Retinal S-Antigen in Human Subretinal Fluid

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Studies in experimental models of retinal detachment have proposed that the degree of visual recovery following retinal reattachment depends upon the extent of photoreceptor degeneration. A means of assessing this degeneration would help in establishing postoperative prognosis. S-antigen (S-Ag) is a unique retinal protein found in outer segment disc membranes and photoreceptor cells. In 36 cases of human rhegmatogenous retinal detachment, subretinal fluid (SRF) concentrations of S-Ag, measured by radioimmunoassay, ranged from 43 to 170 ng/ml (serum: 1–28 ng/ml). Analysis of variance showed a positive correlation with the duration of detachment ($P < 0.001$). There was a two-fold increase in S-Ag concentrations during the first 2 weeks of detachment ($P < 0.005$), with constant levels thereafter. These findings reflect progressive photoreceptor degeneration and/or ongoing synthesis of outer segment proteins in the detached retina that stop after the second week of detachment. SRF S-Ag levels may provide a prognostic indicator of visual recovery after reattachment as well as a sensitive measure of retinal metabolic activity during detachment. Invest Ophthalmol Vis Sci 28:2038–2041, 1987

Little is known of the effects of retinal detachment upon the physiology and metabolism of the neural retina. Clinical experience has shown, however, that full visual recovery after macular detachment is not often achieved.[1-3] Furthermore, the longer the macula remains detached, the poorer is the prognosis for visual recovery.[4-6] A recent morphologic study[7] in an animal model of retinal detachment showed that although the ultrastructural relationship between the photoreceptors and the retinal pigment epithelium (RPE) is re-established following surgical reattachment, there are abnormalities even after the shortest periods of detachment. The investigators concluded that if these changes occur in the macular region, they probably interfere with full visual recovery.

We have attempted to identify the types of metabolic changes that occur in the detached human retina by studying a marker specific for the retina. S-antigen is a protein that is known for its autoantigenic properties[8,9] and is found only in retinal photoreceptors and pineal cells.[10,11] Specific antibodies for S-antigen have been produced immunochemically,[11,12] thereby enabling quantitative analysis by radioimmunoassay (RIA). Our objectives were to determine whether S-antigen could be detected in the subretinal fluid (SRF) of humans with rhegmatogenous retinal detachment and whether S-antigen levels in the SRF correlated with various clinical data, particularly the duration of retinal detachment.

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Materials and Methods

Patient Population

Patients were screened and entered into the study in accordance with the guidelines for human research of the Department of Ophthalmology, Hotel-Dieu de Paris and the Centre de Recherche d'Ophtalmologie, Universite Pierre et Marie Curie, Paris, France. Thirty-six consecutive patients who underwent surgery for rhegmatogenous retinal detachment and who were willing to participate in the study served as the database. There were 27 men (mean age 52.3 years) and 9 women (mean age 54.2 years). Aphakia was present in 12 patients and high myopia in eight patients; two had both aphakia and high myopia. By carefully interrogating the patient for the onset of symptoms, an approximation of the time of occurrence of retinal detachment was established as previously described.[13] Because of the relatively imprecise nature of this estimation, intervals were established that reflect this approximation within reasonably accurate limits. Thus, the duration of detachment prior to surgery was classified as less than 4 days (patient symptoms began "two or three days ago"), 4–7 days (patient said "nearly a week ago"), 1 to 2 weeks, 2 to 4 weeks, or greater than 1 month

Table 1. Clinical characteristics and subretinal fluid S-Ag levels in the study population

Patient	*Age*	*Sex*	*Aphakia*	*High myopia*	*Cryopexy*	*Extent*	*Duration*	*S-Ag*
PG	29	M	−	+	3	4	4 d	47.0
ML	45	F	−	+	2	4	1–2 wk	170.0
MV	29	M	−	+	B	3	4–7 d	120.0
FD	82	M	+	−	1/2	3 1/2	1–2 wk	151.2
MT	59	M	+	−	B	2 1/2	1 mo	156.3
HD	53	F	−	+	1/2	2 1/2	4 d	43.0
DK	50	M	−	+	2	3	1 mo	133.7
AJ	61	M	+	−	1/2	2 1/2	4 d	97.6
SE	35	F	−	+	1	1 1/2	2–4 wk	106.9
RT	59	M	−	+	1/2	3 1/2	2–4 wk	126.9
PF	67	F	−	−	3	4	4 mo	122.0
PG	29	M	−	+	2	3	4 d	117.6
MT	59	M	+	−	B	2 1/2	1 mo	159.9
JV	69	M	+	−	2	3	4–7 d	98.0
UB	50	M	+	−	1/2	3 1/2	1 mo	117.7
LA	46	M	−	−	2	2	1 mo	102.5
AL	70	M	−	−	1/2	4	2–4 wk	125.5
MM	54	M	−	−	1/2	3	4–7 d	141.0
ME	33	F	+	−	1/2	1 1/2	4–7 d	77.6
PJ	54	F	+	−	B	3	1 mo	154.4
JS	58	M	−	−	1/2	2	1–2 wk	135.6
PG	72	M	−	−	2	2 1/2	1 mo	143.2
MN	57	F	−	−	2	1 1/2	4–7 d	115.8
JB	53	M	−	−	1/2	1 1/2	4–7 d	145.3
CD	71	F	−	−	1/2	1 1/2	1–2 wk	162.2
AB	49	M	+	−	2	3 1/2	1 mo	153.5
JM	41	M	−	−	2	3 1/2	1–2 wk	155.6
NM	53	M	−	−	2	2 1/2	2–4 wk	145.6
GM	51	F	−	−	2	2 1/2	1 mo	111.3
RD	74	M	+	+	1	3 1/2	4–7 d	117.9
RP	76	F	+	−	2	4	2–4 wk	108.2
FP	63	M	+	+	B	3	1 mo	112.1
FH	6	M	−	−	1	1	1 mo	131.1
TM	71	M	+	−	4	4	1 mo	141.4
MT	59	M	+	−	B	2	2–4 wk	119.7
CD	13	M	−	−	2	2 1/2	1 mo	112.2

