Immunopathology of the lens

II. Humoral and cellular immune responses to homologous lens antigens and their roles in ocular inflammation

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SUMMARY It has been possible for the first time to demonstrate antibodies to homologous lens proteins in rabbits without the addition of adjuvant. By means of immunofluorescence and immunoperoxidase methods it has been possible not only to show for the first time that homologous lens antibodies cross-react with extraocular tissues but that the cross-reacting antigens are related to the cell mitochondria, microsomes, and the proteins associated with contractile organelles.

The rabbits did not produce antibodies to γ -crystallins even when the whole lens homogenate was injected with Freund's complete adjuvant. This suggests that γ -crystallins are non-antigenic in homologous situations, and this may be related to both B- and T-cell tolerance. Alternatively, the failure of γ -crystallins to induce antibody production may be due to intermolecular antigenic competition with other crystallins. The presence of mycobacteria in an adjuvant is essential for an antibody response to be detectable by agar diffusion techniques. The response to homologous lens antigens, both in magnitude as well as in duration, varied in different rabbits, which suggested to us that a central control mechanism involving the immune response (Ir) genes may play an important role. Antibodies to homologous lens proteins as detected by immunofluorescence and immunoperoxidase methods were shown to be of the IgG class. This is the first time that the kinetics of the immune response to the same homologous lens antigen in saline with or without incomplete or complete adjuvant has been examined and their relative merits compared.

Systemic homologous immunisation followed by discission of the lens led to a marked Arthus type reaction in and around the lens, but a typical granulomatous phakoallergic endophthalmitis was not produced. It seems likely that the rabbit is not suitable for the production of an experimental model of this condition.

It is now well established that the mammalian lens contains several soluble antigenic proteins (e.g., pre- α -, α -, β -, β s- and γ -crystallins) which can and do stimulate both antibody- and cell-mediated immune responses when animals are injected with lens proteins from unrelated species.

A positive humoral immune response to such heterologous lens protein was first demonstrated by Uhlenhuth (1903) and later confirmed by Hektoen (1922), Woods and Burky (1927), Manski *et al.* (1965), Clayton *et al.* (1968), and its study extended more recently by such techniques as immunofluorescence, immunoperoxidase, and lymphocyte transformation tests (Rahi *et al.*, 1977). Our latest studies have shown that the nature as well as the

amplitude of the immune response varies in different animals largely according to whether the antigen is injected alone or with an adjuvant containing mycobacteria.

Since circulating antibodies can be induced without the incorporation of an adjuvant by repeated injections of large amounts of saline homogenates of heterologous lens, it had been speculated that an immune reaction to lens protein may lead to intraocular inflammation and formation of cataract. Since homologous rather than heterologous material is more likely to resemble autologous material, attempts were made to immunise animals with homologous lens proteins in order to study their effect on ocular tissues, but these experiments proved unsuccessful (Romer and Gebb, 1912; Morax and Pollack, 1914; Hektoen, 1922;

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Woods and Burky, 1930; Swift and Schultz, 1936). It was soon realised that the antigenic determinants on homologous lens proteins were not accessible to the immunologically competent cells unless the nature of these molecules was altered by chemical treatment. Thus Hektoen and Schulhof (1924) found that homologous lens proteins after fractionation with acetic acid induced an antibody response in rabbits. In a later study Woods and Burky (1930) confirmed this finding but stated that it is only the α -crystallin, the most acidic and heaviest of all the lens proteins, which after acid treatment becomes antigenic in homologous situations.

The antigenicity of the homologous lens protein has been re-examined by other workers, who found that saline homogenates of homologous lens antigen do not lead to any antibody response (Halbert and Manski, 1965; Manski, 1973). It is only when the homologous antigen is combined with Freund's complete adjuvant that the animals show any evidence of an immune response (Halbert *et al.*, 1957a, b). Furthermore, these workers also found that the antibody so raised did not cross-react with extraocular tissues of the rabbit, which is in contrast to the wide reactivity of the heterologous lens antibody (Halbert and Fitzgerald, 1958). The response to homologous lens antigens was later confirmed by Sourek and Klenka (1960). A review of the literature suggested to us that the following aspects of humoral and cellular immune responses to homologous lens antigens had not been adequately investigated.

