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Spontaneous and stimulated lymphocyte transformation test in homozygous children with cystic fibrosis

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Spontaneous and non-specific mitogenic stimulated lymphocyte transformation tests of cellular immune function were carried out. The phytohaemagglutinin, concanavalin A and pokeweed mitogen stimulated lymphocyte transformation was significantly diminished in the group of cystic fibrosis (CF) homozygotes as compared to the controls, as an indication of the impaired reactivity of the T-lymphocytes. The spontaneous phytohaemagglutin stimulated lymphocyte transformation ratio was diminished, too.

A significantly increased frequency of HLA B18 antigen has been found in CF homo- and heterozygotes (László: unpublished data). An association was described between certain HLA B antigens and the immunoreactive (Ir) gene [12].

Associations have also been demonstrated between the HLA antigens and the level of T-lymphocyte reactivity [10].

Jersild et al [8] observed a strong correlation between multiple sclerosis (MS) and LD-7a, a mixed lymphocyte culture determinant closely linked to HLA-A7 patients with MS. Finkelstein et al [4] detected a correlation between the T-cell mediated aberration and the distribution of HLA-A7 in patients with MS.

The aim of this study was to test the cellular immune function of CF homozygotes with the lymphocyte transformation test (LTT).

Patients and Method

Spontaneous and stimulated LTT-s were carried out in 10 healthy persons and in 13 CF homozygotes by means of *Bain's* [1] method. Mean age of the control patients was 32 years with a range of 20–46 years.

LTT test: mitogen-stimulated lymphocyte transformation was performed in microtitre plates (Greiner) using 2×10^5 lymphocytes in each culture. Phytohaemagglutinin (PHA), concanavalin A (ConA) and pokeweed mitogen (PWM) were used to measure the mitotic capacity of the lymphocytes. PHA was used in three amounts: 5, 10 and 50 μl from a 1 : 10 diluted stock solution; the doses of ConA

TABLE I

LTT in CF-homozygotes

Patient	HLA-B		PHA-stimulated		ConA-stimulated	PWM-stimulated	Spontaneous LTT
1. Sz. K.		18	cpm	29082	6102	—	499
			abs./spont.	64.77	13.59	—	
2. V. Cs.			cpm	21046	3867	—	739
			abs./spont.	28.47	5.23	—	
3. Sz. O.		12	cpm	11131	24962	12634	1517
			abs./spont.	7.33	16.45	8.32	
4. Sz. T.	15,	5	cpm	11600	17677	4617	2330
			abs./spont.	4.97	7.58	1.98	
5. V. H.	18,	7	cpm	4244	3932	7261	236
			abs./spont.	17.98	16.66	30.76	
6. I. K.	40,	18	cpm	16229	13290	13313	361
			abs./spont.	44.95	36.81	36.87	
7. R. Cs.		12	cpm	8874	13949	17540	2051
			abs./spont.	4.32	6.79	8.54	
8. M. K.	18,	18	cpm	13400	17110	—	762
			abs./spont.	17.58	22.4	—	
9. R. T.	27,	14	cpm	20428	16212	—	686
			abs./spont.	29.77	23.63	—	
10. M. M.	38,	8	cpm	1623	14233	13120	161
			abs./spont.	10.08	88.40	81.49	
11. M. E.	35,	8	cpm	1057	5258	6587	266
			abs./spont.	3.97	19.70	24.76	
12. K. Á.			cpm	2132	9974	3770	1371
			abs./spont.	1.55	7.27	2.74	
13. H. A.	18,	13	cpm	1094	270	781	266
			abs./spont.	4.11	1.1	2.93	
cpm $\bar{x}$				10918	11295	8847	865
S.D. $\pm$				8962.3	7078	5527.9	723.9
Student's *t*-test				$p < 0.05$	$p < 0.05$	$p < 0.005$	$p > 0.05$
abs/spontaneous $\bar{x}$				18.450	20.431	22.043	
S.D. $\pm$				19.04	22.56	25.85	
Student's *t*-test				$p < 0.05$	$p > 0.05$	$p < 0.05$	

were 5 and 2.5 μg/ml; and that of PWM was 5 μl from a 1 : 2 diluted stock solution. The individual optimum values are expressed in the Tables. All tests were performed in triplicate.

Cultures were labelled with 1 μCi of tritiated thymidine for 5 h on day and harvested for scintillation spectrometry. Results were expressed as cpm/2×10^5 lymphocytes.

RESULTS

The ^{3}H-thymidine uptake of lymphocytes (spontaneous and stimulated with PHA, ConA and PWM) in the group of CF homozygotes and in the controls are shown in Tables I and II and in Fig. 1. The result of spontane-

TABLE II

Lymphocyte transformation tests in the control group

	No	PHA stimulated	ConA stimulated	PWM stimulated	Spontaneous LTT
1.	cpm	12382	1186	19263	187
	abs./spont.	66.21	6.34	103.01	
2.	cpm	19538	7347	21823	760
	abs./spont.	25.7	9.66	28.71	
3.	cpm	15823	27137	36020	1120
	abs./spont.	14.12	24.22	32.16	
4.	cpm	38618	36125	10299	722
	abs./spont.	53.48	50.03	14.26	
5.	cpm	20701	35069	35478	565
	abs./spont.	36.6	62.06	62.79	
6.	cpm	26101	12821	15627	378
	abs./spont.	67.44	33.91	41.34	
7.	cpm	20706	23551	27570	569
	abs./spont.	36.39	41.39	48.45	
8.	cpm	19372	23326	24497	806
	abs./spont.	24.03	28.94	30.39	
9.	cpm	12885	16556	28702	600
	abs./spont.	21.47	27.59	47.83	
10.	cpm	18536	23456	29054	826
	abs./spont.	22.44	28.39	35.17	
cpm $\bar{x}$		20466.2	20657	24833.3	653.3
S.D. $\pm$		7538.9	1128.8	8265.8	257.7
abs./spontaneous					
	$\bar{x}$	36.78	31.25	44.41	
	S.D. $\pm$	19.21	16.9	24.4	

ous LTT in CF patients did not differ from that for the controls, whereas the T–B mitogen stimulated LTT showed a significant decrease in the CF group.

