Immune complexes in retinitis pigmentosa

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SUMMARY In a group of 46 patients with retinitis pigmentosa (RP) we studied the presence of circulating immune complexes (CIC) and the alterations in the complement system. Our results showed the presence of CIC in 43.5% of the patients studied, reduced levels of the complement components C3 and C4 ($p < 0.001$), and of the haemolytic activity CH50 ($p < 0.001$) when compared with a control group consisting of a 100 healthy subjects. We found a statistically significant correlation between the values of C3 and CIC ($p < 0.01$), C4 and CIC ($p < 0.01$), and between CH50 and CIC ($p < 0.001$). These findings indicate that the CIC may play a role in the pathogenesis of primary retinitis pigmentosa.

There have been few studies to date on the possible presence of immunochemical alterations in retinitis pigmentosa (RP). In 1966 Fessel¹ described for the first time the presence of a rheumatoid factor and raised IgM in six out of 10 cases of RP. In 1973 Rahi² found raised levels of IgM in patients with RP, whereas Spalton *et al.*³ detected raised levels of this immunoglobulin in only five out of 17 subjects with RP and rheumatoid factor in only two out of 17 patients. The levels were normal for the remaining immunoglobulins tested as well as for the C3.

The existence of an immune response to the antigens of the pigment epithelium and to the rod outer segments⁴ could provoke an inflammatory reaction with retinal oedema in patients with degenerative processes of the retina determined genetically,³ where an increase in the vascular permeability in subjects with diverse genetic types of RP takes place.⁵

The increase in permeability and oedema in RP have led some authors³ to believe in the possible existence of circulating immune complexes (CIC). These authors have suggested the possible formation and deposit of these at a local level. The deposit of CIC in situ would produce an inflammation where the CIC would play a major role in provoking tissue injury.⁶ On the other hand there is some evidence that CIC play an important part in the pathogenesis of a wide variety of inflammatory autoimmune disorders,⁷ even having been detected in apparently healthy subjects.⁸

Although CIC in humans do not necessarily involve a pathogenic action they frequently reflect an alteration in the immune system. The adherence of CIC to mononuclear T and/or B cells influences the crucial cell to cell interaction which is necessary for the immune response⁹ and which has been found to be altered in subjects with RP.^{10,11}

All these considerations prompted us to carry out this study of the possible presence of CIC in 46 patients with RP in whom we also determined the C3 and C4 levels as well as the haemolytic units of complement (CH50).

Material and methods

Patients. We studied a group of 46 patients with RP, 29 males (63%) and 17 females (37%), aged between six and 51 years (average age 26 years). For the diagnosis of RP the following symptoms and signs were taken into account: loss of field of vision, arteriolar narrowing, pigment accumulation, poor adaptation to light and/or darkness, and abolished electroretinogram.

Controls. The control group consisted of 100 healthy subjects: 58 males (58%) and 42 females (42%) aged between 16 and 48 years (average age 25 years). The controls were free from ocular alterations and immunological abnormalities of whatever origin. They were subjected to the same immunological studies as the patient group with the exception that the determination of the possible presence of CIC and the values of these were carried out in only 70 of the 100 subjects.

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Study of the levels in mg/dl of the complement components C3 and C4. These were determined with an ICS Beckman nephelometer using antisera from Kallestad (Austin, Texas, USA). The main characteristics of this technique are described in Buffone.¹²

Determination of the number of the haemolytic units of complement (CH50). This modified version of Lachmann's technique, fully described in Weir,¹³ is based on the time that is necessary to obtain a 50% lysis using a known quantity of complement. Briefly, 15 µl of serum of the patient or control is mixed with 750 µl of complement fixing diluent (CFD) pre-warmed to 37°C, with the addition of 250 µl of 0.2% sheep red blood cells (EA). The mixture is aspirated into the cuvette of a spectrophotometer (Beckman 34C), which records a curve of haemolysis until 100% lysis is reached, followed at 600 nm of optical density (OD). A curve is then plotted with the use of normal human serum (NHS) as measured by the Mayer technique. We represented the units of CH50 in the abscissae and the time to obtain 50% haemolysis in the ordinates. After calculating the time of 50% haemolysis of a problem serum we can extrapolate the CH50 units of this serum.

Study of the presence of CIC. The technique described by Harkiss and Brown¹⁴ was used. 0.3 ml serum from patients or controls was added to 50 µl of borate buffer and to 50 µl of EDTA of 0.2 M. The solutions were mixed in PS-3 tubes (Aulabor, Barcelona, Spain). To these tubes 0.1 ml of a polyethylene glycol (PEG) solution of 12.5% was added before mixing and was left for 90 mins at 4°C. After centrifugation at 1700 g for 10 min the supernatants were removed, and the pellets were washed with 1 ml of 2.5% PEG solution. After further centrifugation the pellets were resuspended in 30 µl of warm CFD to which were added 15 µl of NHS (fresh or frozen at -70°C). At this stage two control tubes were introduced, 30 µl CFD at 37°C plus 15 µl of NHS. These were incubated for 30 min at 37°C and then deposited in an ice bath. The tubes were made up to

