

Hypersensitivity towards Alpha-Crystalline in the Heterochromia Syndrome

Helga Hammer and M. Olah

Department of Ophthalmology (Head: Prof. Á. Kahán),
University Medical School, Szeged

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Summary. In 4 cases of heterochromic cyclitis with cataract, 5 cases of herpetic iridocyclitis, and 5 cases of senile cataract, hypersensitivity to both alpha-crystalline and uveal pigment was investigated by lymphocyte-transformation and leukocyte-migration tests. In 3 cases of heterochromic cyclitis with cataracts, cellular hypersensitivity to alpha-crystalline was proven. Basing on these findings, the role of hypersensitivity to lens proteins in the etiology of heterochromic cyclitis is suggested.

Zusammenfassung. Mit Hilfe des Lymphocyten-Transformations- und des Leukocyten-Migrations-Testes wurden vier Patienten mit durch Cataract komplizierter Heterochromie-cyclitis, fünf Patienten mit herpetischer Iridocyclitis und fünf Patienten mit Cataracta senilis auf eine Überempfindlichkeit gegenüber alpha-Crystallin sowie Uveapigment geprüft. Im Laufe der Untersuchungen konnte bei drei Patienten mit durch Cataract komplizierter Heterochromiecyclitis eine Überempfindlichkeit cellulären Typs gegenüber alpha-Crystallin nachgewiesen werden. Aufgrund dieser Ergebnisse ist anzunehmen, daß bei der Entstehung der Heterochromiecyclitis eine Autosensibilisierung gegenüber Linseneiweiß eine ätiologische Rolle spielen kann.

Heterochromia of the iris with signs of chronic cyclitis was first described in 1906 by Fuchs, bearing his eponyme even nowadays [1]. At first glance this frequently unilateral disease is manifesting in a lighter-coloured, mostly bluish iris of the afflicted eye, and keratic precipitates are revealed by slitlamp examination with an atrophy of the peripheral parts of the iris according to the area of the dilator muscle fibers resulting in attenuation of the stroma and of the pigment layer. This decay of normal architecture is causing itself heterochromia of the iris. In the chamber angle, neovascularisation and tissue deposits are seen, which may induce glaucoma by obstructing the trabecular meshwork. Often the lens is also involved in the disease process leading to cataract, which is ascribed to nutritive damage. Histologic changes consist of infiltration of the iris by lymphocytes, plasma cells, and eosinophils, and of hyalinic degeneration of its arterioles. Until now the etiology of Fuchs's syndrome has been unclear. Earlier in this century damage to the melanocytes [2], later a defect in sympathetic innervation [3, 4], and more recently the allergic or toxic nature of the lesion [5] were supposed. Parkins and Wood demonstrated anti-lens antibodies by complement fixation test in patients suffering from heterochromic uveitis [6].

In the present work, hypersensitivity, both towards uveal and lenticular antigens, was investigated in patients suffering from heterochromic cyclitis with cataract. Lymphocyte-transformation and leukocyte-migration tests, widely used to verify the antigen involved, were performed simultaneously. In the former, the

Table 1. Clinical data of cases investigated

Case number	Sex	Diagnosis	Heterochromia	Cyclitis	Cataract	I.O.P. (Hg mm)
1.	♀	Heterochromic cyclitis with cataract	+	+	+	22
2.	♀	Heterochromic cyclitis with cataract	+	+	+	20
3.	♀	Heterochromic cyclitis with cataract	+	+	+	20
4.	♂	Heterochromic cyclitis with cataract	+	+	+	18.5
5.	♀	Herpetic uveitis	—	+	—	26
6.	♀	Herpetic uveitis	—	+	—	18.5
7.	♀	Herpetic uveitis	—	+	—	20
8.	♂	Herpetic uveitis	—	+	+	24
9.	♂	Herpetic uveitis	—	+	—	17
10.	♀	Senile cataract	—	—	+	22
11.	♀	Senile cataract	—	—	+	18.5
12.	♀	Senile cataract	—	—	+	26
13.	♂	Senile cataract	—	—	+	17
14.	♂	Senile cataract	—	—	+	20

small lymphocytes of the hypersensitive patient cultured in the presence of the respective antigen were transformed into lymphoblasts. In the leukocyte-migration test, the white blood cells of the hypersensitive patient drawn into a capillary migrate out from the latter under secured conditions. This migration is inhibited, however, by the presence of the antigen of the delayed-type hypersensitivity. By these tests applied in ophthalmology, hypersensitivity to uveal antigens in sympathetic ophthalmitis, Vogt-Koyanagi-Harada disease and some other types of uveitis [7-11], while in lens-induced uveitis, hypersensitivity to alpha-crystalline [12] was demonstrated.