Although it is widely held that homologous mammalian lens protein is nonantigenic when given without adjuvant, no serious attempt has been made to re-examine this aspect of the immune response of animals using such modern methods as immunofluorescence and immunoperoxidase labelling techniques.

Furthermore, we are not aware of any study in which the kinetics of the humoral immune response to the same homologous lens antigens in saline with or without incomplete or complete Freund's adjuvant have been examined and where the relative merits of agar precipitation, haemagglutination, immunofluorescence, and immunoperoxidase methods have been critically compared.

It is now known that the antibodies in man and other mammals belong to several distinct classes of immunoglobulins. It is also known that various serological techniques employed to detect specific antibodies to a given antigen demonstrate preferentially one class of immunoglobulin. For example, the agar precipitation test detects mainly high affinity IgG, the haemagglutination test mainly IgM, and complement fixation test only the comple-

ment fixing IgG and IgM. Immunoperoxidase and immunofluorescence methods on the other hand can, in addition, detect with equal efficiency other classes of immunoglobulins. By using monospecific antisera it should now be possible to be certain which immunoglobulin is mainly involved in homologous lens immunity.

It has only recently become clear that delayed tissue allergy is mediated by lymphocytes which are processed in the thymus and called T-lymphocytes. The delayed hypersensitivity can now be measured by a very precise *in-vitro* technique called the lymphocyte transformation test. So far as we are aware no such tests have been employed in the past to detect immune responses in animals injected with homologous lens proteins without any adjuvant. Since an immunological investigation would be incomplete without examining the T-lymphocyte reactivity, the assertion of Manski (1973) that homologous lens antigens on their own fail to induce an immune response needs further examination. Furthermore, we are not aware of any study in which the lymphocytes of animals sensitised with homologous lens antigens in saline, with or without incomplete or complete Freund's adjuvant, have been examined and the response correlated with antibody levels. Although cross-reactivity of heterologous lens antisera has been widely studied, adequate data on similar cross-reactivity of homologous lens antisera are lacking, and furthermore no attempt has been made at microscopical localisation of the cross-reacting antigen in ocular and extraocular tissues. The present study, therefore, was undertaken in an attempt to provide some answers to these problems and to bridge some of the gaps in our knowledge concerning the antigenicity of the homologous lens proteins, the nature of the immune response and its role in ocular inflammation.

Materials and methods

The study was performed on New Zealand adult albino rabbits using litter mates where possible. Rabbit total lens proteins in saline, with or without incomplete or complete Freund's adjuvant, were given intramuscularly at different sites at 10-day intervals. Each injection contained 15 mg of lens protein. The animals were bled on the first day of immunisation and also at the time of subsequent injections. After the final injection animals were skin-tested and the injection site was biopsied 48 hours later. They were anaesthetised and dissection of the right lens was performed with a Ziegler's knife. They were killed a week later and blood was collected from the heart in preservative-free heparinised containers for lymphocyte transforma-

tion tests. Both eyes were enucleated for histological examination. Proper controls were set up as with the previous study on heterologous lens antigens (Rahi *et al.*, 1977).

Immunological investigations

The *Ouchterlony* test was performed in 1.5% agar in barbitone buffer (pH 8.2) at 4°C. The agar plates were washed 1 week later and after drying at 37°C were stained with 0.1% nigrosine.

Immuno-electrophoresis and *Osserman* tests were performed on 10 × 10 cm agar plates. The pH of the barbitone buffer in the agar was 8.2 (I=0.05) whereas in the electrophoresis tank it was 8.6 (I=0.075). The technique used was essentially the same as previously described (Rahi and Chignell, 1975).

The *passive haemagglutination* test was performed using formalised sheep red cells (Oxoid). These were first treated with 1:20 000 analytical grade tannic acid solution in phosphate buffer saline (pH 7.2, 0.15 M) and then with homologous lens proteins which were previously adsorbed with untanned cells. The antigen concentration used was 2 mg for each millilitre of tanned sheep red cells. The cells thus sensitised were carefully washed with the buffer containing 1% normal rabbit serum which was previously heat-inactivated and preabsorbed. The final concentration of red cells was adjusted to 1.25%. The test was performed on a microtitre assay system (Cooke Laboratory). Controls were set up with sensitised red cells in buffer alone and with nonimmune serum obtained from the rabbits before initial immunisation. Controls were also set up using unsensitised cells with or without antiserum.

