SEROLOGICAL STUDIES IN SYMPATHETIC OPHTHALMITIS*

BY

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ELSCHNIG (1910a, b; 1911) and Elschmig and Salus (1911) postulated that sympathetic ophthalmitis might be the result of an auto-immune reaction to uveal pigment. This suggestion is supported by the findings of Collins (1949; 1952), Vannas, Nordman, and Teir (1960), and Aronson, Hogan, and Zweigart, (1963a, b, c), who showed that uveitis of experimental animals might be produced by immunizing them with homologous uveal tissue. Woods (1925) reported that the subcutaneous injection of bovine uveal pigment produced local erythema and induration in the skin of patients with sympathetic ophthalmitis. This reaction occurred very seldom in patients with penetrating injuries of the uveal tract who did not develop sympathetic ophthalmitis. It did not occur in control individuals who had no eye injury. Biopsy specimens of the injection site in patients with a positive skin reaction showed a marked cellular response with phagocytosis of the injected pigment (Friedenwald, 1934). In patients showing no skin reaction, there was almost no cellular response, and very little phagocytosis of pigment.

Doubt has been cast on the specific role of uveal pigment by Friedenwald (quoted by Hogan and Zimmerman, 1962), who noted that the same cellular reaction could be obtained in the skin of patients with sympathetic ophthalmitis by the injection of uveal tissue from albino animals. Also Aronson and others (1963a) induced uveitis in apparently albino guinea-pigs by immunization with homologous uveal tissue with adjuvant. A greater proportion of albino animals developed uveitis with injected uveal tissue obtained from other albino guinea-pigs than with that obtained from normal pigmented guinea-pigs. This suggests that uveitis induced by immunization with homologous uveal tissue may occur in response to antigens other than the mature pigment granule.

Woods (1921) was unable to detect complement-fixing antibodies for purified bovine uveal pigment antigen in the sera of six patients with sympathetic ophthalmitis. Similar negative findings had been reported by Fuchs and Meller (1914). The present communication reports an attempt to demonstrate circulating auto-antibodies for uveal tissue in the sera of five patients with sympathetic ophthalmitis.

Material and Methods

The interaction of antigen and antibody may be demonstrated *in vivo* and *in vitro* by many techniques. In this study three techniques were used in an attempt to demonstrate circulating antibodies for uveal tissue in patients with sympathetic ophthalmitis.

* Received for publication March 10, 1964.

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(1) *Immunofluorescent Studies*.—Antibody molecules may be conjugated with a suitable fluorochrome (e.g. fluorescein isothiocyanate) without significant alteration of the chemical and physical properties of either substance. If antibody conjugated with fluorochrome is allowed to react with a tissue section, the specific uptake of antibody, and hence the cytological localization of an antigen, may be demonstrated by an appropriate microscopic technique.

(2) and (3) *Complement-Fixation and Passive Cutaneous Anaphylaxis Tests*.—Complement-fixation may be demonstrated by the conventional *in vitro* technique. A newer *in vivo* technique, which probably also depends on complement-fixation (Osler, Hawrasiak, Ovary, Siqueira, and Bier, 1957), involves the induction of passive cutaneous anaphylaxis in suitable experimental animals. In this test, small quantities of serum thought to contain antibody are injected intradermally. Several hours later, a large quantity of antigen is given intravenously, together with Evans blue dye. Antigen-antibody interaction may be accompanied by vasodilatation and increased vascular permeability at the site of serum injection. This allows the blue dye to leak out locally into the tissues, where it can be seen macroscopically.

Sera.—These were obtained from five patients with sympathetic ophthalmitis and from controls of the same sex and approximately the same age. Serum specimens were stored at $-20^{\circ}\text{C}.$, and were thawed at $37^{\circ}\text{C}.$ when required.

