Autoimmunity in hereditary retinal degeneration.

I. Basic studies

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SUMMARY One hundred and sixteen patients with retinitis pigmentosa (RP), 64 patients with other eye diseases, and 36 control subjects with no known eye disease were examined for antiretinal autoimmune activity. Sera were screened by indirect immunofluorescence on normal donor human eye sections to detect antibodies to human retinal antigens. Forty three of 116 RP patients (37%), 21 out of 64 non-RP patients with other eye diseases (33%), and 1 out of 42 controls (2%) had antibodies reacting with donor eye retinal antigens. Lymphocytes were tested by an *in vitro* transformation assay to detect cell mediated immunity to retinal antigens. Sixteen RP patients (19%), 11 non-RP patients (18%), and four controls (10%) showed lymphocyte sensitisation. Autoimmune responses were detected in many degenerative ocular disorders, but it is not known if they play a contributory pathogenic role.

Over the past 10 years various studies have examined the role of the immune system in retinitis pigmentosa (RP), a group of hereditary retinal degenerative disorders characterised by pigmentary retinopathy, progressive visual field loss, and nyctalopia. In 1973 Rahi reported that these patients had raised levels of serum IgM, though no specificity was assigned to the antibody.¹ Spiro *et al.* failed to confirm Rahi's findings but suggested that the discrepancy may have been from variations in methods, antisera, environmental influences, and composition of the RP groups used in the two studies.² Spalton *et al.* using immunofluorescence techniques showed the binding of sera from RP patients to rat photoreceptor cells.³ Keltner *et al.* recently reported one case of photoreceptor degeneration in a cancer patient who had serum antibodies reactive with normal retinal tissue on the immunofluorescent complement fixation test.⁴

A number of studies have been made to look at cell mediated immunity in retinal degenerations. Lymphocytes from 17 out of 23 patients with retinal degenerative disease were cytotoxic to retinoblastoma cells in tissue culture, which indicates immune reactivity against retinal antigens by these patients.⁵ Retinoblastoma cells have previously been shown to contain some antigens present on normal photoreceptor cells.⁶ Rocha and Antunes showed cell

mediated immunity in 34 out of 50 RP patients with the use of the migration inhibition factor test.⁷ Brinkman *et al.* using transformation and migration inhibition assays showed sensitisation to retinal antigens of lymphocytes from RP patients.⁸ A study by Heredia *et al.* showed sensitisation to retinal antigens in 26 out of 45 RP patients and presented evidence that lymphocytes from these patients also had a reduced response to phytohaemagglutinin *in vitro* and had diminished suppressor cell activity.⁹

While autoimmune activity has been well recorded in RP patients, it is not known whether it is specific to certain types of RP or how commonly it occurs in other types of retinal degeneration and ocular disease. To determine the extent and specificity of immune mechanisms active in a number of retinal degenerative disorders two tests of immune function were evaluated in blood samples from patients with RP, patients with non-RP ocular disease, and normal control subjects. The RP type was diagnosed as specifically as possible for each patient.

Materials and methods

Patients with progressive panretinal degenerations were evaluated with a comprehensive medical, ocular, and family history (pedigree analysis); ophthalmological examination; and tests consisting of Goldmann visual fields, electroretinogram, dark

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adaptation final rod threshold, fundus photography, and fluorescein angiography.

The patients with hereditary retinal degenerations were classified as precisely as possible by the above tests and examination results by means of previously published methods and rationale¹⁰⁻¹⁴ and placed in RP subgroups of: rod-cone and cone-rod degeneration by inheritance, choroideremia, preserved para-arteriole retinal pigment epithelium RP, pigmented paravenous retinochoroidal atrophy, and Usher syndrome.

Other retinal degenerations tested included congenital stationary nightblindness, Stickler's syndrome, luetic chorioretinitis, choroiditis, and sympathetic ophthalmia. These diseases were tested as an aid to understanding the specificity of the immunological responses. The control group consisted of volunteers with no known eye problems.