The *indirect immunofluorescence* test was performed using the standard technique. A Zeiss epifluorescence microscope equipped with an interference filter was used for the present study. Frozen sections of a composite block consisting of liver, stomach, kidney, parotid, and diaphragm from a rat and the stomach of a mouse were used to detect cross-reacting antibodies in homologous lens antisera. Rabbit, guinea-pig, and rat eye sections were used to detect an immune reaction not only with the lens but also with other ocular structures. The sera were tested at an initial dilution of 1:2 and both polyvalent (Wellcome) and IgG specific (Dako) anti-rabbit immunoglobulins labelled with fluoresceine (FITC) were used for the present study.

The *indirect immunoperoxidase* test was performed on frozen sections of the composite block and of the eyes as used for immunofluorescence examination. The sections were first treated with 1:20 normal pig serum and washed in phosphate buffer saline (pH 7.2) to inhibit non-specific reactions and then

with 1:4 homologous lens antiserum. After washing in PBS with magnetic stirrer the sections were treated with 1:20 peroxidase-labelled anti-rabbit IgG serum (Dako) which was raised in pigs. The washed sections were incubated for 20 minutes in freshly prepared diaminobenzidine tetrahydrochloride (DAB) reagent in a concentration of 6 mg/10 ml Tris buffer saline and containing 2 drops of 10 vol solution of hydrogen peroxide. The sections were later washed in buffer and counterstained with haematoxylin.

Lymphocyte transformation test. The lymphocytes were separated from the rabbit blood by the Ficoll-Hypaque technique (Fotino *et al.*, 1971). One million lymphocytes were cultured in 1 ml T.C.199 (Wellcome) prepared in HEPES buffer and enriched with heat-inactivated 10% fetal calf serum. Several such cultures were set up in sterilin tubes. To each of the experimental tubes 0.1 ml of 1% sterile homologous lens solution was added followed 48 hours later by 2 µCi of tritiated thymidine (23 000 mCi/mmol Amersham). The control tubes, in which there was no lens protein, were similarly treated with the radioactive thymidine and incubated overnight. After centrifugation the proteins in the cellular deposits were precipitated with 5% trichloroacetic acid and filtered on glassfibre filter paper which was washed in methanol and transferred into scintillation vials and air dried. 10 ml of a toluene-based scintillation fluid was added to each vial and the radioactivity was measured in a Packard tricarb liquid scintillation counter. The result was expressed as a transformation index, which represents the ratio of the radioactivity in the experimental tube to that in the control tube (i.e., E/C). In a positive reaction the transformation index is greater than 1.

Results

Serum collected from all rabbits immediately before immunisation was negative for lens antibodies as shown by the agar precipitation, haemagglutination, immunofluorescence, and immunoperoxidase tests. Antibodies to homologous lens proteins as shown by haemagglutination technique appeared on the 10th day of immunisation (though in low concentration) when the antigen was given with Freund's complete adjuvant. They appeared on the 20th day of immunisation when antigen was given with incomplete adjuvant, whereas when antigen was given in saline alone antibodies were not detectable until 30 days. The antibody concentration, however, was low.

The final homologous lens haemagglutinin titre in 1 rabbit 100 days after repeated immunisation rose to 1:20 480 with Freund's complete adjuvant.

Table 1 *Antibody response in a rabbit injected with homologous lens proteins in Freund's complete adjuvant*

Methods of antibody detection	Days after initial immunisation					
	10	20	40	60	80	100
Gel diffusion (Ouchterlony)	-	-	+	+	+	+
Immunoelectrophoresis	-	-	+	+	+	+
			(α - β)	(α - β)	(α - β)	(α - β)
Passive haemagglutination	+	+	+	+	*	+
	(1:10)	(1:40)	(1:640)	(1:2560)		(1:20 480)
Indirect immunofluorescence	$\pm$	+	+	+	*	+
Immunoperoxidase	*	+	+	+	*	+