M = Male, F = Female.

"+" Denotes the presence of and "−" the absence of aphakia or high myopia (−8 Diopters); the numbers in the "Cryopexy" column refer to the approximate numbers of quadrants treated with cryopexy at surgery; the numbers in the "Extent" column refer to the number of quadrants involved in the retinal detachment; in the "Duration" column: 4 d = less than 4 days; 4–7 d = 4 to 7 days; 1–2 wk = 1 to 2 weeks, 2–4 wk = 2 to 4 weeks, 1 mo = greater than 1 month (see text for details); S-Ag concentrations are expressed in ng/ml.

(patient said "a couple of months ago"). The extent of detachment (number of quadrants), the size of retinal break (in disc diameters), the presence of aphakia and high myopia (greater than −8D), and the amount of cryopexy applied during surgery (in number of quadrants) were documented. Venous blood was sampled at surgery for analysis of serum S-antigen levels.

Subretinal Fluid Acquisition

In all cases transcleral cryopexy was performed, a buckling element was positioned without scleral dissection, and subretinal fluid (SRF) was drained via a sclerotomy following diathermy application to the exposed choroid. A 21-gauge angiocatheter was positioned at the sclerotomy site, and a syringe was used to collect the draining SRF as has been previously described.[13] Specimens that were visibly contaminated with blood were discarded. Samples were centrifuged at 10,000 rpm for 10 min to remove any cellular elements, and the supernatant was stored at −20°C until analyzed.

Radioimmunoassay (RIA) For S-Antigen

Subretinal fluid samples and serum from each patient were independently analyzed by RIA to quantitate the levels of S-antigen. Liquid-phase RIA of SRF was performed by incubating 1/10 diluted SRF with rabbit anti-S-antigen antiserum and with ^{125}I-labelled hydroxyapatite-purified S-antigen (specific activity = 32 μCi/μg). The purified, unlabeled antigen was used as a standard. The free tracer was separated from the bound by using goat anti-rabbit serum as a second antibody. After centrifugation and washing, the radioactivity in the pellet containing the bound tracer was counted in a multidetector gamma counter.

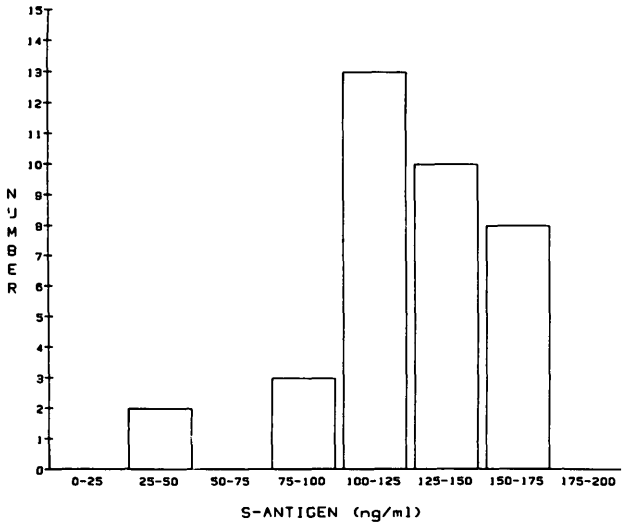

Fig. 1. S-antigen (S-Ag) levels in human subretinal fluid (SRF). This frequency distribution of S-Ag levels by 25 ng/ml intervals (abscissa) demonstrates a broad range of values with a peak at 100–125 ng/ml.

A plot of B/Bo (B = activity of the bound tracer, and Bo = activity of the tracer in the absence of unlabeled antigen) versus log antigen concentration gives a standard curve allowing determination of the amount of S-antigen expressed as nanograms per milliliter of SRF.

Statistical Analysis

Correlations were performed between the subretinal fluid (SRF) concentrations of S-antigen (S-Ag) and the duration of retinal detachment by one-way analysis of variance.[14] The significance of the F-value obtained by this test was evaluated using standard statistical tables. The significance of the difference in SRF S-Ag levels at the first and third time point in this study were then evaluated using the non-paired, two-tailed parametric t-test.

