Immunoglobulins in human aqueous humour

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SUMMARY The immunoglobulin concentrations in human aqueous humour from 44 patients aged 35 to 85 years with cataracts were measured by a standard immunodiffusion method. IgG was found in all the samples (mean level 7.0 mg/100 ml). IgD, IgA or IgM could not be detected. There was no significant difference in IgG levels in aqueous humour between the two sexes, in different age groups, and in the different types of cataracts.

The immunoglobulin content in internal secretions such as cerebrospinal fluid, aqueous humour, and synovial fluid differs from that in external secretions such as tears, saliva, nasal and bronchial fluids, colostrum, breast milk, perspiration, and jejunal juice (McClellan *et al.*, 1973; Tomasi and Bienenstock, 1968). Though considerable data have recently been collected on the immunoglobulin level in human tears (Sen *et al.*, 1976), very little work has been done to establish the level in the aqueous humour of human eyes. Difficulties are obvious in obtaining samples from living absolutely normal human eyes. On the other hand the validity of the results of necropsy studies on the composition of immunoglobulins in aqueous is in doubt, as it is known that the composition of aqueous changes rapidly after death (Hemmingsen and Øther, 1967; Dieckhues, 1967; Allansmith *et al.*, 1973).

It was therefore decided to study the immunoglobulin levels in aqueous humour in living eyes with senile cataractous changes so that the available data could be used as control for further studies in various ocular diseases.

Subjects and methods

44 cases with cataractous changes were included in the study. All of them reported to Irwin Hospital for extraction of the lens. The mean age of these patients was 55.9 years (SD 10.0, range 35 to 85 years). There were 21 females with mean age 54.3 years (SD 8.8, range 40 to 70 years), and 23 males with mean age 60.0 years (SD 10.8, range 35 to 85 years). 14 cases showed early senile cataractous changes, 13 moderate senile cataractous changes, and 17 nearly mature senile cataracts. Hypermature

cataracts were excluded from this study, as it was feared that some of them might have unsuspected minimal leakage of lens matter into the aqueous. All the cases were otherwise healthy both systemically and on local examination, including slit-lamp biomicroscopy. When the posterior segment could not be examined preoperatively owing to the presence of advanced cataractous changes, it was examined after operation to rule out any lesion.

Samples of aqueous humour were collected from the eyes just before the extraction of the lens under local anaesthesia, care being taken not to contaminate aqueous with blood. A small stab incision was made with a keratome at the limbus, and aqueous humour was drawn into a tuberculin syringe fitted with a fine cannula. The total quantity of sample obtained was 200 μ l approximately. The samples were stored in screw-capped vials at -20°C until needed. The IgA, IgG, IgD, and IgM in the samples were measured by a single radial immunodiffusion method (Mancini *et al.*, 1965) using monospecific goat antisera against heavy chain human IgA, IgG, IgD, and IgM. The respective reference standards, except that of IgA, were obtained from Meloy Laboratories, USA. The reference standard of secretory IgA was prepared from human colostrum.

Antihuman lambda and anti-human kappa antisera (Meloy Laboratories) were used to detect lambda and kappa light chains in the aqueous humour. The IgG was characterised against anti-gamma chain (WHO), anti-Fc, and anti-Fab (Nordic, Denmark) antisera. Human myeloma IgG (λ) obtained from Meloy Laboratories served as control.

Results

IgG was invariably found in all the samples. The mean level was 7.0 ± 4.2 mg/100ml (range 1.5 to

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17.6 mg/100 ml). There was no significant difference statistically ($P < 0.10$) between the IgG level in the males (6.5, SD 4.6 mg/100 ml, range 1.5 to 17.6 mg/100 ml) and that in the females (7.6, SD 4.2 mg/100 ml, range 2.4 to 17.0 mg/100 ml), though the level in females tended to be higher. The distribution of IgG level in different age groups is given in Table 1. There was no statistically significant difference in the IgG level between the various age groups. The distribution of IgG level in different types of cataracts is given in Table 2. There was also no significant difference statistically in the IgG level in aqueous humour in different types of cataracts. IgA, IgM, or IgD were not detected in any of the aqueous samples.

The study of the distribution of the types of light chains among the IgG molecules present in the aqueous shows that they had predominantly lambda chains. Thus lambda chains were detected in 80% of samples, kappa chains in 8% of samples, and both chains in 8% of samples.

The IgG in aqueous has been characterised by various monospecific antisera. It gave sharp precipitin lines with anti-gamma chain and anti-Fc and anti-Fab antisera. Myeloma-IgG (λ), which served as control, also gave precipitin lines with these three antisera. It is therefore concluded that IgG molecules in aqueous have intact heavy and light chains. The latter is predominantly of the lambda type.

Discussion

IgG is the predominant immunoglobulin in the internal secretions, the ratio of IgG to IgA being 5:1 approximately, with a smaller proportion of IgM (McClellan *et al.*, 1973). However, data on the immunoglobulin level in living human eyes are scanty. Audain *et al.* (1974) studied the immunoglobulin content of human aqueous from 5 senile cataractous eyes. They found IgG levels in all the samples, the values ranging from 5 to 8.5 mg/100 ml. IgA levels in two samples were 4 and 4.5 mg/100 ml (absent in three other cataract cases), and they failed to detect IgM and IgD in any sample. The normal average concentrations of immunoglobulins

Table 1 IgG level in mg/100 ml in the aqueous humour of cataract cases in different age groups

Age group in years	No. of cases	IgG Mean $\pm$ SD	Coefficient of variation in percentage	Range
35-44	7	8.2 $\pm$ 4.5	54.8	2.4-17.0
45-54	11	5.4 $\pm$ 2.5	46.3	1.5-11.0
55-64	18	7.1 $\pm$ 3.2	45.0	2.4-15.0
65 and above	8	7.8 $\pm$ 5.2	66.6	1.5-17.6

Table 2 IgG level in mg/100 ml in the aqueous humour of cataract cases in different types of cataracts

Cataract type	No. of cases	IgG Mean $\pm$ SD	Coefficient of variation in percentage	Range
Early senile changes	14	6.5 $\pm$ 4.3	66.1	1.5-17.6
Moderately advanced senile changes	13	7.8 $\pm$ 5.2	66.6	1.5-17.0
Mature senile cataract	17	6.0 $\pm$ 3.1	51.6	2.4-16.0

in the human aqueous as determined by Schmut and Zirm (1974) were IgG 13.6 mg/100 ml, IgA 4.0 mg/100 ml, and IgM 3.9 mg/100 ml. They reported IgM in 40% of the cases. In our series of samples from cataractous eyes the only immunoglobulin that could be detected was IgG, the level being 7.0 mg/100 ml and range 1.5 to 17.6 mg/100 ml.

Zirm and Schmut (1973) found a significant increase in concentration of immunoglobulin during ageing but no obvious sex-related difference. Our data show that there is no significant difference in IgG level in aqueous in cataractous eyes in the different age groups studied and in various types of cataracts.

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