+ Positive. $\pm$ Weak positive. - Negative. * Not tested. (α - β) = Antibodies to crystallins in α - β regionTable 2 *Antibody response in a rabbit injected with homologous lens proteins in Freund's incomplete adjuvant*

Methods of antibody detection	Days after initial immunisation					
	20	30	40	60	80	>80
Gel diffusion (Ouchterlony)	-	-	-	-	-	-
Immunoelectrophoresis	-	-	-	-	-	-
Passive haemagglutination	+	+	+	+	+	+
	(1:30)	(1:30)	(1:50)	(1:320)	(1:160)	(1:160)
Indirect immunofluorescence	*	*	*	*	*	$\pm$
Immunoperoxidase	*	*	*	*	*	$\pm$

+ Positive. $\pm$ Weak positive. - Negative. * Not testedTable 3 *Antibody response in a rabbit injected with homologous lens proteins without adjuvant*

Methods of antibody detection	Days after initial immunisation					
	30	60	70	80	100	>100
Gel diffusion (Ouchterlony)	-	-	-	-	-	-
Immunoelectrophoresis	-	-	-	-	-	-
Passive haemagglutination	+	+	+	*	+	+
	(1:5)	(1:320)	(1:320)		(1:160)	(1:160)
Indirect immunofluorescence	*	*	*	*	+	+
Immunoperoxidase	*	*	*	*	+	+

+ Positive. - Negative. * Not tested

When the antigen was given either alone or with incomplete adjuvant the haemagglutinin titre was always very low. It rose temporarily to 1:320 on the 60th day but fell to 1:160 on the 100th day of immunisation (Tables 1, 2, 3). With complete adjuvant the lens haemagglutinin titre generally showed a sustained rise, but with incomplete adjuvant or when the homologous antigen was given in saline alone the plateau was reached much earlier. The kinetics of the immune response are shown in Fig. 1.

At least 3 precipitin lines were seen in Ouchterlony plates (Fig. 2) when serum from rabbits injected with homologous lens proteins in Freund's complete adjuvant were tested. Immunoelectrophoresis showed these lines in the α - β region. No precipitin lines were seen, however, when the antigen was given alone or with incomplete adjuvant. The level of the immune response was subject to individual variation even when the antigen was given with Freund's complete adjuvant. Thus 1 rabbit had a haemagglutinin titre of only 1:5 even after 14 weeks of repeated

Fig. 1 Kinetics of average antibody response in 3 groups of rabbits injected with homologous lens in saline (A), in incomplete (B), and in complete (C) Freund's adjuvant. The arrows represent immunising injections

immunisation, whereas another rabbit similarly treated gave a titre of 1:20 480. In 1 rabbit the haemagglutinin titre after 100 days was 1:320 and remained stationary at that level even after repeated immunisation for another 50 days. The biological variations in homologous lens antibody responses are shown in Fig. 3 and Tables 4 and 5.

Immunofluorescence and immunoperoxidase tests were weakly positive for lens antibodies when sera from rabbits immunised with saline homogenate alone or with incomplete adjuvant were tested. A strongly positive result was obtained, however, when sera from animals injected with lens homogenate in complete adjuvant were used. These tests

showed that homologous lens antibodies belong to the IgG class (Fig. 4). The possibility that they belong to the IgM class, however, and also to other classes cannot be excluded.

In addition to reactivity within the lens cortex sera from rabbits showing high-titre haemagglutinins showed weak cross-reactivity with the iris (Fig. 5), the ciliary body, the corneal epithelium, and the inner retina. Diffuse as well as coarse granular reactions were also noted with gastric parietal and chief cells, hepatocytes, and renal tubular epithelium

Fig. 2 Ouchterlony test: The central well contains lens proteins from a rabbit. The peripheral wells contain sera from 4 rabbits immunised with homologous lens in Freund's complete adjuvant. There are a varying number of precipitin lines

Table 4 Biological variations in antibody response in a group of 7 rabbits injected with homologous lens proteins in Freund's complete adjuvant, 40 days after regular immunisation at 10-day intervals