10 ml of peripheral venous blood was drawn into plain tubes for serum samples and 30 ml into heparinised tubes for isolation of lymphocytes. Blood was drawn from 116 RP patients, 64 non-RP patients with other eye disorders, and 42 normal volunteers without eye disorders who had given informed consent.

Isolation of serum and lymphocytes. Blood drawn for serum samples was allowed to clot and was centrifuged at 2000 rpm for 10 min. Then the serum was removed and stored at -20°C until testing.

To isolate lymphocytes blood was centrifuged at 2000 rpm for 10 min. The buffy coat was removed, diluted with an equal volume of RPMI 1640 (Flow Laboratories, Inglewood, California), and layered on to 5 ml cushions of Ficoll-paque (Pharmacia Fine Chemicals, Piscataway, New Jersey). After centrifugation at 2000 rpm for 20 min the layer of cells at the interface was recovered, washed three times in RPMI 1640, and stored in freezing medium (RPMI 1640, 10% DMSO, 10% glycerol, 10% sterile, heat inactivated human AB serum) at -70°C until testing.

Immunofluorescence. Serum samples were tested by the method of Sainte-Marie¹⁵ to detect circulating antibodies to retinal antigens. A positive control consisted of human retinal sections incubated with rabbit antibody to human retina. A negative control consisted of human retinal sections incubated with saline in place of serum in the first step. Direct staining of sections with fluorescein conjugated goat anti-human IgG and IgM (Meloy, Springfield, Virginia 22151) was carried out to determine that there were no immunoglobulins bound to the retina prior to incubation with sera. Sera from patients and controls were examined for the presence of antibodies by means of fluorescein conjugated goat antihuman IgG and IgM.

In vitro transformation assays. Lymphocytes were

tested by in vitro transformation assays with the use as antigens of crude human retinal extract (250, 100, and 50 $\mu\text{g/ml}$; 20 $\mu\text{l/well}$), and a bovine rod outer segment preparation (250, 100, and 50 $\mu\text{g/ml}$; 20 $\mu\text{l/well}$).¹⁶ Mitogens used to test functional viability of lymphocytes were concanavalin A (Miles-Yeda Ltd, Rehovot, Israel), 100 $\mu\text{g/ml}$; 20 $\mu\text{l/well}$, and phytohaemagglutinin (Difco Laboratories, Detroit, Michigan), 100 $\mu\text{g/ml}$; 20 $\mu\text{l/well}$. ($\mu\text{g/ml} = \text{mg/l}$.) The lymphocytes were incubated with the mitogens for three days or with antigens for six days at 37°C in a 5% CO_2 atmosphere. Tritiated thymidine (specific activity 25 mCi/mmol) 10 $\mu\text{Ci/well}$ (Amersham, Arlington Heights, Illinois) was added four hours prior to harvesting. Results are expressed as a stimulation index (SI) calculated as:

$$\text{SI} = \frac{\text{counts per minute in presence of antigen}}{\text{counts per minute in absence of antigen}}$$

This ratio allows for comparison of results from one assay to another. A stimulated index >2.0 was considered an indication of cell mediated activity against retinal antigens.⁸

The results of the immunofluorescence and sensitisation tests were analysed statistically for the three groups (RP patients, patients with non-RP eye problems, controls with no known eye problems) with the use of the χ^2 test and analysis of variance. Statistical review was done by Coralee Yale of the UCLA Department of Biostatistics.

Results

Table 1 summarises the results of indirect immunofluorescence testing of sera from patients and controls. Forty three of 116 RP patients (37%) showed antibodies to retinal antigens. The antibodies were not restricted to one particular type of retinitis pigmentosa but rather were seen in all types examined so far. Twenty one of 64 patients with non-RP eye disorders (33%) also developed antibodies to retinal antigens in their serum. One of the 42 normal controls (2%) had circulating antiretinal antibodies. The intensity of staining varied from weak to strong, but even weakly stained sections could be distinguished readily from those in which staining was absent (Figs. 1-3). Staining was seen most frequently in the rod outer segments, though sera from several patients also reacted with cell membranes either in the outer nuclear layer or, more often, throughout the entire retina.

