

# Immunological studies in retinitis pigmentosa associated with retinal vascular leakage

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**SUMMARY** Seventeen patients with retinitis pigmentosa and vascular leakage were studied for humoral immunological abnormalities. Apart from raised levels of IgM in 5 patients no other abnormalities were found.

Retinal vascular leakage in patients with retinitis pigmentosa (RP) is well recognised (Spalton *et al.*, 1978; fytche, 1972; Grizzerd *et al.*, 1978), and we have recently studied a number of these patients (Spalton *et al.*, 1978).

Increased vascular permeability was shown in patients with all genetic types of RP (Spalton *et al.*, 1978) and has been shown in several other genetically determined conditions such as dominant macular oedema (Deutman *et al.*, 1976), Goldman Favre syndrome (Fishman *et al.*, 1976), and Batten's disease (Spalton *et al.*, 1978).

Retinal arterial narrowing is a well recognised feature of RP. Work on rats with hereditary degeneration or iodoacetate visual cell destruction suggested that the consequent loss of retinal thickness exposed the retinal vessels to increased oxygen tension and subsequent vasoconstriction (Dantzker and Gerstein, 1969; Gerstein and Dantzker, 1969). This is followed by arterial and capillary closure and destruction of vascular endothelium (Ashton, 1977). These experiments may provide a homologue for genetically determined retinal degeneration in man, and a similar process could result in vascular leakage in RP. However, in our patients peripheral vascular leakage occurred mostly from major veins without detectable capillary closure, suggesting that this explanation is inadequate. Alternatively, oedema may be a non-immune response to toxic products liberated by degenerating retina or pigment epithelium. Finally, the oedema may be caused by inflammation. The patterns of leakage in RP are similar to those in aphakic macular oedema and posterior uveitis, in which inflammation is thought to be the basis of the oedema. Therefore, it was reasonable to seek evidence to support the concept

that an inflammatory response could cause retinal oedema in RP.

Retinal outer segments and pigment epithelium are known to be antigenic (Rahi, 1970; Wacker and Kalsow, 1974; Rahi *et al.*, 1974; Meyers, 1974; Reich D'Almeira and Rahi, 1974), and an immune response to these antigens may cause an inflammatory reaction and retinal oedema in patients with genetically determined retinal degeneration. We have screened a group of 17 patients who had RP and retinal vascular leakage for humoral immunological disturbances that might account for the increased vascular permeability.

## Methods

From a group of 17 patients with retinitis pigmentosa and retinal vascular leakage a single 10-ml specimen of blood was withdrawn prior to fluorescein angiography. This group of patients formed part of a larger series in which the inheritance and distribution of vascular leakage had been studied and reported on (Spalton *et al.*, 1978).

The serum was analysed for levels of IgG, IgA, and IgM by standard techniques of radial immunodiffusion using ICL plates. WHO standard sera provided reference curves.

The C<sub>3</sub> fraction of complement was measured by the standard Mancini technique, and C<sub>3</sub> breakdown products were sought by counter immunoelectrophoresis (Rocket technique) (Laurell, 1966).

Immunofluorescence tests were performed on composite-block frozen sections consisting of liver, kidney, stomach, diaphragm, and parotid from rat and the stomach of a mouse to detect antibodies to cell nuclei (and nucleoli), mitochondria, microsomes, smooth and skeletal muscle, and reticulin fibres. Frozen sections of rat eyes were used to detect

antibodies to photoreceptors and other retinal structures by immunofluorescent methods.

Haemagglutination tests for thyroglobulin and thyroid microsomal antibodies were performed using commercial preparations. IgM rheumatoid factor was measured using the Rheumaton (Wellcome) technique.

## Results

Of the 17 patients with RP the inheritance was autosomal dominant in 4 and X-linked in 3; 2 other patients had affected relatives, but there was not enough information to distinguish between dominant and X-linked inheritance. Of the 8 patients with no family history 2 were children with severe disease and were likely to have inherited it in an autosomal recessive manner. One of the patients with no family history had unioctular retinitis pigmentosa. All patients apart from 1 had leakage of fluorescein in the posterior pole; 7 patients had peripheral leakage of dye (see Table 1).

Rheumatoid factor was present in 2 patients; antinuclear antibody in 5 (Fig. 1); smooth muscle antibody in 6 (Fig. 2); gastric parietal cell antibody in 5 (Fig. 3); 6 patients had 2 or more antibodies present. The prevalence of these antibodies is slightly higher (especially smooth muscle antibody) than in a normal population (see Table 2). No

patients had antithyroid, antimicrosomal, anti-mitochondrial, skeletal, or reticulin fibre antibody.

The levels of  $C_3$  were within the normal range, and there was no evidence of complement breakdown products in the blood of any of the patients.

Levels of IgG were within the normal range in all patients, and 1 patient had a low IgA level. Five patients had levels of IgM above the normal upper limit (+2 SD).