Methods of antibody detection	Rabbits studied						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Gel diffusion (Ouchterlony)	-	-	+	-	+	-	-
Immunoelectrophoresis	-	-	+	-	+	-	-
			(α - β)		(α - β)		
Passive haemagglutination	+	+	+	+	+	+	-
	(1:60)	(1:30)	(1:640)	(1:5)	(1:640)	(1:40)	
Indirect immunofluorescence	+	±	+	*	+	±	-
Immunoperoxidase	*	*	+	*	+	*	*

+ Positive. ± Weak positive. - Negative. * Not tested. (α - β) = Antibodies to crystallins in α - β region

Table 5 Biological variations in antibody response: n a group of 7 rabbits injected with homologous lens proteins in Freund's complete adjuvant, 100 days after regular immunisation at 10-day intervals

Methods of antibody detection	Rabbits studied						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Gel diffusion (Ouchterlony)	-	-	+	-	+	-	-
Immunoelectrophoresis	-	-	+	-	+	-	-
			(α - β)		(α - β)		
Passive haemagglutination	+	+	+	+	+	+	+
	(1:320)	(1:320)	(1:1280)	(1:40)	(1:20 480)	(1:320)	(1:5)
Indirect immunofluorescence	+	+	+	+	+	+	-
Immunoperoxidase	+	+	+	+	+	+	-

+ Positive. $\pm$ Weak positive. - Negative. * Not tested. (α - β) = Antibodies to crystallins in α - β region

(Figs. 6, 7). This suggests the presence of anti-mitochondrial, antiribosomal (and antimicrosomal) antibodies in homologous lens antisera, since a similar fluorescence has been described with human sera containing specific antibodies to mitochondria, ribosomes, and microsomes (Taylor *et al.*, 1962; Anderson *et al.*, 1967; Wright, 1975). A strong antiserum from one animal reacted with smooth muscle fibres in rat stomach, suggesting the presence of antibodies to contractile muscle proteins (Fig. 8). The lymphocyte transformation test (T-cell reactivity) was strongly positive in a large proportion of the rabbits immunised with homologous total

Fig. 3 Biological variations in antibody response in 7 rabbits injected with homologous lens protein in Freund's complete adjuvant, 40 days (■) and 100 days (□) after regular immunisation at 10-day intervals

Fig. 4 Indirect immunofluorescence test with an unfixed section of rat lens. Serum from an immunised rabbit reacts with the lens epithelium and the cortex to produce bright fluorescence. The fluorescein-labelled antiserum was IgG specific. $\times 275$

Fig. 5 Lens antiserum containing cross-reacting antibodies. Note the fluorescence of rat iris. $\times 350$

Fig. 6 *Indirect immunoperoxidase test with sections of the rat liver. Antilens serum reacts with the cytoplasmic contents (e.g., mitochondria and ribosomes) to produce dark reaction products. The nuclei have been counterstained with haematoxylin $\times 900$*

lens protein in Freund's complete adjuvant. The transformation index varied from 7 to 20 and generally correlated well with antibody titres as tested by agar precipitation and haemagglutination methods. The animals immunised with saline homogenate alone or in combination with incomplete adjuvant also showed evidence of T-cell reactivity. The mean transformation index was 2.5 and 3.5 respectively.

Skin biopsies from immunised rabbits following homologous lens injection showed a non-granulomatous reaction which consisted of lymphocytes, polymorphs, eosinophils, and macrophages (Fig. 9). The inflammatory reaction was more

marked when antigen was given with complete rather than incomplete adjuvant. Animals which were injected with saline extract alone showed minimal infiltration. The needled eyes showed evidence of non-granulomatous inflammation. The lens was infiltrated with polymorphs, eosinophils, macrophages, and occasional lymphocytes and plasma cells (Fig. 10). A similar inflammatory exudate was present in the vitreous and also in the anterior chamber. The iris, however, showed only a mild inflammatory reaction. The eye from one of the rabbits immunised with homologous antigen in Freund's adjuvant, however, showed a fairly large number of plasma cells, lymphocytes, neutrophils,

Fig. 7 *Lens antiserum containing antibodies to mitochondria and ribosomes. Note the bright granular fluorescence of some of the renal tubules. Indirect immunofluorescence test. $\times 650$*

