affected depended not only on severity of reaction, but also on the time after onset when histological examinations were made.

Although the antigens used to elicit uveitis were shown to be retinal specific,6,11 the data show that involvement of the retina occurred secondary to extensive choroidal infiltration. Among possible reasons for this phenomenon, the proximity of uveal circulation to the retina and retinal antigens and the metabolic dependence of especially outer layers of the retina on uveal circulation may be significant factors. The initial signs of retinal atrophy were not unlike those induced by administration of antimalarial drugs12 or by occlusion of choroidal vessels by latex microspheres,13 both of which are associated at least in part with choroidal disturbances. Subsequent changes resembled a necrotic process, in which the retina was extensively infiltrated by neutrophilic granulocytes. Whether these changes resulted from marked physiologic disturbances in the retina due to impaired uveal circulation or to an antigen-antibody reaction in the retina is unknown.

The cytologic events during the development of uveitis may have possible implications for the mechanism of uveal immunopathology. Differences in cytologic events associated with immediate and delayed types of hypersensitivity are recognized, and these events in the skin14 and uveal tract15 of the guinea pig have been described. The initial cellular infiltration, which was observed in the ciliary body and iris, consisted predominantly of lymphocytes. The exudation of cells from the uvea into the anterior vitreous had already occurred and contained a significant number of neutrophils in addition to lymphocytes. This finding suggested an early
transient granulocytic infiltration which was supplanted, perhaps within hours, by round cell infiltration. Lymphocytic infiltration of the uvea persisted for 2 or 3 days after onset and was followed by marked plasmacytosis. A similar transition from granulocytic to lymphocytic infiltration occurred within 24 hours in the delayed hypersensitive response of sensitized guinea pigs tested intradermally with retinal extract, and it has also been described for the tuberculin reaction elicited in the uvea and skin of this species. Although the intraocular cellular events are consistent with those of delayed hypersensitivity, direct evidence for mediation of the observed immunopathology by passive transfer of either cellular or humoral factors is still lacking.

There is increasing evidence that the model produced here with retinal tissue and that produced previously with uveal tissue may be a single experimental entity. Serologic cross-reactions were noted between uveal and retinal tissues in preliminary investigations which were subsequently attributed to the difficulty in complete removal of all of the retina from the choroid. Indeed, except under the most rigorous conditions of dissection, the
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Fig. 8C. Severe choroiditis with marked disorganization and destruction of the retina (×120).

soluble retinal antigen which elicited uveitis was recovered in slight and inconsistent amounts in both the vitreous and uveal harvests. In our investigations, the amount of such antigen recovered in the harvest of uvea or vitreous was inadequate to elicit uveitis or antibody production in either guinea pigs or rabbits by a single immunization. Repeated immunization of both species with concentrated extracts of uvea and retinovitreous was recently reported by Aronson to elicit uveitis in both species and to stimulate production of antibody which demonstrated a serologic reaction of antigenic identity between the two kinds of extracts.

Nevertheless, there are differences between the results presented here and those ascribed previously to uveal immunization. The ocular lesions produced here with retinal tissue were of much greater severity than those produced with uveal tissue in the guinea pig either in Collins' original studies, or in our confirmatory report. The antibody responses observed were described as uveal specific and in unpublished investigations did not cross-react with the soluble retinal antigen. Furthermore, in contrast to present results, manifestations of uvea-induced uveitis were observed only by histology and consisted of choroiditis and mild cyclitis without iris involvement. No clinical manifestations were observed, either because vitreous exudation was not produced by the mild uveal reaction or it was transient. Although the present results resemble the severity and distribution of ocular lesions produced with uveal tissue by Aronson and co-workers, the significant difference in the incidence of uveitis which they found on using albino and pigmented uvea was not found in the present study using retina from albino and pigmented guinea pigs (Table I). In our opinion, resolution of these differences by sensitive tools of immunology are required to determine whether 1 or 2 models of uveitis have been obtained.

REFERENCES