## Discussion

It has been previously reported that about a quarter of patients with RP have raised levels of IgM

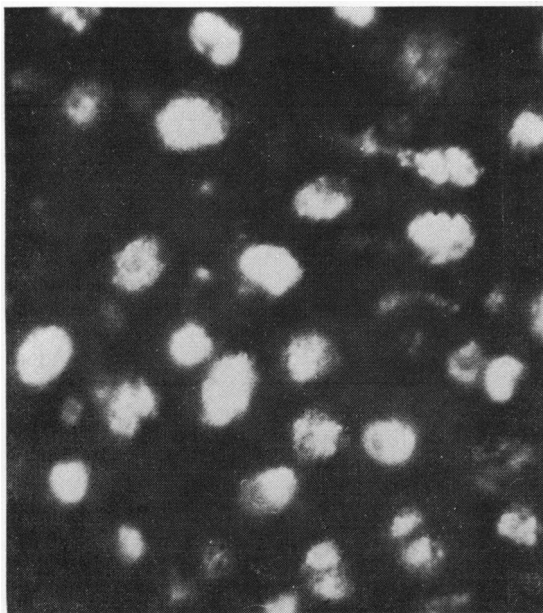


Fig. 1 Frozen section of rat liver showing nuclear fluorescence due to antinuclear antibody in patient's serum  $\times 640$



Fig. 2 Frozen section of rat stomach showing fluorescence of muscularis mucosa due to smooth muscle antibody in patient's serum  $\times 450$

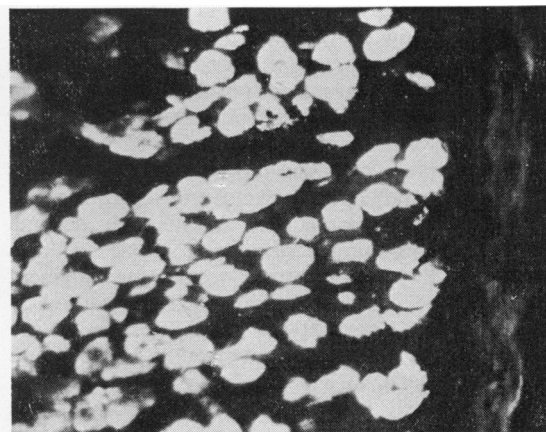


Fig. 3 Frozen section of mouse stomach showing bright fluorescence of gastric parietal cells due to specific antibody in patient's serum  $\times 450$

Table 1 Analysis of patients

Patient	Age	Sex	Genetic type of RP	Leakage Central	Peripheral	Immunoglobulins IU/ml			C <sub>3</sub> mg%	Titre of rheumatoid factor	Antinuclear antibody IU/ml	Gastric parietal cell antibody	Smooth muscle antibody
						IgG	IgA	IgM					
1	26	F	AD	+	-	148	135	235	118	—	—	—	1:10
2	21	F	AD	+	+	195	125	370	126	—	—	1:40	1:10
3	43	M	AD	+	+	130	92	200	133	1:10	16	—	—
4	49	M	AD	+	-	155	76	185	94	—	—	—	—
5	22	M	XL	+	-	125	120	230	138	—	—	—	1:40
6	23	M	XL	+	-	128	66	250	133	—	—	—	1:40
7	23	M	XL	+	-	180	140	190	94	—	—	—	—
8	10	F	AR	+	+	130	100	115	126	—	4	1:40	—
9	6	M	AR	+	+	140	150	210	110	—	—	—	—
10	25	F	S	-	+	105	37	280	111	—	—	—	—
11	27	M	S	+	-	165	145	210	103	1:10	—	—	—
12	29	F	S	+	+	175	152	310	132	—	—	—	1:40
13	20	F	S	+	-	160	200	470	136	—	4	1:10	—
14	21	F	S	+	-	205	141	508	160	—	4	1:10	1:10
15	44	F	AD/XL	+	-	165	110	440	185	—	4	1:10	—
16	55	M	AD/XL	+	+	190	300	290	130	—	—	—	—
17	21	M	S	+	-	110	130	125	—	—	—	—	—

AD—Autosomal dominant. XL—X-linked. AR—presumed autosomal recessive. S—sporadic

Table 2 Normal values from 100 healthy adults (Rahi et al., 1976; Davies et al., 1976)

IgG	mean	121	range	70–282	IU/ml
IgA	mean	125	range	64–300	IU/ml
IgM	mean	151	range	68–300	IU/ml
C <sub>3</sub>	mean	123	range	67–180	mg/100 ml
Autoantibodies present in healthy adults (age 20–50 years)					
Smooth muscle antibody present in 16% of healthy adults					
Antinuclear antibody present in 15% of healthy adults					
Rheumatoid factor present in 9% of healthy adults					
Gastric parietal cell present in 3% of healthy adults					
Antireticulin antibody present in 2% of healthy adults					

(Rahi, 1973), and our series would appear to confirm this since the level of IgM was raised in 5 of 17 patients. The significance or explanation of this result is unknown. It does not relate to the degree of vascular leakage, as only 3 of the 7 patients with peripheral leakage had raised levels, and the patients with raised IgM did not show excessive leakage. It is of interest that 2 of the patients had proved dominant inheritance, and all 5 patients were female. Increased levels of IgM have also been

found in patients with uveitis, especially if associated with vasculitis (Rahi et al., 1976).

