Acta Paediatrica Academiae Scientiarum Hungaricae, Vol. 15 (1), pp. 31—39 (1974)

Cell-mediated immune response in patients with Down's syndrome

By

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(Received September 29, 1973)

Skin tests and in vitro lymphocyte stimulation assays were done in 21-trisomic children and controls. Skin tests with tuberculin, monilia extract and in vitro stimulation of lymphocytes by PHA showed no significant difference.

An impaired intradermal reactivity to PHA and a weakening of the reaction between 24 and 48 hrs was found. There was no correlation between the PHA responses in vivo and in vitro.

Down's syndrome (DS) or trisomy-21 is the most frequent chromosomal abnormality causing mental deficiency. The condition is well-known for its being associated with an increased susceptibility to infections [21] and, in addition to the congenital heart anomalies, it is the most important cause of the high mortality of children with DS [11, 12]. Furthermore, a number of data has been published about the increased incidence of leukaemia among DS patients [8, 9, 13, 15]. Persistent Australia antigen (HAA) positivity is also more frequent in this group of patients [4, 10, 24]. Results are contradictory as to the serum immunoglobulin levels in DS [17, 19], so recently the cellular immune reactions have been emphasized as the possible causative factors in the high infection frequency of DS children [14, 18, 25]. Bonforte et al. [5] could not, however, demonstrate any difference in cell-mediated immune responses between normal controls and 21-trisomic patients. Apart from this latter report we could not find any data concerning the intradermal application of phytohaemagglutinin (PHA) in DS children.

The present study deals with our own experience using tuberculin, monilia extract and PHA for skin tests and the in vitro lymphocyte stimulation assays carried out in 21-trisomic children and controls.

Material and Methods

Selection of patients. The studies were carried out in 55 BCG-vaccinated DS patients. All were 21-trisomic kariotypically. The control group consisted of 33 children, who had been admitted for a minor operation or for a general examination. In this group, the history and the clinical and laboratory data have ruled out the presence of any haematologic, immunologic or tuberculous disorder.

Sex distribution was the same in both groups. Mean age was 8.01 (3—15) years

in the DS group and 7.72 (3—14) years in the control group.

The following studies were carried out.

1. *Tuberculin skin tests.* First, 0.1 ml of a 1 : 100 dilution (= 100 U) of commercial old tuberculin (Human, Budapest) was injected intradermally. The skin reaction was read after 48 and 72 hrs; an induration 10 mm in diameter was considered positive. This skin test was carried out in all of the 33 control children and in 50 patients with 21-trisomy.

The 31 DS children, who were negative with this amount of tuberculin, were tested after 14—30 days again with 1000 U of old tuberculin, and in 11 patients, who were still negative for this dose, we repeated the skin test with 1000 U of purified protein derivative (PPD Powder, Human, Budapest) after the same interval. The controls were not studied with the higher doses of tuberculin.

2. *Candidin (monilia extract) skin test.* 0.1 ml of a 1 : 1000 dilution of Candidine (Pasteur Institute, Paris, Batch No. 44743) was applied intradermally in all of the 33 controls and in 51 DS patients. Evaluation took place after 24 and 48 hrs in the same way as with tuberculin.

3. *PHA skin tests.* 1 and later 10 μg of PHA (Purified Phytohaemagglutinin, Borroughs Wellcome and Co., MR 68, Lot K 6117) was injected intradermally in 0.1 ml isotonic saline solution, and the skin reaction was measured after 24 and 48 hrs. The criterion of positivity was a hyperaemic induration at least 5 mm in diameter. Testing with 1 μg was done in 32 control cases after 24 hrs and in 30 controls after 48 hrs. With 10 μg of PHA the reaction was evaluated in 24 control cases. 29 DS children were tested for both PHA doses.

Carrying out these skin tests with the three substances, isotonic saline was injected intradermally to exclude aspecific inflammatory reactions. The tests were applied on the back, below the scapula.