Transformation assays. Results of in vitro transformation assays (Table 1) indicated sensitisation to retinal antigens in 16 out of 86 RP patients (19%), 11

out of 64 patients with other eye diseases (17%), and 4 out of 42 controls (10%). There was no difference in mitogen response between patients and controls.

Table 1 Patients with autoantibodies to retinal antigens and/or sensitisation to retinal antigens

Patient	Diagnosis	Immuno- fluorescence staining		Stimu- lation index
		IgG	IgM	
Retinitis pigmentosa (RP) type*				
1	Preserved para-arteriolar retinal pigment epithelium (PPRPE)	+	—	1.6
2	PPRPE	—	+	1.4
3	PPRPE	+	+	NT
4	PPRPE	—	+	1.4
5	Simplex rod-cone	+	+	2.7
6	Simplex rod-cone	—	—	2.2
7	Simplex rod-cone	+	+	2.5
8	Simplex rod-cone	—	—	2.3
9	Simplex rod-cone	+	—	2.7
10	Simplex rod-cone	+	—	1.4
11	Simplex rod-cone	—	+	1.7
12	Simplex rod-cone	+	+	NT
13	Simplex rod-cone	+	+	1.1
14	Simplex rod-cone	+	+	NT
15	Simplex rod-cone	+	—	2.1
16	Simplex rod-cone	—	—	2.1
17	Simplex rod-cone	+	+	0.3
18	Multiplex rod-cone	—	+	1.6
19	Simplex cone-rod	—	+	2.5
20	Simplex cone-rod	+	+	2.0
21	Simplex cone-rod	—	—	2.8
22	Simplex cone-rod	—	+	2.8
23	Autosomal recessive rod-cone	+	+	1.1
24	Autosomal recessive rod-cone	—	+	1.6
25	Autosomal recessive rod-cone	+	+	1.6
26	Autosomal recessive rod-cone	—	+	1.4
27	Autosomal recessive rod-cone	—	—	3.4
28	Autosomal recessive cone-rod	—	+	2.1
29	Autosomal dominant rod-cone	+	+	1.7
30	Autosomal dominant rod-cone	—	+	1.5
31	Autosomal dominant rod-cone	+	+	1.7
32	Autosomal dominant cone-rod	—	+	NT
33	X-linked recessive rod-cone	—	+	1.5
34	X-linked recessive rod-cone	+	+	1.1
35	X-linked recessive rod-cone	—	—	2.3
36	X-linked recessive rod-cone	—	+	1.5
37	X-linked recessive rod-cone	—	+	1.5
38	X-linked recessive cone-rod	+	—	1.1
39	Choroideremia	+	—	1.1
40	Choroideremia	+	—	2.4
41	Choroideremia	+	—	NT
42	Choroideremia	—	+	1.5
43	Choroideremia	+	—	2.4
44	Usher syndrome	+	—	1.1
45	Usher syndrome	—	+	1.6
46	Usher syndrome	—	—	3.8
47	Usher syndrome	—	+	1.6
48	Usher syndrome	—	+	1.5
49	Simplex cone-rod	—	+	1.5
50	Simplex cone-rod	—	+	1.2
51	Leber's amaurosis	—	+	1.4
52	Congenital RP with macular colobomata	+	+	NT

Nine of the RP patients showing sensitisation in the transformation assays also developed circulating antiretinal autoantibodies. Five of the non-RP

Table 1 Patients with autoantibodies to retinal antigens and/or sensitisation to retinal antigens—continued