Methods

The investigated cases were 4 patients suffering from heterochromia, cyclitis, and cataract, and as controls 5 cases of senile cataract, and 5 cases of herpetic uveitis. Their main clinical data are summarized in Table 1.

Antigens. Alpha-crystalline prepared from bovine lenses [13] and bovine uveal pigment [14], and as aspecific mitogen phytohaemagglutinine M (DIFCO) were used.

The lymphocyte-transformation test was performed as described previously [7]. Leukocytes were separated from the erythrocytes by sedimentation in venous blood with drawn with citrate, and 10^6 /ml lymphocytes were cultured in 10% pooled AB-Rh-positive plasma and each of 100 μ g/ml penicillin and streptomycin containing Parker 199 medium. From each case 4 different cultures were made: the first one containing 40 μ g/ml alpha-crystalline, the second 2 μ g/ml uveal pigment; the third was stimulated by 100-fold diluted phytohaemagglutinine; the fourth served as control, the antigens being applied in concentrations for optimal stimulation as determined in our previous studies [7, 12]. The rate of blastic transformation was measured by the increase of DNA synthesis indicated by the augmented 3 H-thymidine incorporation from the medium. The result was considered positive if the incorporation of 3 H-thymidine increased at least to twofold in the respective antigen-containing culture as compared to that without antigen.

Table 2. Results of lymphocyte-transformation and leukocyte-migration tests

Case number	Diagnosis	Transformation index			Migration index	
		due to Alpha-crystalline	Uvea pigment	PHA	due to Alpha-crystalline	Uvea pigment
1.	Heterochromic cyclitis with cataract	4.3	1.2	48.6	0.71	0.96
2.	Heterochromic cyclitis with cataract	3.7	1.1	71.3	0.63	1.07
3.	Heterochromic cyclitis with cataract	1.4	1.1	50.7	0.93	0.98
4.	Heterochromic cyclitis with cataract	5.2	0.9	28.9	0.76	0.95
5.	Herpetic uveitis	1.3	1.4	33.0	0.92	1.14
6.	Herpetic uveitis	0.8	1.0	46.1	1.09	1.02
7.	Herpetic uveitis	1.2	0.9	57.3	0.97	0.93
8.	Herpetic uveitis	1.1	0.9	52.4	1.00	0.95
9.	Herpetic uveitis	0.9	1.3	62.9	0.96	0.87
10.	Senile cataract	1.0	1.2	42.2	1.08	1.04
11.	Senile cataract	1.0	0.8	24.5	0.91	0.94
12.	Senile cataract	0.8	0.9	43.5	1.05	0.98
13.	Senile cataract	1.4	1.3	39.0	1.03	1.08
14.	Senile cataract	1.1	0.9	57.8	0.90	1.07

PHA = phytohaemagglutinine.

The leukocyte-migration test was performed according to the method of Søborg and Bendixen [15], slightly modified. The leukocytes were drawn into a capillary, fixed to the bottom of a glass container, and covered with Parker 199 medium. After being cultured for 24 hs under these conditions, the motile cells migrated out from the capillary. Three different cultures were made from every case: the first contained 40 $\mu\text{g/ml}$ alpha-crystalline, the second 2 $\mu\text{g/ml}$ uveal pigment; the third served as control. When the culture period was over, the cluster of the emigrated cells was projected onto a screen using a projecting microscope; its boundary was drawn by pencil and its area determined by planimetry. The rate of inhibition decreasing emigration was expressed by the migration index, the ratio of the area immigrated by the cells from the culture containing the antigen and of that without any antigen. This index was considered positive below the value of 0.85.

Results

The results of both the lymphocyte-transformation and leukocyte-migration tests performed with the cells of patients suffering from heterochromic cyclitis and cataract are juxtaposed in Table 2 with those having herpetic cyclitis or senile cataract (control cases) in which different antigens were used.