4. *In vitro lymphocyte stimulation by PHA.* A 10—15 ml volume of blood was obtained by venipuncture into heparinized, sterile tubes, and allowed to sediment at 37 °C for 1 hour. The buffy coat was centrifuged at 1200 r. p. m. for 20 min on a Ficoll-Uromiro gradient. The obtained leukocytes were washed 3 times with TC-199 medium. Each culture vial contained 2.5×10^5 lymphocytes in 0.25 ml TC-199 medium with 20% inactivated AB-serum, 100 U/ml of penicillin G, and 100 μg/ml of streptomycin. For stimulation, 2 μg of PHA (PHA—P, Difco, Lot 553372) was used in each tube. The culture vials were gassed with 5% CO_2 in air, closely stoppered, then incubated at 37 °C for 70 hrs, at which time 1 μCi of ^{3}H-thymidine was added to each culture (^{3}H-thymidine-methyl, specific activity 10 Ci/mM, Amersham, England) and the cells were incubated for further 2 hrs. The vials were then immersed in ice and the suspension was filtered through Whatman GFC filter disks. The cell-residue was washed with methanol and 5% trichloroacetic acid. The dried filter disks were placed into Packard tubes and ^{3}H-thymidine incorporation was measured using a Packard Tricarb Scintillometer. The results are given in counts per minute (CPM) after background subtraction.

The results were evaluated statistically using Student's t test.

Results

1. *Tuberculin skin tests.* In 9 controls and 36 DS children the reaction to 100 U of old tuberculin was negative after 48 hrs. Measuring the reaction in millimeters and regarding the traceless response as zero, the mean values were similar in the two groups. After 72 hrs, 6 controls and 22 DS patients had a negative response. The mean size of the reactions did not differ significantly at this time either.

In the 36 DS children, who were negative for 100 U of old tuberculin at 48 hrs, the skin test was repeated

with a tenfold dose, then with PPD. 8 cases remained completely negative after 72 hrs.

2. *Candidin skin test.* After both 24 and 48 hrs, 21 controls of 33 gave a negative response. In the trisomic group, 31 of 51 tested children were negative after 24 hrs, and 34 after 48 hrs. The mean size of the reaction was almost the same in both groups.

3. *PHA skin tests.* All of the control patients showed a positive skin reaction to 1 μg of PHA after both 24 and 48 hrs. Among the DS children, 4 patients had a negative response after 24 hrs and this number increased to 13 by 48 hrs. The mean size of the reaction was significantly lower in the trisomic than in the control group.

To 10 μg of PHA, all the controls gave a positive response, while in the DS group 2 cases were negative after 24 hrs and 7 cases after 48 hrs. The size of the reaction was significantly smaller again in the DS group than in the controls. (Table 1,)

4. *In vitro lymphocyte stimulation by PHA* (Fig. 1). Spontaneous thymidine incorporation was the same in DS patients as in controls. Addition of 2 μg of PHA to the cultures resulted in a 15 fold average increase in ^{3}H-thymidine incorporation in the control group with a wide scattering. A similar increase was observed in the trisomic group, and no significant difference was found between the two groups.

Discussion

As the earliest information concerning the humoral immunity status of DS patients, Siegel [21] reported a decreased antibody response, while other authors [17, 19] found that any or all of the 3 main classes of immunoglobulin may be decreased, unaltered or increased. It has been suggested that all changes in the immunoglobulin levels were secondary [19, 23].

In the present study only the cellular immunity of DS children was studied, which is postulated to have a great importance in the increased incidence of infections, the virus persistence and susceptibility to leukaemia in such patients [24, 25].

Of the functions of the thymus-dependent (T-) lymphocytes we studied the delayed-type hypersensitivity skin reactions and the stimulation by PHA of cultured lymphocytes in vitro.

Sutnick et al. [25] examined skin reactions using 6 substances and found a low index of skin reactivity in DS. This hyporeactivity was significant only with PPD and mumps antigen, while the other 4 reactions were unaltered. As already mentioned, Bonforte et al. [5] could observe no difference in skin reactions of DS patients.