Fig. 8 *Indirect immunoperoxidase test with unfixed sections of the rat stomach. Lens antiserum from a rabbit reacts with the chief and the parietal cells (A) and the smooth muscle fibres between the glands (B), suggesting the presence of antibodies to mitochondria, ribosomes, and smooth muscle proteins. The nuclei have been counterstained with haematoxylin. $\times 900$*

and eosinophils in the ciliary body (Fig. 11) and to a lesser degree in the iris. Epithelial hyperplasia of the lens (Fig. 12) with subcapsular fibrosis (Fig. 13) was a common feature of all eyes. As in the skin the reaction in the needled eyes of those animals where saline homogenates of homologous lens without any adjuvant were injected was minimal

and consisted mostly of vasodilatation in the iris and the ciliary body.

Discussion

The present study has shown for the first time that homologous lens protein can induce a detectable

Fig. 9 *Section of the skin biopsy from an immunised rabbit injected with homologous lens protein. It shows an inflammatory reaction consisting of lymphocytes, polymorphs, eosinophils, and occasional macrophages. Haematoxylin and eosin. $\times 550$*

Fig. 10 The needed lens of an immunised rabbit is diffusely infiltrated by degenerate polymorphs, eosinophils, macrophages, and occasional lymphocytes. Haematoxylin and eosin. $\times 650$

immune response without the addition of any adjuvant. The degree of the immune response, however, was low and the specific antibodies were detected only by such sensitive techniques as the haemagglutination test. This is in contrast to heterologous lens proteins which induce an antibody response that can easily be detected by simple Ouchterlony and immunoelectrophoretic methods (Rahi *et al.*, 1977).

Although Halbert *et al.* (1957a, b) successfully demonstrated an antibody response to homologous lens proteins when injected with Freund's complete adjuvant, the present study has examined for the first time the role of adjuvant in the production of both cellular and humoral immune responses. It

shows that both types of immune responses to homologous lens proteins in rabbits are much stronger when the antigen is given with Freund's complete adjuvant than when it is given alone or with incomplete adjuvant. It would appear, therefore, that in contrast to heterologous lens antigens the presence of mycobacteria is essential for a good humoral immune response to homologous lens proteins. This suggests that rabbits show a degree of immunological tolerance to lens proteins which can be overcome by using adjuvants rich in mycobacteria. Furthermore, since we failed to demonstrate any precipitating antibodies to γ -crystallins even when Freund's complete adjuvant was used, it seems probable that animals are tolerant to at least

Fig. 11 The ciliary body is infiltrated by eosinophils, lymphocytes and plasma cells. Haematoxylin and eosin. $\times 650$

Fig. 12 *The needed lens of an immunised rabbit shows epithelial hyperplasia. Inflammatory cells surround the lens capsule. Haematoxylin and eosin. $\times 550$*

these homologous lens proteins. The reason why rabbits react to heterologous but not to homologous γ -crystallins may be related to some differences in the antigenic determinants and their accessibility to the immune system. It would appear that, so far as homologous γ -crystallins are concerned, rabbits probably show a state of tolerance which is likely to involve both B- and T-lymphocytes. This subject has been discussed in detail in our earlier study on heterologous lens proteins, and although in most textbooks lens proteins are still considered to be

sequestered antigens, which means that animals should be intolerant to all of them (i.e., α -, β -, and γ -crystallins) and should be able to produce antibodies without the addition of adjuvants, there is now evidence that they are probably tolerant to homologous γ -crystallins as evinced by the conspicuous absence of γ -precipitin lines in the immunoelectrophoretic study. It is possible, however, that the absence of an immune response to homologous γ -crystallins was due to an intermolecular antigenic competition (Michaelis, 1902) because of

Fig. 13 *Sections show an injured lens with marked subcapsular fibrosis and extracapsular inflammatory cells. Haematoxylin and eosin. $\times 550$*

the presence of α - and β -crystallins in the injected lens homogenate. It is now known that the inhibition of the immune response to one antigen by the simultaneous administration of another antigen is T-lymphocyte dependent and is probably related to the antigen specific T-cell products which through the agency of macrophages play a decisive role in the activation of B-lymphocytes which eventually transform into antibody-producing plasma cells (Taussig, 1975).