The spontaneous and mitogen stimulated LTT values were expressed as a ratio (Fig. 2). The mean spontaneous LTT/PHA-stimulated LTT ratio in the CF group proved significantly lower than that for the controls. The corresponding ratio of PWM was diminished, too.

DISCUSSION

The LTT with non-specific mitogens is of value in malignancy: a good correlation is exhibited between the diminished response to PHA and the survival of patients with lung cancer [7]. PHA reactivity is generally low in severely ill tumorous patients [3, 6] and especially in cases with rapid progression [5], while in the early stage the response may be the same as in healthy subjects [2].

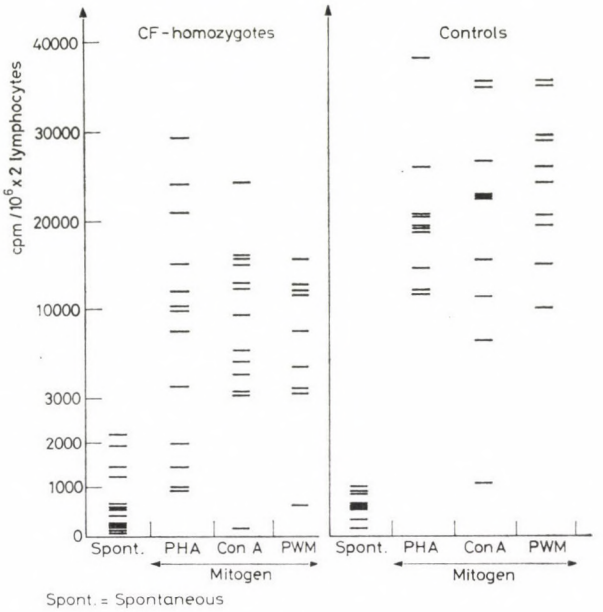
CF-homozygotes
Controls
cpm/10⁶x2 lymphocytes
40000
30000
20000
10000
3000
2000
1000
0
Spont.
PHA
Con A
PWM
Mitogen
Spont. = Spontaneous

FIG. 1

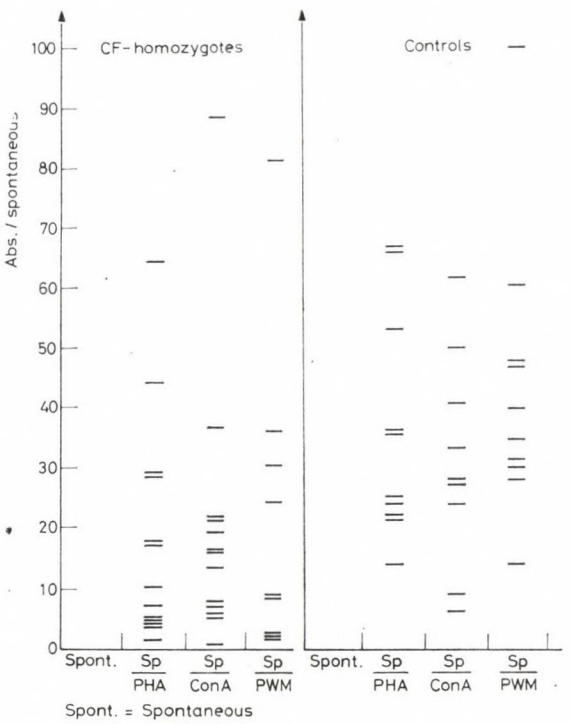
CF-homozygotes
Controls
Abs./spontaneous
100
90
80
70
60
50
40
30
20
10
0
Spont.
Sp/PHA
Sp/ConA
Sp/PWM
Spont. = Spontaneous

FIG. 2

HLA antigens and the general capacity of lymphocytes to respond to specific allogeneic and non-specific PHA stimulation have been investigated [11]. Antigens All, w19, Bw27 and Bw35 seemed to be linked with low responder characteristics, while A9, 10, 28 and B18 were associated with a high responsiveness to PHA reactivity.

Jersild et al [9] suggested that the increased antimeasles virus antibody titre of MS patients reflected the existence of an immune response locus linked to HLA-A3, 7 and w18. Petrányi et al [12] described a significant correlation between HLA-A3, 7 haplotype and the lack of spontaneous lymphocytotoxic activity, and found a correlation between the low lymphocytotoxic activity and the hyporesponsiveness to PHA of the lymphocytes.

LTT-s were performed with three different mitogens: ConA, PHA and PWM stimulating the T-lymphocytes, mainly the T-lymphocytes and the B-lymphocytes, respectively, through a helper T cell effect. We found significant differences between CF patients and controls with all three mitogens.

We first detected a diminished response to non-specific mitogens (PHA, ConA, PWM) in the lymphocytes of CF-homozygotes, as a sign of an impaired cellular immunity. Accordingly we asssume that the frequent infections in the CF patients might be explained by a reduced reactivity of the T-lymphocytes.

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