Iris Tissue.—This was obtained at operation from patients undergoing iridectomy. Tissue blocks (approx. 2×2 mm.) were transferred to a drop of ovalbumin on the surface of a microtome chuck. This was then immersed in liquid nitrogen ($-180^{\circ}\text{C}.$) for 4 to 5 seconds. Tissue blocks, mounted in this way, were stored in air-tight polythene containers at $-40^{\circ}\text{C}.$ Sections were cut in a freezing microtome (Pearse) at a temperature of $-20^{\circ}\text{C}.$, and transferred to thin microscope slides (0.8 to 1 mm.). Sections were rapidly thawed by placing the tip of the finger beneath the section on the under surface of the slide. Mounted sections were air-dried at $22^{\circ}\text{C}.$ for one hour before fluorescent "staining".

Immunofluorescent Studies.—A two-stage technique was used. Iris sections were covered by a drop of patient's serum and placed in a humidified vessel at $22^{\circ}\text{C}.$ for one hour. As much as possible of the serum was then decanted, and the preparation washed for 30 min. in three changes of phosphate buffered saline (pH 7.4). The section was then covered by a drop of anti-human globulin conjugated with fluorescein isothiocyanate and incubated at $22^{\circ}\text{C}.$ for one hour. The preparation was again washed for 30 min. in three changes of buffered saline (pH 7.4).

Preparations were mounted in buffered glycerol (glycerol 96 per cent., buffered saline (pH 7.4) 4 per cent.) and examined by dark-ground ultra-violet microscopy. A mercury vapour lamp (Osram HBO 200) was used as a source of illumination. By the use of appropriate filters (Chance-Pilkington 0X1), the specimen was examined in light of approximately 3,600 Å. Under these conditions, fluorescein isothiocyanate fluoresces a bright apple-green.

Serum and conjugate controls were processed and examined in parallel with each test specimen. All tests were carried out in triplicate.

Passive Cutaneous Anaphylaxis Tests.—Serial dilutions in 0.95 per cent. NaCl of serum from patients with sympathetic ophthalmitis were made. 0.1 ml. aliquot of each dilution was individually inoculated intradermally into the epilated back of a white guinea-pig. Similar dilutions of control "normal" serum were inoculated into the same animal. The guinea-pig was then left for 6 hr. to allow capillary permeability at the site of the injections to return to normal.

An intravenous injection consisting of 3 ml. Evans blue and 2 ml. bovine choroidal tissue antigen was then given. The guinea-pig was observed over a period of 2 hr. A positive reaction is indicated by the development of obvious blue tissue staining around the site at which serum has previously been inoculated.

Complement-Fixation Tests.—These were carried out by the method of Hallett, Wolkowicz, Leopold, Canamucio, and Wijewski (1962). The antigen used was prepared from bovine choroidal tissue.

Case Histories

Case 1, a boy aged 7 years, sustained a penetrating injury of the left eye on June 24, 1962. Extensive keratic precipitates were first noted in the right eye on August 2, 1962. A clinical diagnosis of sympathetic ophthalmitis was made and the left eye was enucleated immediately.

Serum specimens were obtained on August 9, 1962 (uveitis active) and October 11, 1963 (uveitis quiescent, subsequently active). The patient was receiving systemic steroid therapy on both occasions.

The pathology of the enucleated left eye was reported as follows:

On macroscopic examination: The soft and shrunken globe showed a vertical corneal scar from 12 to 6 o'clock. The intra-ocular contents were disorganized and haemorrhagic.

On microscopic examination: The corneal laceration site has been closed by cellular fibrous tissue which is continuous with the subjacent iris stroma. On either side the anterior chamber is maintained, and the angles are open. The iris, ciliary body, and detached retina form a disorganized mass of tissue obliterating the posterior chamber. No lens remnant is recognized. Throughout the uveal tract, but particularly in the region of the ciliary body, there are inflammatory foci, which consist of islands of epithelioid cells, separated by zones of lymphocytes with fairly numerous eosinophils, and giant cells are also present. Structures resembling Dalen-Fuchs nodules are seen in the ciliary body region, but these are distorted by the general disorganization of the anterior segment. The retina is totally detached, but uninvolved in the inflammatory process. There is early gliosis of the optic nerve.