The kinetics and the variability of the immune response studied in several groups of animals further emphasises the role of immunological tolerance. It is known that the immune responsiveness to a variety of antigens is controlled by the immune response (Ir) genes, which are associated with the major histocompatibility region of the chromosome (Ellman *et al.*, 1971). Furthermore, it seems likely that mycobacteria present in the complete adjuvant modify the response to homologous lens proteins possibly by their direct non-specific effect on the T-lymphocytes. This effect, however, does not seem to influence the response to γ -crystallins, but this requires further critical examination.

A comparative study of the 5 different methods for the detection of homologous lens antibodies, which have been performed for the first time on the same serum sample, shows clearly that the best method for detecting antibodies to lens proteins is the passive haemagglutination technique, which becomes positive before the other tests. For reproducible results, however, it is necessary to use freshly homogenised lenses, which should be preadsorbed with sheep red cells before the lens homogenates are used to coat the tanned red cells. The serum to be tested should be preabsorbed and heat-inactivated immediately before the test.

Immunofluorescence and immunoperoxidase tests are less sensitive. Ouchterlony and immunoelectrophoretic techniques are least sensitive, being positive only when the homologous lens haemagglutinin titres were 1:640 or more. The one advantage of the immunoelectrophoretic technique, however, was in its capacity to demonstrate that antibodies to homologous γ -crystallins did not appear even when the whole lens solution was injected with Freund's complete adjuvant. The immunofluorescence and immunoperoxidase tests had one advantage over the haemagglutination and the immunoelectrophoretic tests in being able to detect the chemical class of the immunoglobulin involved in an immune reaction to homologous lens proteins. It is now possible, therefore, to state with certainty that IgG is involved in this reaction, although it does not exclude the possible role of other immunoglobulins.

Although Halbert and Fitzgerald (1958) failed to detect by agar diffusion techniques any cross-reactivity between homologous lens antisera and extraocular tissues, our study, using much more sensitive methods of immunofluorescence and immunoperoxidase, show clearly not only that such cross-reactivity exists but that the cross-reacting antigens are related to mitochondria, ribosomes, and the proteins associated with contractile organelles. It is of interest that the lens epithelium and the superficial lens fibres contain all or most of these organelles (Hogan *et al.*, 1971). Furthermore, these organelles, although not discernible electron microscopically in the deeper regions of the lens, might be present in inspissated structureless forms. That this may be so is supported by the evidence that ribonucleoproteins can be extracted from the lens nucleus (Maraini *et al.*, 1964).

The reason why we obtained a higher lymphocyte transformation index with homologous immunisation as compared to our previous heterologous study is probably related to the fact that in the present study the rabbits were injected with total lens extract instead of soluble proteins only.

Systemic immunisation with homologous lens protein followed by discission of the lens did not lead to a granulomatous phakoallergic endophthalmitis.* The lenses, however, were infiltrated by polymorphs, eosinophils, lymphocytes, and plasma cells, suggesting an Arthus-type reaction. The lens epithelial hyperplasia and subcapsular fibrosis which were found in almost every eye were produced either by injury or perhaps as a result of an immune reaction analogous to that which leads to the formation of a retrocorneal membrane in a failed graft.

The fact that a granulomatous reaction was not present in spite of marked delayed hypersensitivity, as evinced by a strongly positive lymphocyte transformation test, suggests that the rabbit is not a suitable experimental model for lens-induced uveitis. Furthermore, this is borne out by the fact that in spite of a marked cellular infiltration in and around the injured lenses, the iris and the ciliary body showed only a minimal inflammatory reaction. Goodner (1964) immunised rabbits with homologous lens antigens in Freund's complete adjuvant and similarly failed to demonstrate typical lens-induced uveitis even when lens material was injected into the vitreous of the previously sensitised animals. In a study using heterologous and homologous lens antigens we have found that guinea-pigs and rats

*The term phakoallergic endophthalmitis has been used throughout the text as a preferred alternative to such terms as lens-induced uveitis, endophthalmitis phako-anaphylactica or phakogenic endophthalmitis. The reasons for this are discussed in a separate communication.

show a greater propensity to develop phako-allergic endophthalmitis. These studies will be reported in detail elsewhere.

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