0.75 ml with CFD. Afterwards 0.25 ml of a suspension of 0.2% EA (at 37°C) was added to each sample, and the decrease in turbidity due to lysis of the red cells was followed at 600 nm OD using a Beckman 34C spectrophotometer with an automatic sample changer with cuvettes at 37°C to be monitored continuously. The CH50 units were determined for each sample and control. The results were initially expressed as residual CH50 units and subsequently as percentages of complement consumption (CC) with respect to 0% of the consumption of the control tubes.

$$\% \text{ CC} = 100 \times \left(1 - \frac{\text{Units CH50 test solution}}{\text{Units CH50 control}} \right).$$

Results

Table 1 shows the mean values and deviations in mg/dl of the complement components (SI conversion: mg/dl × 10 = mg/l). The C3 levels for the controls were 129.80 ± 23.51 mg/dl and for the group of 46 patients with RP 106.67 ± 21.63 mg/dl, showing a statistically significant difference (p < 0.001). The C4 levels were 29.93 ± 8.65 mg/dl for the control group and 22.09 ± 8.18 mg/dl for the patient group (p < 0.001). For the control group the CH50 units were 37.13 ± 7.38, whereas the patient group registered 29.61 ± 7.61 (p < 0.001). The study of the presence of CIC gave a mean and standard deviation of 8.10 ± 5.78 for the control group and 20.35 ± 14.55 (p < 0.001) for the patient group. If we consider the presence of CIC positive, where the percentage of CC is greater than the mean plus two standard deviations of the control group, we end up with 43.5% of patients with levels of CC greater than 20% (Fig. 1).

If the C3 values in mg/dl are compared with the CIC values (in CC%) in the patient group, a significant correlation may be observed between the diminution in C3 and the increase in CIC (r = -0.4185, p < 0.01, Fig. 2). Likewise, a significant correlation exists between the diminution in C4 and the increase in CIC (r = -0.407, p < 0.01, Fig. 3) and between the diminution in CH50 units and the increase in percentage of complement consumption (r = -0.536, p < 0.001, Fig. 4).

Discussion

It is generally accepted that the presence of CIC has a limited diagnostic value, and that this does not always characterise all immune complex disease.¹⁵ In order to speak of an immune complex disorder it is necessary to be able to associate the clinical activity of the disease with variations in the level of CIC, haemolytic activity of the complement, functional activity of the complement components, or the presence of raised values of the degradation products of the complement.⁷

Table 1 Means values and deviations of C3, C4, CH50, and CIC of controls (C) and patients (P) with RP

	C	P	
	n=100	n=46	
C3 in mg/dl	$\bar{x}$ = 129.80 SD = 23.51	$\bar{x}$ = 106.67 SD = 21.63	p < 0.001
C4 in mg/dl	$\bar{x}$ = 29.93 SD = 8.65	$\bar{x}$ = 22.09 SD = 8.18	p < 0.001
CH50 haemolytic units/ml	$\bar{x}$ = 37.13 SD = 7.38	$\bar{x}$ = 29.61 SD = 7.61	p < 0.001
CIC in % CC	n=70 $\bar{x}$ = 8.10 SD = 5.78	$\bar{x}$ = 20.35 SD = 14.55	p < 0.001

SI conversion: mg/dl × 10 = mg/l.

Fig. 1 Levels of immune complexes (complement consumption in percentages) of controls C and patients P with RP.

Since the CIC contain C1q, C1r, C1s, C3, and immunoglobulins it is difficult to demonstrate the presence and identify the antigen, and therefore we have to consider the possibility that at times the CIC

Fig. 2 Correlation between the values of C3 and CIC in the patient group.

Fig. 3 Correlation between the values of C4 and CIC in the patient group.

may result from the interaction of immunoglobulin molecules without antigen participation.¹⁶ Autoantibodies are directed against antigenic sites located in the immunoglobulin constant region.¹⁷ Other autoantibodies are directed against the idiotype determinants of the immunoglobulins¹⁸ (antigenic sites located in the hypervariable region of the immunoglobulins). The existence of these does not always give rise to a pathogenic action, since it is generally accepted that the repertoire of a person's antibodies comprises a network of idiotype and anti-idiotype antibodies in which the immunoglobulins function both as antibodies for some immunoglobulins and as antigens for other immunoglobulins.¹⁹ Therefore the antibodies (independent or as CIC) play a part in the immune homeostasis, where the CIC may have opposing roles, both enhancing and inhibiting the immune response.²⁰

Fig. 4 Correlation between the values of CH50 and CIC in the patient group.

From our results we can infer the existence of CIC in 43.48% of the patients with RP under study, and this may have a certain pathogenic significance in this disorder owing to the existence of a correlation (see 'Results') between the levels of these and the fall in the C3 and C4 complement components and CH50 haemolytic activity. Thus, these CIC activators of the complement could produce the release of anaphylotoxins (C3a and C5a) of the C3 and C5 complement components, leading to the release of histamine by mast cells and basophils, with the subsequent increase in the vascular permeability²¹ in the ocular structures,⁵ in which small particles of deaggregated CIC could diffuse on solubilisation after union with C3.²²

In conclusion, the affirmation of the presence of CIC in RP may have a certain prognostic value in the evolution of this process if, as in other diseases, it can be proved that the presence of CIC at the time of diagnosis leads to fewer remissions and a poorer prognosis than in those cases where they are not detected.²³

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