In the transformation test, the presence of alpha-crystalline incited a more than twofold increase of ^3H -thymidine incorporation into the lymphocytes of three patients belonging to the heterochromic group, whereas in those of the fourth of this group and of all control cases it remained unaffected. The fourth heterochromic patient with lymphocytes not exhibiting blastogenic transformation in the presence of alpha-crystalline differed neither in clinical signs or history from

the three reacting cases. Even in this fourth case, extraction of the cataract, general and local glucocorticosteroids and mydriasis resulted in complete recovery as it did in the remaining three heterochromic patients; and even this fourth case is free of any signs of cyclitis after one year without treatment.

The effect of uveal pigment did not differ in the heterochromic and control cases, nor did those of phytohaemagglutinine differ. The latter stimulant incited a more than twentyfold increase of ^3H -thymidine incorporation in every case, whereas uveal pigment did not evoke blastic transformation in the lymphocytes of the cases.

As evident from Table 2, results of the lymphocyte-transformation test and those of the leukocyte-migration procedure were in full accord with each other. Leukocyte migration was inhibited by alpha-crystalline in the same three heterochromic patients the lymphocytes of which underwent blastic transformation when excited by the same antigen. Conversely, with the leukocytes of the fourth heterochromic patient having unresponsive lymphocytes to the mitogenic stimulation by alpha-crystalline, as well as with those of the controls, migration indices above 0.85 were found. In all the investigated cases, uveal pigment did not inhibit the migration of leukocytes whatsoever.

Discussion

Lens proteins differ in regard to their chemical structure, electrophoretic, and immunologic behaviour. According to the investigations of Burky, Woods and Woodhall [16], alpha-crystalline is the main organ-specific protein able to sensitize animals by adequate administration. Even in humans its delivery from the lens consequent to injuries, extracapsular extractions of cataracts and across the capsular dehiscences of hypermature cataracts may induce the formation of anti-lens antibodies [17-20].

In normal conditions alpha-crystalline is to be found only in the lens, however, among the uveal antigens some have been demonstrated to cross-react with lenticular proteins [21-22]. According to the investigations of Sandberg, Fölling and Class [23] performed with radioimmune assay, the concentration of alpha-crystalline is less than $0.006 \mu\text{g/ml}$ in the aqueous of patients having cataract, however, they found more than $0.01 \mu\text{g/ml}$ alpha-crystalline in the aqueous of two patients with heterochromia and cataracts.

Delivery of crystallines from the lens into the eye elicits lens-induced uveitis manifesting in an inflammation of the uveal tract of non-infective origin, its etiology being the hypersensitivity to crystallines. This assumption is in full accord with the clinical course: in contrast to the doubtful outcome of other uveitic cataract extractions, the favourable prognostics of removing heterochromic cataracts have been fully realized for a long time when: the source of antigen is removed, cyclitis must disappear. Beyond the clinical argument, this assumption is verified by finding in the sera and aqueous of the patients antibodies that react specifically with crystallines. Hypersensitivity to alpha-crystalline was demonstrated by lymphocyte-transformation and leukocyte-migration tests in lens-induced uveitis [12].

In the course of the present investigations, in three out of four cases of heterochromic cyclitis with cataracts, alpha-crystalline proved to incite blastic transformation in lymphocyte cultures, and to inhibit the migration of leukocytes, demonstrating in these patients the cellular hypersensitivity to alpha-crystalline. On the other hand, none of these cellular reactions were brought about by uveal pigment.

Based on the present findings, and on the observations of Sandberg, Følling and Class [23], heterochromic cyclitis with cataract, or at least one part of these cases is suggested to be due to a prolonged rise of a yet unknown cause of the alpha-crystalline concentration in the aqueous which induces auto-immune reactions. The triggered auto-aggressive reactions may occur not only with alpha-crystalline, they may interact with uveal antigens cross-reacting with crystallines and induce iridocyclitis thereby. Heterochromia, the rise in I. O. P., and even the cataract may be secondary to the inflammation of the uveal tract; moreover, in heterochromia cataract may be due to the hypersensitivity towards crystallines too.

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Helga Hammer, M.D.
Department of Ophthalmology
University Medical School
P.O. Box 407
H-6701 Szeged, Hungary