For skin testing, tuberculin was applied as bacterial antigen. According to Hungarian data [22], 45% of non-tuberculous, BCG-vaccinated children are negative with 100 U of old-tuberculin, which was the first dose applied in our study. Our DS group at 48 hrs showed a 72% negativity against 27% in the controls. After 72 hrs the frequency of negative responses (44%) corresponded to the mentioned figure, while in the control

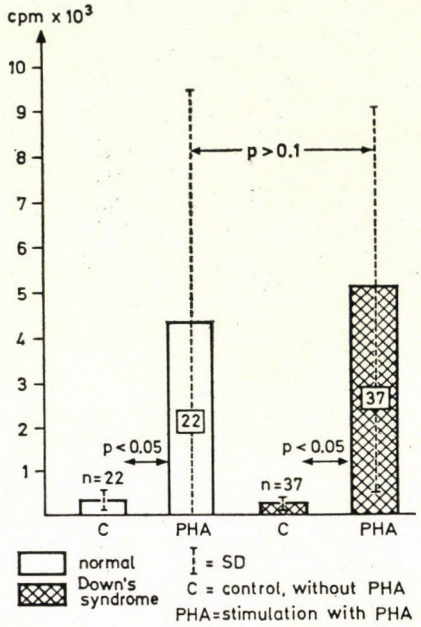

FIG. 1

group 19% were negative. We cannot explain this latter finding.

As to the possibility of distinguishing between reactions on the basis of the size of response, this has failed there having been no significant difference in this respect between the two groups. It was, however, remarkable that scattering was much wider in the DS group than in the controls.

Since high doses of tuberculin are not being used for screening, no conclusions could be drawn from the persistent negativity in 14% of the trisomic patients.

On testing with monilia extract, there was no difference between the two groups in either the form of the response or its size.

The intradermal PHA test looks back to a much shorter history than the application of specific antigens. SCHRECK and STEFANI [20] were the first to study the skin reaction to PHA in humans. Recently it has been shown [2, 3, 7] that the intradermal injection of PHA resulted in a delayed-type skin reaction, the histologic appearance of which corresponded to other delayed-type reactions of the skin. All normal subjects including premature infants respond to the mitogen within 24 hrs [6]. Among the cases reported in literature we found only 14 patients with expressed immunodeficiency, who failed to respond to intradermal PHA [6, 16].

In our study all controls gave a positive skin reaction to PHA in both doses. In the DS group, 4 cases were negative to 1 μg of PHA after 24 hrs

Table I

Results of skin tests in children with Down's syndrome and controls

Stimulant	Dose	Time (HRS)	Control				Down's syndrome				P (Control-Down's s.)
			n	+	—	$\bar{x} \pm SD$ (mm)	n	+	—	$\bar{x} \pm SD$ (mm)	
Tuberculin	100 U	48	33	24	9	7.87±5.91	50	14	36	6.56±6.57	$p > 0.05$
	OLD-	72	33	27	6	8.51±5.32	50	28	22	5.94±6.20	$p > 0.05$
	1000 U	48					31	18	13		
	OLD-	72					31	15	16		
	PPD	48					11	4	7		
		72					11	3	8		
Candidin	1 : 1000	24	33	12	21	3.36±4.50	51	20	31	3.27±4.41	$p > 0.05$
		48	33	12	21	3.51±4.94	51	17	34	3.04±4.50	$p > 0.05$
PHA	1 μg	24	32	32	0	10.32±3.90	29	25	4	6.15±3.23	$0.01 > p > 0.001$
		48	30	30	0	9.73±3.14	29	16	13	2.86±2.91	$p < 0.001$
	10 μg	24	24	24	0	12.66±3.41	29	27	2	8.53±3.05	$p < 0.001$
		48	24	24	0	12.06±2.30	29	20	7	4.70±3.45	$p < 0.001$

and further 9 after 48 hrs. Repeating the test with a tenfold dose, 2 children persisted in remaining negative at 24 hrs, and by 48 hrs the number of negative cases increased to 7 (Table I). Among the above-mentioned negative cases (6) only 1 patient with congenital rubella failed to respond to both 2 and 10 μg of PHA.

Comparing the size of the reaction, the DS children showed a significant hyporeactivity to both doses of intradermal PHA. The difference was even more expressed after 48 hrs, as the reaction showed a significant ($p < 0.001$) decrease in size from 24 to 48 hrs, with both doses of PHA (Fig. 2). In the control group, too, the size of the reaction decreased after 24 hrs, similarly as in the cases of AIRO et al. [2], but less than in the trisomic patients ($p > 0.05$). In the DS group, the size of the reaction to the other two antigens showed a decreasing tendency, while such a change was not observed in the controls.