Patient	Diagnosis	Immuno-fluorescence staining		Stimulation index
		IgG	IgM	
<i>Patients with non-RP ocular disease†</i>				
1	Choroiditis	+	+	1.4
2	Stickler syndrome	+	+	2.3
3	Measles retinopathy	+	—	1.0
4	Sympathetic ophthalmia	+	—	1.0
5	Regional RPE/choroidal atrophy	+	—	3.4
6	Luetic chorioretinitis	+	+	1.1
7	Luetic chorioretinitis	—	+	1.4
8	Congenital stationary night blindness	+	—	2.3
9	Macular oedema	+	—	0.4
10	Cystoid macular oedema	—	—	3.0
11	Macular hole	+	—	0.1
12	Macular degeneration	—	—	2.2
13	Macular degeneration	—	+	2.0
14	Cataract	+	+	1.5
15	Cataract	+	+	2.3
16	Cataract/retinal detachment	—	+	1.2
17	Glaucoma/macular degeneration	—	—	2.0
18	High myopia	—	+	1.1
19	High myopia	—	—	3.8
20	Detached retina	—	+	1.6
21	Retinal break	—	—	2.8
22	Toxoplasmosis	+	+	1.5
23	Toxoplasmosis	+	+	1.3
24	Fundus flavimaculatus	—	—	2.5
25	Diabetic retinopathy	+	—	1.3
26	Chorioretinitis	+	—	0.7
27	Uveitis	+	+	1.1
<i>Normal‡</i>				
1		—	+	2.1
2		—	—	3.0
3		—	—	3.3
4		—	—	2.2

NT = No cells available for testing.

*There were 64 patients with negative indirect immunofluorescence and stimulation index <2.0 with the following diagnoses: PPRPE (1), pigmented paravenous retinochoroidal atrophy (5), simplex rod-cone (15), multiplex rod-cone (1), simplex cone-rod (7), recessive rod-cone (9), dominant rod-cone (2), dominant cone-rod (1), X-linked rod-cone (7), choroideremia (4), Usher syndrome (8), congenital RP with macular colobomata (2), sector RP (1), Albers-Schönberg (1), RP/diabetes/renal failure (1).

†There were 37 patients with negative indirect immunofluorescence and stimulation index <2.0 with the following diagnoses: Congenital stationary night blindness (1), macular oedema (1), macular/retinal degeneration (9), cataract (2), retinal tear (1), fundus flavimaculatus (1), diabetic retinopathy (3), birdshot chorioretinitis (2), surface wrinkling retinopathy (1), dominant optic atrophy (1), vitreous degeneration (1), hydroxychloroquine toxicity (1), retinal vasculitis (4), carrier X-linked (1), chorioretinitis (3), panretinal degeneration (1), RPE degeneration (1), high myopia (1).

‡There were 38 normal volunteers with no indirect immunofluorescence or stimulation index >2.0.

patients and one control also showed both sensitisation and positive immunofluorescence. Results of both tests are summarised in Table 2.

Antibodies reactive with retinal antigens were present in significantly more RP and non-RP patients ($p = 0.001$) when each group was compared with the

Fig. 1 Indirect immunofluorescence on normal human retina showing IgM autoantibodies in serum from patient with PPRPE. Heavy staining of rod outer segments and a generalised lighter staining of outer nuclear cell membranes (arrows) can be seen ($\times 235$).

Fig. 2 Indirect immunofluorescence on normal human retina showing IgG autoantibodies in serum from patient with X-linked recessive cone-rod degeneration. Note strong staining (arrows) of cell membranes in outer nuclear layer ($\times 235$).

Fig. 3 Indirect immunofluorescence on normal human retina showing lack of staining with serum taken from normal control. Autofluorescence of the cones is evident in this section ($\times 235$).

Table 2 Summary of immune responses to retinal antigens

	RP patients*	Non-RP patients†	Normal controls
Autoantibodies	43/116 (37%)	21/64 (33%)	1/42 (2%)
Sensitisation	16/85 (19%)	11/64 (18%)	4/42 (10%)
Autoantibodies and sensitisation	9/86 (10%)	5/64 (8%)	1/42 (2%)

* RP = Retinitis pigmentosa.