Healed corneal laceration. Leucoma adherens. Retinal detachment. Sympathetic ophthalmitis.

Case 2, a man aged 46 years, sustained a penetrating injury of the right eye on July 25, 1957. After four operations on the right eye, including the insertion and subsequent extraction of a Strampelli lens, an iritis of the left eye was first noted on January 6, 1959. A clinical diagnosis of sympathetic ophthalmitis was made and the right eye was enucleated.

A serum specimen was obtained on August 28, 1962 (uveitis active). The patient was receiving systemic steroid therapy at that time.

The pathology of the enucleated right eye was reported as follows:

Naked-eye: specimen is an eyeball with 1 cm. optic nerve attached. In one area the cornea is thickened and adherent to the ciliary body. The lens remnant is soft and no trace of an acrylic lens has been found. The posterior chamber is normal apart from an increase in the depth of the choroid. The optic nerve stump is normal.

Histology: the area of corneal thickening shows well-scarred granulation tissue, with some infiltration by lymphocytes, plasma cells, and pigment-carrying macrophages in the region of the ciliary body. The epithelium over the front of the cornea and the endothelium behind it are normal.

Between the retina and sclera there is a wide band of dense cellular infiltration, containing lymphocytes, strands of "epithelioid-like" histiocytes, and pigment-carrying macrophages. No caseation or giant cell-epithelioid cell systems were seen. The appearance here is that of choroiditis of the type seen in sympathetic ophthalmitis.

Case 3, a boy aged 14 years, sustained an apparently non-penetrating injury of the left eye on October 23, 1959. An iridocyclitis of the right eye developed 5 months later. In July, 1960, a clinical diagnosis of sympathetic ophthalmitis was made and the left eye was enucleated.

A serum specimen was obtained on October 16, 1963 (uveitis quiescent). The patient was receiving no therapy at that time.

The pathology of the enucleated left eye was reported as follows:

A left globe with blood-stained cornea. Horizontal sections.

The upper half of the cornea is blood-stained over a wide area, sparing the peripheral zone. The anterior chamber has been very largely obliterated but it persists as a small space nasal to the corneal centre. Posteriorly, it is bounded by a layer of fibrous tissue which extends nasally to the angle and which is firmly united to the lens capsule and the anterior iris. Laterally, the iris lies up against the back of the cornea where it shows some drawn-out processes on its posterior surface, the result of lenticular adhesion. The lens is dislocated nasally and also somewhat downwards. The retina is essentially in position though in the disc region there is some organized transudate, projecting at one point well into the retina.

In the iris and ciliary body a prominent granulomatous reaction is evident, the lining epithelium being split at numerous points by masses of epithelioid tissue with a variable and often rich content of large heavily pigmented cells. A diffuse cellular reaction is present in the choroid, being more pronounced on the nasal side; this is largely composed of small mononuclear cells, enclosing here and there islands of epithelioid cells.

There is evidence of previous contusion with recurrent hyphaema, the latter being now represented by organized fibrous tissue. The inflammatory reaction is typical of sympathetic ophthalmitis. Diagnosis: sympathetic ophthalmitis.

Case 4, a man aged 21 years, sustained a penetrating injury of the left eye on November 5, 1947. A foreign body was present in the anterior chamber, the entry wound being in the conjunctiva near the limbus. The foreign body was removed and an iridectomy performed immediately. The patient attended again with a severe iridocyclitis of the left eye on December 22, 1947, and the left eye remained irritable in spite of treatment. On January 2, 1948, fine keratic precipitates were noted in the right eye, and a clinical diagnosis of sympathetic ophthalmitis was made.