The in vitro stimulation test showed no difference between the two groups apart from a wider scatter in the DS group. Pertaining data are difficult to compare in view of differences in methods and doses. HAYAKAWA et al. [14] observed a hyperreactivity to 1 μg of PHA in DS patients, whereas RIGAS et al. [18] found a hyporeactivity to the same dose and so did AGARWAL et al. [1] even when applying 25 μg of PHA.

There seems to be evidence of a dissociation of the PHA response in vitro from the in vivo skin reactions to this substance [2, 6].

The skin reactions and stimulation in vitro were compared in DS patients who were negative for 1 μg of PHA at 24 hrs (Table II). Only one of them had completely negative skin tests. Among the 4 children, 3 were tested by PHA stimulation in vitro and the stimulation index was above 8 in all of them. Analyzing the question from the point of view of stimulation, of 37 in vitro assays in DS children, 9 were reacting weakly with a stimulation index of 8 or less (Table III). All these children responded to old tuberculin and 2 were also positive with monilia extract. All the 9 weak reactors displayed a strong positivity with 1 μg of PHA.

There are several theories to explain the dissociation of the PHA response in vitro and in vivo. RIGAS et al. [18] postulated two cell populations different in reactivity. According to BONFORTE et al. [6], the in vitro techniques are measuring the response of all the cells, whereas only a fraction of the cells has to react to give a positive skin reaction in vivo. This hypothesis offers an excellent explanation of the positive reactions in vivo and the negative results in vitro in chronic lymphocytic leukaemia [2] and sarcoidosis [6]. Nevertheless, this explanation is not satisfactory for our present results, as in our DS group the response to PHA in vivo was inhibited, which, according to the above hypothesis, would require less responding cells.

In our experiments, the hyporeactivity in one of the skin tests and the unaltered function of the lympho-

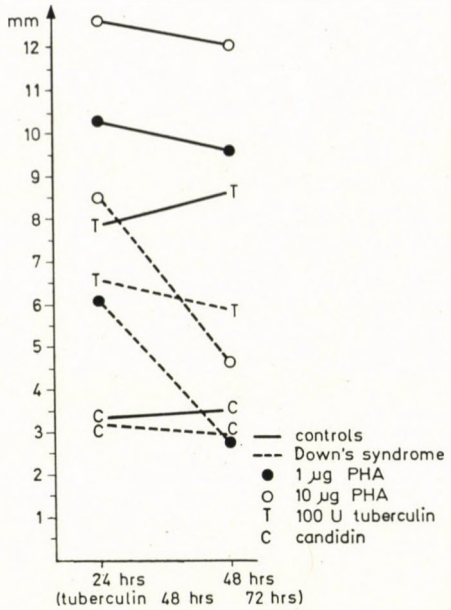

FIG. 2

TABLE II

Skin tests and in vitro stimulation index in patients with Down's syndrome who were negative for 1µg of PHA at 24 hrs

	Candidin	100 U TUB.	1000 U TUB.	PPD	STIM. cpm/ /Control cpm
1 *	—	—	—	—	18
2	—	—	+	∅	9
3	—	—	+	∅	38
4 *	—	+	∅	∅	∅

TUB. = Old Tuberculin
∅ = Not tested
* = Negative for 10 g of PHA

cytes in vitro, provided no conclusive basis for supposing or excluding an impairment of cell-mediated immunity in DS. There were, however, a remarkable hyporeactivity and in addition a weakening of the skin reaction to PHA with time in DS children. Further research is needed to clarify the details of an eventual cellular immune deficiency in Down's syndrome.

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We are greatly indebted to Dr. M. M SZONDY and Dr. L. HORVÁTH for kind permission to examine their patients, and to Miss. A. Molnár and Mrs. E. Zahalka for skilled technical assistance.

TABLE III

	Candidin	Tuberculin			PHA	
	1 : 1000	100 U	1000 U	PPD	1 μg	10 μg
1	−	−	+	∅	+	+
2	−	−	+	∅	+	+
3	−	−	+	∅	+	+
4	−	+	∅	∅	+	+
5	−	+	∅	∅	+	+
6	−	−	+	∅	+	+
7	+	−	+	∅	+	+
8	+	+	∅	∅	+	+
9	−	+	∅	∅	+	+

∅ = not tested

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