Rheumatoid factor was present in only 2 patients, a smaller proportion than in some series (Fessel, 1962), and neither of these patients had raised levels of IgM. This discrepancy might be due to differences in technique. We have used a modified haemagglutination method which is known to be highly specific.

The proportions of patients who were found to have increased titres of antibodies, especially to smooth muscle but also to gastric parietal cells and nuclear antigens, appear to be higher than that found in a series of 100 normal adults (Rahi et al., 1976), but the numbers are small. It is unlikely that these antibodies would play an aetiological role, and they are probably formed in response to the release of antigenic proteins into the blood from damaged tissues. None of our patients was found to have antithyroid, antimitochondrial, antimicrosomal, skeletal muscle, or reticulin fibre antibody. Two twins were recently described with RP, endocrine problems, and high titres of thyroid antibodies (Nye and Howell Evans, 1977); it would appear

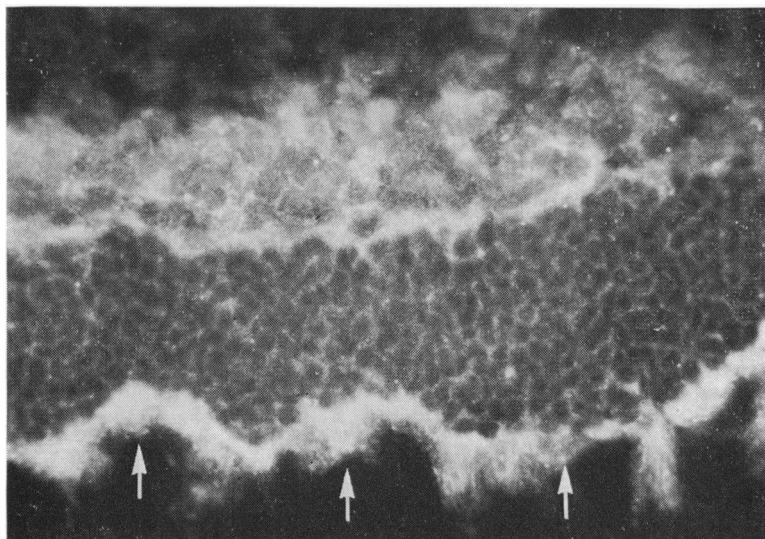


Fig. 4 Frozen section of rat retina treated with a 1 : 5 dilution of the patient's serum and fluorescein labelled anti-immunoglobulin G showing fluorescence of the photoreceptors (arrows)  $\times 380$

likely that the last would be more directly related to their endocrine problems than their RP.

We have attempted to show the presence of antibodies to rat photoreceptors in the serum of our patients by immunofluorescence, since it is known that rod outer segments are strongly antigenic and immunisation will induce uveoretinal lesions in guinea-pigs and rabbits (Wacker and Kalsow, 1974; Rahi *et al.*, 1974; Meyers, 1974). All our patients showed positive immunofluorescence to photoreceptors at 1:5 dilution of serum (Fig. 4), but a large proportion of sera from healthy adults contain low titres of smooth muscle antibody and also show similar fluorescence. It was not possible, therefore, to distinguish whether the immunofluorescence was due to photoreceptor antibody or to smooth muscle antibodies cross-reacting with contractile organelles in the photoreceptors. The absorption of smooth muscle antibody from the sera is technically difficult owing to its diverse nature.

Immune complexes are known to be associated with vasculitis in many diseases such as systemic lupus erythematosus, rheumatoid arthritis, and erythema nodosum. All our patients had normal levels of  $C_3$ . This is not surprising, as large amounts of complexes must be formed before the kinetics are disturbed enough to lower serum levels. It is more significant that we found no evidence of  $C_3$  breakdown products, a relatively more sensitive test for indicating the activation of  $C_3$ . Nevertheless, our results do not exclude the presence of very small amounts of immune complexes. Indeed, if these were being formed and deposited locally, circulating complexes might be absent and their presence detected only by tissue studies.

Apart from raised levels of IgM in 5 patients we found no immunological disturbance to account for the vascular leakage, although it is not completely possible to exclude immune complex disease without tissue specimens, and cell mediated immunity has not been studied. It is possible that a toxic metabolite of degenerating retina or of pigment epithelium might cause vascular leakage by disturbing vascular endothelium, since this phenomenon is seen to be common to a number of genetically determined retinal dystrophies. Certainly our results do not suggest a consistent humoral immunological basis.

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