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Immunological Studies in Patients Suffering from Sympathetic Ophthalmitis

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Abstract. In nine sympathetic ophthalmitis patients, the serum IgG, IgA, and IgM concentrations were found to be in the normal range, as were the proportions of active and total T-lymphocytes, the proportions of C3-binding and mouse erythrocyte-binding receptor-bearing B lymphocytes, the lymphocyte-stimulating effects of phytohemagglutinin and concanavalin A, the leukocyte migration-inhibiting effect of the purified protein derivative of tuberculin, and the suppressor activity of the periphearal blood mononuclear cells. The β_{1C} (C3 component of complement) level was increased in every patient. These results indicate that sympathetic ophthalmitis may also develop without damage to the immune system.

Introduction

In vitro lymphocyte functional assays have for many years been one of the major tools in the study of the mechanisms of immunity. Considerable progress has been made toward an understanding of the pathophysiology of immune-mediated diseases by the use of these assays.

Sympathetic ophthalmitis (SO) is a bilateral granulomatous uveitis of unknown etiology, believed to represent a form of autosensitization of ocular tissues following a penetrating injury to one eye. Circulating antibodies against uveal pigment (Mills and Shedden 1965; Aronson et al. 1964), delayed type hypersensitivity processes against this antigen (McPherson and Woods 1948; Kahán et al. 1964; Hammer 1971, 1974), and the lymphocyte-stimulating activity of the soluble uveal-retinal antigen (Wong et al. 1971) and of the homologous retinal pigment (Marak et al. 1971) have all been demonstrated in SO.

In order to acquire a better understanding of the immunological pathogenesis of SO, in the present work we have investigated the following: the proportions of the circulating T-lymphocytes (E-rosette-forming) and B-lymphocytes (mouse erythrocyte rosette-forming and C3-receptor-bearing); the serum concentrations of IgG, IgA, IgM, and β_{1C} (C3 component of complement); the lymphocyte-stimulating effects of phytohemagglutinin (PHA) and concanavalin A (con A); the leukocyte migration-inhibiting effect of the purified protein derivative (PPD) of tuberculin; the suppressor activity of the peripheral blood mononuclear cells (PBMC).

Methods

Patients. Nine patients were examined, and the SO diagnosis was based on the perforating eye injury featured in the case history, the characteristic clinical picture, the hypersensitivity to uveal pigment, and (in five of nine cases) histological examination of the enucleated eye.

Quantitative Estimations of IgG, IgA, IgM, and β_{1C} Globulin. These estimations were performed using inverse radial immunodiffusion techniques (Berkó and Husz 1976).

Peripheral Blood Mononuclear Cells. These cells were isolated from venous blood containing sodium citrate by density gradient centrifugation, as described by Böyum (1968). The cell suspension contained 91%–97% of living cells that were not stained by trypan blue.

Membrane Markers. The frequencies of total T- and active T-lymphocytes were determined as E-rosette-forming cells with conventional rosette techniques (Kerman and Geis 1976). The B-lymphocytes were detected as mouse erythrocyte-rosette-forming (MERF) lymphocytes (Dobozy et al. 1976) and as C3-receptor-bearing cells (Huber and Wigzell 1975).

Assessment of PHA or con-A Stimulation. For the assessment 10^6 PBMC per ml was added to 2.5 ml 10% AB Rh-positive human plasma containing TC-199 medium. The cell suspensions were cultured for 3 days at 37° C. The cultures were stimulated with 5 µg/ml PHA M (Difco) or 30 µg/ml con A (Serva) and two cultures served as controls. The extent of the stimulating effect was measured by means of ^{3}H-thymidine incorporation. The amount of thymidine incorporated into the DNA was detected with a liquid scintillation method. The stimulating effect was expressed as the difference in cpm/10^6 lymphocytes for the mitogen-containing cultures and control cultures.

The Leukocyte Migration Inhibitory Effect of PPD. This effect was determined as described earlier (Hammer 1974). Separated leukocytes were withdrawn into a capillary tube. The migration of cells was assessed by planimetry after culturing for 24 h. A migration index (the quotient of the migration data for antigen-containing and media control cultures) was used to express the inhibitory effect of 1 µg/ml PPD.

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Assessment of Suppressor Activity of PBMC. The suppressor activity of PBMC was estimated by ^{3}H-thymidine incorporation (Breshnihan and Jasin 1977). Briefly, the washed PBMC were resuspended in TC-199 medium supplemented with L-glutamine and 10% heat-inactivated fetal calf serum. Test cultures were set up in triplicate, each tube containing 3×10^6 cells in 3 ml medium. The lymphocytes were stimulated with 1 µg/ml con A, either at the initiation of the culture or after 24 h of incubation at 37° C. The incorporation of ^{3}H-thymidine was assayed by adding 1 µCi/ml ^{3}H-thymidine (1 Ci/mmol) to the cultures for a 20-h period, 76 h after the addition of con A. The radioactivity was measured in a scintillation counter. The amount of ^{3}H-thymidine incorporated into the cells was expressed as total cpm/10^6 lymphocytes.

The suppressor index (SI) was calculated via the formula:

$$SI = \frac{\text{cpm}/10^6 \text{ lymphocytes of 24-h preincubated ultures}}{\text{cpm}/10^6 \text{ lymphocytes of cultures stimulated at 0 h}}$$

Baselines of unstimulated cultures were subtracted from the counts for the stimulated cultures.

Statistics. The significance of the differences was calculated with Student's *t*-test.

