Autoimmunity and the retina

1. Antigenic specificity of photoreceptor cells

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The first suggestion that the retina might be antigenic was made by Hess and Römer (1906). This was later confirmed by Burke, Sullivan, Petersen, and Weed (1944), who demonstrated changes in the retina and pigment epithelium after injecting adult lens antiserum into chick embryo. Maisel and Langman (1961) studied the distribution of lenticular antigens in various tissues of the chick eye and described at length how the cornea, uvea, and pigment epithelium of the retina share some antigens in common with the lens. The uveitogenic nature of the retina was first suspected by Wacker and Lipton (1965) in guinea-pigs, and was later confirmed in rabbits (Wacker and Lipton, 1968; Aronson, 1968) and rhesus monkeys (Lerner, Stone, Meyers, and von Sallmann, 1968; von Sallmann, Meyers, Stone, and Lerner, 1969). The precise antigenic locus (or loci) in the retina, however, is as yet undefined, in part because "fractionation of retinal tissue to approach isolation of an antigenic agent has not been attempted" (von Sallmann and others, 1969).

This present study, therefore, was designed to investigate the antigenic properties of the outer limbs of the photoreceptor cell, as it is these which are most commonly affected in retinal dystrophies of doubtful aetiology.

Material and methods

IMMUNIZATION

Dissected retinæ from fresh bovine eyes were homogenized in cold saline, distributed in 0.2 ml. aliquots, and stored at -30°C . Each retina (wet weight 600 mg.) yielded 1 ml. tissue extract.

The animals were healthy rats and rabbits, free from any eye disease. Each experimental animal was given a subcutaneous dose of the extract (reconstituted to 1 ml. in saline) emulsified in Freund's complete adjuvant (H_{37}Ra : Difco) injected into the back once a week for 6 weeks. Control animals were given only the adjuvant. One week after the final injection the animals were aseptically bled and the sera obtained were inactivated, adsorbed, and finally stored at -30°C .

PREPARATION OF TEST ANTIGEN

Outer limb suspensions of photoreceptor cells were prepared according to the method of Saito (1938). The modified technique of Collins, Love, and Morton (1952), although better for biochemical studies, is time-consuming and was therefore not considered necessary for the present investigation. The dissected retina was suspended in three times its weight of freshly prepared 45 per cent. sucrose in McIlvaine's citrate-phosphate buffer (pH 7) and shaken for 30 to 45 sec. until greasy. It was then centrifuged at 2,000 r.p.m. for 15 min. The outer limbs separated out in the supernate were recovered after discarding the sediment and recentrifuging the supernate (mixed with four times its volume of the buffer) at 3,000 r.p.m. for 30 min. The sediment was re-suspended in cold saline, homogenized, and then stored in sterile vials at -30°C . for future use as antigen for agar diffusion and haemagglutination tests.

THE TEST

Antibodies against whole retinal extracts and photoreceptor cells were tested by tanned red cell agglutination and two-dimensional double-diffusion tests. Agar plates were prepared with 1.5 per cent. Noble agar in barbitone buffer (pH 8.2; $I = 0.075$) containing 0.1 per cent. sodium azide. Diffusion was allowed to occur at 4°C. Immunoelectrophoresis of rabbit antiserum was carried out on similar agar plates against antirabbit IgG.

Results

- (i) Sensitized tanned red cells showed agglutination with test sera from immunized rabbits and rats. There was no agglutination with buffer alone or with non-immune serum obtained from animals receiving only the adjuvant. Furthermore, no agglutination was observed with non-sensitized tanned red cells in immune sera.
- (ii) On the second day precipitation lines (single with photoreceptor cells and double with whole retinal extracts) were observed by naked eye in the agar plate, and these became heaviest (and showed a reaction of identity) on the fourth day (Figs 1 and 2).

FIG. 1 *Double diffusion gel precipitation: Antisera (A) rabbit and (B) rat demonstrate a single precipitin band against bovine photoreceptor outer limbs (OL). Sera from normal rats (C) and rabbits (D) show no precipitin line.*

FIG. 2 *Agar diffusion precipitin reaction: Antisera (A and B) rabbit and (C and D) rat demonstrate closely placed double bands against whole bovine retinal extract (WR).*

No precipitation was observed with non-immune sera. Occasional faint secondary (Liesegang) lines were seen after long incubation in some of the sectors of the agar plate, but these were probably artefacts produced by environmental changes. Homogenates from rat and bovine retinæ cross-reacted and showed a reaction of partial identity (Fig. 3).

- (iii) Sera from immunized rabbits showed a high level of IgG (Fig. 4).

Comments

The experiment was designed as a prelude to further investigations into the role of antigenic determinants of photoreceptor cells in the pathogenesis of retinal degenerations. Wacker and Lipton (1968), working with whole retinal extracts, suggested the presence of two antigenic substances, one soluble and the other particulate, responsible for immune

FIG. 3 Reaction of partial identity between rat (A and B) and bovine (C and D) retinæ. (RS) indicates rabbit antiserum against bovine retina.

FIG. 4 Immunelectrophoresis: Holes (A and B) contain sera from immunized and normal rabbits respectively. Trough (T) contains monospecific serum against rabbit IgG. Comparatively large precipitin line near (A) indicates high level of IgG in immunized rabbit.

reactions in guinea-pigs. Aronson (1968) prepared his antigen from whole retinal extract after dialysis and centrifugation and suggested that antigenic substance was present in the supernate. Whether there is one or more than one antigenic substance in the retina—a complex cellular structure functionally and anatomically—is a matter for thorough investigation. It seems certain, however, that one of the antigenic substances is located in the outer limbs of the photoreceptor cells. This assumption is further supported by the fact that some of the immunized animals (Wacker and Lipton, 1965, 1968; Aronson, 1968; Lerner and others, 1968) showed necrosis and degeneration of the outer layers of the retina. Although it has been suggested that these changes were secondary to choroiditis, it is difficult to exclude an antigen-antibody reaction in the retina, especially when retinal immune reactions, quite independent of choroiditis, have been demonstrated in rhesus monkeys (von Sallmann and others, 1969). Yamamoto and Yamori (1966) could not demonstrate with certainty antibodies against bovine opsin extracted with digitonin in rabbit immune sera, but they were of the opinion that the results obtained could be improved if saponin extracts were used, since digitonin extracts are unsuitable for antigen-antibody reactions.

Conclusions

The antigenicity of the outer limbs of the photoreceptor cells has been investigated in rabbits and rats. Specific antibodies have been demonstrated by haemagglutination

and precipitation techniques. It is suggested that the role of antvisual cell antibodies in the pathogenesis of retinal dystrophies should make an interesting and possibly a valuable study.

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