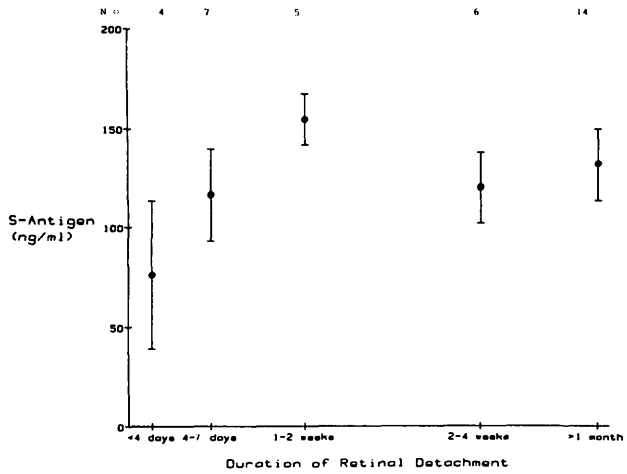

Fig. 2. Correlation of subretinal fluid (SRF) S-antigen levels (ordinate) with the approximate duration of retinal detachment (abscissa). There was a significant increase in SRF S-antigen levels during the first 2 weeks ($P = 0.003$), which thereafter reached a plateau at about 130 ng/ml. Bars represent the standard deviation about the mean. The number of samples (n) for each mean is indicated at the top.

Results

S-antigen was detected in all 36 SRF samples (Table 1); the concentration ranged from 43 to 170 ng/ml. Most samples had levels greater than 100 ng/ml, and there was a peak at 100–125 ng/ml (Fig. 1). Figure 2 illustrates the relationship between SRF levels of S-antigen and the duration of retinal detachment. The one-way analysis of variance for this data had an F value = 8.385. This F power test result was significant at the $P < 0.001$ level. As can be seen in Figure 2, S-Ag levels doubled during the first 2 weeks of detachment, an increase that was statistically significant ($P < 0.005$). At 2–4 weeks and at >1 month of detachment, the S-antigen levels remained stable at about 130 ng/ml. Serum levels of S-antigen ranged from 1 to 28 ng/ml and did not correlate with the SRF levels.

There was no correlation between S-antigen levels and the amount of cryopexy applied prior to SRF drainage ($r = 0.09$, $P = 0.62$). The extent of retinal detachment in terms of the number of quadrants involved did not correlate with SRF S-antigen levels ($r = 0.05$, $P = 0.73$), confirming the results of a previous study.[13] There was no correlation with the size of the retinal break and no significant differences in SRF S-antigen levels of aphakes and high myopes, as compared with other patients.

Discussion

This study demonstrates for the first time the presence of retinal S-Ag in the sub-retinal fluid (SRF) of patients with rhegmatogenous retinal detachment. SRF levels of S-Ag increased significantly during the first 2 weeks of detachment and remained stable thereafter. It is interesting that a study[13] of opsin in human subretinal fluid found decreasing concentrations with increasing duration of retinal detachment. However, only 16 cases were studied in that investigation and only two of these cases had a duration of detachment of 2 weeks or less. Thus, the dynamics of SRF protein accumulation during the acute phase of retinal detachment could not be adequately evaluated by that study.

Morphologic studies[15] have shown that in retinal detachment there is loss of photoreceptor outer segments in the retina and the appearance of these

structures in the SRF. Studies[16] of SRF protein kinase activities found a plateau at about 2 weeks of detachment duration, leading these investigators to conclude that 2 weeks after detachment, there is no further substantial degradation of rod outer segments. Thus, both S-Ag and protein kinase accumulation in the SRF could result from ongoing outer retinal degeneration that ceases after the second week of detachment. Phospholipase[17] and acid phosphatase[18] activities in SRF have been implicated in photoreceptor degradation during retinal detachment and could be responsible for the increasing concentration of retinal S-Ag in SRF.

Alternatively, ongoing synthesis of photoreceptor discs without pigment epithelium phagocytosis and consequent shedding of discs into the subretinal space could contribute to the increasing SRF S-antigen levels observed during the first two weeks of detachment. This hypothesis is supported by studies[19] demonstrating that the retina can continue to synthesize protein under a variety of conditions. Cessation of protein synthesis after 2 weeks may result from the cumulative noxious effects of separating the outer retina from its primary source of oxygen and nutrients, the choriocapillaris and RPE. This would result in stabilization of the SRF S-antigen level, as observed in this study.

It is quite possible that both of these hypothetical mechanisms contribute to the presence and accumulation of S-antigen in SRF, but to varying degrees at different times following detachment. Studies in an animal model using the incorporation of radioactive precursors of S-antigen would elucidate the relative contributions of these two mechanisms. Such an experimental model would also afford the opportunity to precisely define the relationship between SRF S-Ag levels and the duration of retinal detachment. The specificity of S-antigen as a product of photoreceptor metabolism renders this protein a useful probe for the study of retinal metabolism during retinal detachment and in other disorders.

Key words: photoreceptors, retinal detachment, S-antigen, subretinal fluid

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