HLA Antigens and Impaired Cellular Response to HSV1 in Behçet Patients

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Introduction

Clinical and immunological features suggest a viral etiology for Behçet's disease (BD), while the association with HLA determinants supports the hypothesis that immunogenic factors are involved.

Although conflicting results have been obtained from virus isolation, recent reports further implicate a virus, namely HSV1, in the etiology of BD [1-2].

More recently, specific antibody and cellular proliferative responses to HSV1 were studied in BD patients [3]. The results showed an impaired humoral and cellular response to HSV1 and varicella-zoster, but not to other viruses, i.e. influenza virus, supporting a specific relationship between HSV1 and BD. Furthermore, BD is strongly associated in different ethnic groups with the HLA class I specificity B51, but also an association with DRw52 II class antigen has been reported [4]. These findings could be of interest for the understanding of BD pathogenesis; in fact, HLA region has been claimed to be involved in immune responsiveness as well as in the pathogenesis of several diseases with an immunopathological background.

In order to test the reported specific impaired cellular response to HSV1 in BD patients and search for any relation with HLA phenotypes, we investigated the lymphoproliferative response against HSV1 in BD patients and in healthy controls.

Materials and Methods

Eighteen BD patients (12 males and 6 females) were typed for HLA A, B, DR and DQ, by the NIH technique using IX Workshop and correlated local antisera (Table I). All patients, considered complete (13) and incomplete (5) types on the basis of the clinical criteria, had ocular lesions. Sixteen healthy subjects served as the control group.

Peripheral blood lymphocytes were separated on Lymphoprep, washed in phosphate buffer saline and resuspended at a concentration of 1×10^6/ml in Iscove medium (Gibco) supplemented with 20% inactivated AB pool sera. Lymphocyte cultures were made in 3040 Falcon microtiter plate, using a 0.1 lymphocyte suspension.

The specific lymphoproliferative response against HSB1 was assessed by adding 0.1 ml of medium containing different concentrations (10 and 50 inactivated viral elements per lymphocyte) of virus. HSV1 (F) strain utilized in our lab had been U.V. inactivated and no cytopathic effect was detected when the strain was tested on an Hep-2 cell monolayer.

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Table I. *HLA typing of BD patients tested for proliferative response to HSV1.*

	Sex	A	B	DR	DQ	Clin.diag.*	Therapy
1	m	2;24	51;/	1;8;w52	w1;w3	i	—
2	m	1;24	17;18	1;/;w52	w1	i	—
3	m	2;3	51;/	2;5;w53	w1;w3	c	—
4	f	2;3	51;39	5;10,w52	w1;w3	c	—
5	m	3;11	51;/	5;8;w52	w1;w3	c	steroid
6	f	1;28	51;44	2;5;w52	w1;w3	i	—
7	f	11;26	8;39	3;5;w52	w2;w3	c	steroid
8	f	2;24	51;/	5;8;w52	w1;w3	c	steroid
9	m	2;11	51;27	ND	w1;w3	c	—
10	f	2;24	51:27	4;5;w52;w53	w3	i	—
11	m	2;/	51;17	7;/;ND;w53	w2;w3	c	steroid
12	f	1;2	7;21	2;3;w52	w1;w2	c	—
13	m	3;24	51;/	4;/;w53	w3	c	—
14	m	2;28	51;/	3;/;w52	w1;w3	c	—
15	m	3;24	51;/	/;/;w53	w3	c	steroid
16	m	11;24	51;15	5;7;w52;w53	w2;w3	c	—
17	m	2;w33	51;14	1;/;w52	w1	i	—
18	m	2;2	51;51	1;2;/	w1	c	—

* c = complete, i = incomplete.

The general immune response capacity was evaluated by mitogenic and alloantigenic (MLR) stimulations.

For the mitogenic response, 0.1 ml of PHA (Difco, 25-50 ug/ml) and PWM (Gibco, 1-2 ug/ml) was added to the lymphocytes.

In the MLR 0.1×10^6 responding lymphocytes and 0.1×10^6 stimulating cells were cultured together in 0.2 ml medium. The stimulating populations were previously inactivated by irradiation (4.500 rads). All cultures were made in triplicate with simultaneous controls.

Eighteen hours before harvesting, 1 μCi H-thymidine (Amersham, England-spec. act. 2 Ci/mmol) was added to each well. The counts per minute (c.p.m.) obtained by liquid scintillation counting, were expressed as the mean of triplicate cultures for the evaluation of the stimulation index (S.I. = cpm stimulated cultures/cpm unstimulated cultures).

Results and Discussion

The proliferative responses against different concentrations of HSV1 in BD patients and controls, expressed as stimulation indexes, are reported in Table II.

Using Student's t test it was shown that our results confirm that there is a statistically significant impairment of the proliferative response to HSV1 in BD patients for both 10:1 ($p < .001$) and 50:1 ($p < .001$) viral concentrations. Moreover, 15 out of the 18 patients (83.33%) showed stimulation indexes lower than the minimum observed in controls. The experiments, performed with both mitogens and alloantigens, show that BD patients have a general immune response capacity comparable to that of healthy subjects, confirming specificity of the cellular defect.

The impaired response does not appear to be related to the clinical type of the disease (complete or incomplete form) or to the therapy.

Worthy of note is the observation that, in spite of the association between BD and HLA antigens B51 and DRw52 reported elsewhere (4), the responsive patients were typed both B51 and DRw52. On the other hand, the specific cellular impairment was shared also by three B51 negative patients and two patients who did not show the DRw52 antigen. In conclusion, our data confirm the association between BD and a

Table II. *Proliferative response of BD and healthy subjects to HSV1.*

	BD		Healthy subjects	
	10/1	50/1	10/1	50/1*
1	3.18**	1.50	7.38	5.28
2	1.02	1.23	11.89	12.31
3	1.81	1.34	42.16	28.32
4	13.51	13.51	15.09	23.01
5	14.19	14.14	52.39	56.80
6	3.36	2.57	12.59	11.20
7	1.55	1.86	28.50	38.21
8	2.17	2.02	11.29	13.78
9	6.26	4.12	9.17	14.79
10	7.57	6.69	28.30	28.08
11	2.47	3.00	26.00	21.80
12	4.01	2.97	21.90	12.59
13	5.29	4.39	26.25	26.48
14	6.64	5.66	38.73	36.33
15	3.48	3.74	17.45	16.92
16	1.78	1.24	4.00	4.10
17	4.56	2.83	8.92	6.09
18	1.24	0.70		
	a	b	c	d

* number of inactivated viral elements per cell
** results expressed as stimulation index = mean activated triplicate cultures / mean cpm non-activated triplicate cultures
Student's test: a:c $p < .001$
Student's test: b:d $p < .001$

low lymphoproliferative response to HSV1. No relation could be found, however, between impaired cellular response to the virus and the HLA phenotype.

Key words

Behçet; HLA antigens.

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