† Non-RP = Eye disease other than retinitis pigmentosa.

control group, but the incidence of antibodies to retinal antigens in the two patient groups did not differ significantly ($p = 0.7622$). The transformation assay, which indicates sensitisation of the cell mediated arm of the immune response, was not significantly different in any of the three groups: RP versus non-RP $p = 0.8668$, RP versus normal $p = 0.3963$, non-RP versus normal $p = 0.4312$. Because of the small number of patients in each diagnostic category so far it was not possible to show significant differences between responses of patients with specific disease types with the use of either test of immunological function.

Discussion

Autoimmune mechanisms have been found to be

associated with many diseases involving degenerative processes (for example, inflammatory bowel disease, multiple sclerosis, thyroiditis, rheumatoid arthritis, autoimmune haemolytic anaemia). Even though they may not be the primary cause of a disease, they can aggravate a degenerative process by immunopathological mechanisms thereby causing the normally protective role of the immune system to turn into a destructive one.

Recent studies in RP evaluating immune responses to retinal antigens suggest the possibility that the immune system may contribute to the retinal degenerative process.^{1-3, 5-9} Our results from the screening of sera to detect antibodies to retinal antigens indicate that autoimmune mechanisms are active in many patients with ocular degenerative disorders. A statistically significant ($p < 0.001$) number of patients with both RP and non-RP eye disorders have circulating antibodies with activity directed towards retinal antigens as compared with normal controls. The transformation assays, which detect sensitised lymphocytes and indicate cellular rather than humoral mechanisms, showed no differences between patients and controls. If immune mechanisms do contribute to retinal ocular degeneration, it is more likely that they do so by the production of cytotoxic antibodies than by cell mediated mechanisms.

Though an analysis of variance was done to evaluate the various ocular degenerative disorders, no pattern has emerged as yet with respect to differences in immune reactivity in the various types of degeneration. The autoimmune response appears to be an associated response with all types of ocular degeneration. We did note, however, several staining patterns on indirect immunofluorescence, in which specific parts of the retina are selectively involved (rod outer segments only, membranes only, or both membranes and rod outer segments). Further studies looking at differential staining patterns may eventually correlate with some disease types.

Patients with pigmented paravenous retino-choroidal atrophy (PPRCA) were grouped arbitrarily with the RP subgroups, since all these patients had a pigmentary retinopathy, and some of them showed a progressive retinal degeneration. However, it should be noted that many PPRCA patients have stationary disease, and the PPRCA is a degenerative pattern in which the common finding in all cases is retinal paravenous degenerative disease.¹⁷ Evaluation of the immune status of the PPRCA group was believed to be particularly pertinent, since the pathogenic mechanism for the paravenous degeneration is not understood.

Evidence of autoimmune reactivity in response to retinal antigens was found in 21 out of 64 non-RP patients and four control subjects in our studies. It is not surprising that such reactions are taking place in eye disorders other than RP, since immune reactions are seen frequently in eye disorders (for example, uveitis, sympathetic ophthalmia). The statistically significant difference between patients and controls showing antiretinal activity suggests that the immune reaction in these patients may be related to their retinal degeneration. The immune reactivity in normal controls and patients with non-retinal ocular problems could reflect a cross reactivity between antigens in the retina and other tissues.

Our preliminary studies thus provide evidence of autoimmune activity in RP of all types as well as other ocular degenerative disorders. Whether these mechanisms play a pathological role clinically has not yet been shown. If autoimmune mechanisms are shown to play a pathogenic role in the degenerative process, and if criteria can be established for therapeutic immunosuppression without endangering the health of the patient, it may be possible to slow the degener-

ative process in selected patients who show strong autoimmune reactivity against retinal components.

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