The bilateral iridocyclitis remained mildly active in spite of treatment until May, 1949. Subsequently the left pupil became eccentric and a few lens opacities developed. A further left iridectomy was performed on December 30, 1955, to enlarge the "pupil". No iridocyclitis developed post-operatively. The bilateral iridocyclitis recurred twice in 1958 and once in 1963, persisting for several months on the last occasion.

A serum specimen was obtained on March 21, 1963 (uveitis active). The patient was receiving topical treatment only at that time.

Case 5, a man aged 79 years, presented with primary acute closed-angle glaucoma of the left eye on January 16, 1962. A left iridencleisis was performed on January 22, 1962. The patient attended again on March 17, 1962, and was found to have a bilateral iridocyclitis. A clinical diagnosis of sympathetic ophthalmitis was made. The iridocyclitis resolved completely in 5 weeks, the treatment including systemic steroids. On June 1, 1962, the patient was re-admitted with primary acute closed-angle glaucoma of the right eye. A right peripheral iridectomy was performed on June 4, 1962, the systemic steroid therapy being continued. Only on one occasion, exactly one month after the second operation, was slight activity noted in the right eye. The left eye remained quiet. The steroid therapy was discontinued in December, 1962, and both eyes have since remained completely quiet without treatment.

A serum specimen was obtained on August 22, 1962 (uveitis quiescent). The patient was receiving systemic steroid therapy at that time.

Results

Immunofluorescent Studies.—No specific fluorescence was observed in any of the preparations studied.

Passive Cutaneous Anaphylaxis.—Anaphylaxis was not demonstrated with any of the serum specimens examined.

Complement-Fixation Tests.—Complement-fixing antibodies for bovine uveal antigen were not detected in any of the serum specimens examined.

Discussion

No circulating antibodies for uveal tissue were demonstrated in the sera of five patients with sympathetic ophthalmitis by the methods described. In three of the patients the clinical diagnosis was confirmed histologically and in two of these the uveitis was active when the serum specimens were obtained.

Of the four patients in the present study who had active disease at the time of obtaining a serum specimen, three were on systemic steroid therapy. There is no evidence to indicate that this would invalidate the *in vitro* tests for antibody.

It is possible that circulating auto-immune antibodies may be present only in a short phase of the disease, and may therefore have been missed in the present study.

The number of specimens of sera available was necessarily small on account of the rarity of sympathetic ophthalmitis in modern practice.

The failure to demonstrate circulating auto-antibody for uveal tissue in this group of patients does not disprove the hypothesis that sympathetic ophthalmitis represents an auto-immune response, as discussed by Woods (1921). It is possible that the condition represents a cellular hypersensitivity and is not dependent on circulating antibodies. This is found in some other types of delayed hypersensitivity reaction, such as the tuberculin reaction (Lawrence, 1949).

On this thesis it follows that cell-fixed antibody might be demonstrable in the uveal tract of eyes affected by active sympathetic ophthalmitis by the immunofluorescent technique. Such cell-fixed antibody might also be found at the injection site after the subcutaneous injection of bovine uveal pigment in patients with sympathetic ophthalmitis (Woods, 1925). The latter possibility affords a practical approach to the further investigation of the pathogenesis of this disease.

Summary

Sera from five patients with sympathetic ophthalmitis were examined by each of three immunological techniques in an attempt to demonstrate antibodies for uveal tissue. The techniques used were fluorescent immunology, passive cutaneous anaphylaxis, and complement-fixation. No evidence of circulating antibodies for uveal tissue was obtained. Possible explanations of these findings are discussed.

We wish to acknowledge and thank Messrs. A. B. Nutt, A. Stanworth, and P. R. Stevens for permission to publish details of their patients and for supplying specimens of sera; Drs. D. R. Barry, G. Meachim, and J. L. S. Smith for permission to publish the pathology reports; Prof. C. P. Beattie for laboratory facilities and advice; and Mr. A. Stanworth for advice on preparing the paper for publication.

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