Results

The serum IgG, IgA, and IgM concentrations for the SO. patients and healthy individuals were almost the same (Table 1). Only in a single case (patient 2) was a pathologically low IgA serum concentration found. In contrast, in every case the β_{1C} content of the serum exceeded the upper limit of the normal range and was significantly higher than for the healthy controls.

On examination of the lymphocyte surface markers in SO, the numbers of active and total T-cells and the proportions of mouse erythrocyte-binding and C3-binding receptor-bearing B-cells were within the normal range in all cases (Table 2).

No pathological changes were found either when a functional study of the T-lymphocytes was made in SO (Table 3). The lymphocyte-stimulating effects of PHA and con A, the leukocyte migration-inhibiting effect of PPD, and the suppressor activity of the PBMC were the same as in the healthy individuals.

Table 1. Serum immunoglobulin levels (g/l)

Patients	IgG	IgA	IgM	β_{1C}
SO1	14.26	2.15	0.644	1.745
2	11.09	0.78	1.12	1.56
3	13.00	2.28	0.545	1.46
4	10.05	2.63	1.21	1.81
5	9.25	2.76	0.35	1.74
6	8.94	3.45	1.60	2.04
7	15.00	2.24	0.87	1.65
8	11.80	1.75	1.24	1.52
9	12.46	2.95	1.16	1.63
Mean ± SD	11.76 ± 2.13	2.33 ± 0.77	0.97 ± 0.40	1.68 ± 0.18
Normal value	12.90 ± 2.79	2.78 ± 1.22	1.26 ± 0.97	0.85 ± 0.20
	$n=97$	$n=96$	$n=96$	$n=81$
	$P>0.05$	$P>0.05$	$P>0.05$	$P<0.001$

Table 2. T- and B-lymphocyte markers

Patients	T-cell	Active T-cell	MERF cell	Cells with C3 receptor
SO1	57	27	12	17
2	58	34	21	22
3	67	30	17	20
4	63	29	15	15
5	74	26	13	19
6	67	28	16	21
7	71	25	18	14
8	63	32	15	17
9	72	29	19	16
Mean ± SD	65.8 ± 6.0	28.9 ± 2.95	16.2 ± 2.9	17.9 ± 2.8
Normal value	68.2 ± 4.7	30.5 ± 5.1	16.3 ± 4.1	17.6 ± 5.3
	$n=70$	$n=31$	$n=126$	$n=28$
	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

Table 3. Stimulating effects of phytohemagglutinin and concanavalin A. leukocyte migration inhibitory effect of purified protein derivative of tuberculin, and suppressor activity of peripheral blood mononuclear cells

Patients	Stimulating effect of		Migration index	Suppressor index
	PHA	con A		
SO1	15,890	5,800	0.81	4.1
2	42,180	4,720	0.87	5.3
3	20,260	6,340	0.63	2.9
4	32,270	3,940	0.73	3.1
5	21,230	5,210	0.59	3.5
6	N.I.[a]	4,860	0.69	4.7
7	30,300	7,320	0.94	2.8
8	28,110	4,240	0.70	3.4
9	27,280	5,040	0.78	4.0
Mean ± SD	27,190 ± 8,213	5,274 ± 1,059	0.75 ± 0.11	3.76 ± 0.85
Normal value	31,740 ± 8,465	5,740 ± 872	0.71 ± 0.09	3.89 ± 1.76
	$n=42$	$n=30$	$n=30$	$n=43$
	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

[a] N.I.: not investigated

Discussion

The importance of the immunologic mechanisms in the etiology of human uveitis has been supported by experimental models illustrating the alterations in immune response in experimental allergic uveitis. In the present study, the humoral and cellular immunity were tested to assess the immunologic status of patients with SO. Examination of the serum IgG, IgA, and IgM concentrations did not reveal any characteristic differences. This result did not confirm the hypothesis of Aronson (1968) that IgM plays a central role in uveitis, but it does conform the observations of Norn (1976), who did not find typical changes in the serum immunoglobulin levels in 300 patients with endogenous uveitis.

We found the serum β_{1C} level to be elevated in every case; Yokoyama et al. (1981) similarly observed significant-

ly increased complement levels in Vogt-Koyanagi-Harada syndrome, another uveitis associated with autoimmune phenomena against pigment. An acquired increase in serum complement level is seen in acute inflammatory diseases, acute stress syndromes, and connective tissue diseases, except in SLE. In contrast, the complement level is generally decreased in autoimmune diseases (Kawai 1973). Our present observations and the results of Yokoyama et al. (1981) indicate that, in contrast to other autoimmune diseases, the complement level is elevated in pigment autoaggressive uveites.

In the lymphocyte marker investigations on the SO patients, the proportions of total and active T-cells. the proportions of mouse erythrocyte-binding and C3-binding receptor-bearing B-cells, the lymphocyte blastic transformations to PHA and con A, and the migration inhibitory effect of PPD were within the normal ranges for all of the patients. These results agree with the observations of Boone et al. (1976), whose lymphocyte subpopulation studies and in vitro functional tests likewise revealed no pathological changes in SO patients.

In all of the patients tested, the suppressor activity of the PBMC was found to be in the normal range. The suppressor cells play an important role in regulating the immune response. Since the immunological responsivity did not exhibit pathological alteration in these patients, the normal suppressor activity is in accordance with this.

No pathological changes were found when either the humoral or cellular immune reactions or the suppressor activity of the PBMC were examined in our SO patients. Our results indicate that SO can also develop without a chronic immunodeficiency, and that the occurrence of autosensitization to the uveal tissues does not lead to damage of the immune system detectable by the methods used.

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