Response to Stimulation *in Vitro* of Lymphocytes from Patients with Down's Syndrome[1] (37616)

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(Introduced by H. Traurig)

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It is well known that patients with Down's syndrome (DS) are more susceptible to respiratory infections than otherwise comparable patients without DS (1). This fact suggests an impairment of immune response either at the level of antibody production or at the level of the cellular immune (delayed hypersensitivity) response. The latter possibility is the subject of this investigation. We have attempted to assess cellular immunity in DS patients, in comparison with controls, by evaluating the response of peripheral lymphocytes to specific antigenic stimulation as well as to phytohemagglutinin (PHA) stimulation *in vitro*. Synthesis of DNA was used as a parameter of response to stimulation. We found that, in response to stimulation by the specific antigen tuberculin (PPD) or by PHA, the lymphocytes of DS patients are not significantly different from those of mentally retarded (MR) patients without DS or from those of normal control (NC) individuals. Our results thus support the findings of Hayakawa *et al.* (2) who reported no evidence of impaired cellular immunity in DS patients.

Methods. Three groups of lymphocyte donors were used: patients with DS, mentally retarded patients without DS, and normal volunteers. Each group comprised 12 individuals, 6 of whom gave a positive skin reaction to PPD and 6 who were negative to PPD.

Culture procedure. Lymphocytes were obtained from 20 ml of heparinized blood by the method of Bach and Hirschhorn (3). The culture medium consisted of TC Medium 199 (Difco), 20% fetal calf serum, *l*-glutamine (25 μg/ml), penicillin (100 units/ml), and streptomycin (100 μg/ml). Each culture contained approximately 5×10^5 mononuclear cells/ml of medium. The final volume of each culture was 3 ml.

Incorporation of isotope. Sixteen hours prior to harvesting the cultured cells, 3 μCi of ^{3}H-thymidine (Tracer Lab, sp act 7.0 Ci/mmole) were added to each culture that was being used for testing its incorporation. At the end of the culture period, the cells were washed 6 times in Hanks' solution to remove the unincorporated isotope and digested in a mixture of perchloric acid and hydrogen peroxide. The resulting solution was added to a PPO–Cellosolve–toluene cocktail and counted in a liquid scintillation spectrometer (Packard Tri-carb, Model 3310) at an efficiency of approximately 20%.

PHA stimulation. For the study of PHA-transformed lymphocytes, each donor furnished cells for a series of 4 cultures. Each series consisted of 2 experimental cultures to which 0.1 ml (100 μg) PHA (M) (Difco) was added and 2 control cultures without additive. ^{3}H-Thymidine was added to all 4 cultures. The cells were harvested at the end of 3 days (the period in which optimum response was obtained). Radioactivity was determined by scintillation counting in counts per minute which were converted to disintegrations per minute (dpm) using the automatic external standardization procedure. The dpm were then tabulated as a stimulation index (dpm in PHA-stimulated cells/dpm in control cultures).

PPD stimulation. The experimental design

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TABLE I. ^{3}H-Thymidine Incorporation in Peripheral Blood Lymphocytes Cultured for 3 Days with PHA Added to the Medium.

Lymphocyte donors		^{3}H-Thymidine incorporation		
		dpm/culture, mean × 10^{-3}		
Type	No. of individuals	PHA stimulated	Unstimulated	Stimulation index[b]
DS	12	162 ± 18[a]	3.5 ± 0.7[a]	47 ± 9[a]
MR	12	135 ± 15	2.6 ± 0.8	52 ± 8
NC	12	99 ± 15	2.8 ± 0.7	36 ± 7

[a] One standard error of the mean.
[b] dpm of stimulated cultures/dpm of unstimulated cultures.

of this series was the same as described for PHA stimulation with the exceptions that the culture period was 6 days (the period in which optimum response was obtained), and the additive was PPD (2 μg/ml medium). Radioactivity was again tabulated as a stimulation index (dpm in PPD-stimulated cells/dpm in control cultures).

Results. Results of the determinations of isotope incorporation in PHA-stimulated cultures expressed as dpm and as stimulation index (dpm of stimulated cultures/dpm of unstimulated cultures) are shown in Table I. Each index given is the mean dpm of 24 PHA-stimulated cultures (two from each 12 individuals)/mean dpm of 24 control cultures taken from the same 12 individuals. The differences in isotope incorporation by cultured lymphocytes among the three classes of donor group studied (DS, MR, and NC) were not significant as determined by Student's t test ($p > 0.05$). Tritiated thymidine incorporation in cultures of lymphocytes taken from DS patients was somewhat higher than in those of normal individuals, but the difference was not statistically significant ($p > 0.05$).

The amounts of isotope incorporation in cultures with PPD added to the medium were calculated in the same way described for PHA stimulation, and the results are shown in Table II. Each stimulation index given is the mean dpm of 12 PPD-stimulated cultures taken from 6 individuals/mean dpm of 12 control cultures taken from the same 6 individuals. The addition of PPD to lymphocytes taken from individuals who gave a positive skin reaction to PPD resulted, as expected, in a significant increase in ^{3}H-thymidine incorporation over that found in donors with

TABLE II. ^{3}H-Thymidine Incorporation in Peripheral Blood Lymphocytes Cultured for 6 Days with PPD Added to the Medium.

Lymphocyte donors			^{3}H-Thymidine incorporation		
			dpm/culture, mean × 10^{-3}		
Type	No. of inividuals	PPD skin test	PPD stimulated	Unstimulated	Stimulation index[b]
DS	6	+	6.5 ± 0.7[a]	2.6 ± 0.9[a]	2.5 ± 0.21[a]
	6	—	2.4 ± 0.5	2.3 ± 0.7	1.0 ± 0.1
MR	6	+	6.0 ± 0.8	2.5 ± 0.8	2.4 ± 0.26
	6	—	2.7 ± 0.6	2.7 ± 0.5	1.0 ± 0.14
NC	6	+	6.7 ± 0.8	2.0 ± 0.8	3.3 ± 0.28
	6	—	2.5 ± 0.8	2.5 ± 0.5	1.0 ± 0.14

[a] One standard error of the mean.
[b] dpm of stimulated cultures/dpm of unstimulated cultures.

a negative skin test (Student's t test, $p < 0.02$).

A comparison of the isotope uptake in PPD-stimulated cultures of cells taken from PPD-positive members of the three donor groups revealed no significant differences among groups ($p > 0.05$). Tritiated thymidine incorporation in cultures of cells taken from DS patients was considerably less than in those obtained from NC individuals, but the difference was not significant ($p > 0.05$).

Discussion. This study finds no evidence of impairment of cellular immunity in DS patients compared with normal individuals or with mentally retarded non-DS patients. There was no significant increase over controls in the transformation of DS patients' lymphocytes in response to stimulation by PHA or PPD as measured by uptake of ^{3}H-thymidine.

Lymphocyte transformation resulting from specific antigenic stimulation has not been studied extensively in diseases in which a reduced response might be expected (*e.g.*, leukemia); instead, the usual procedure has been to assess the immunological competence of lymphocytes on the basis of the magnitude of their response to stimulation by PHA. The morphology of PHA-transformed blast cells *in vitro* is similar to that seen in lymphoid cells *in vivo* in the graft-versus-host reaction (4), in the skin homograft reaction (5), and in contact hypersensitivity (6).

It is generally agreed that a hypoblastic response to PHA by lymphocytes *in vitro* suggests deficient cellular immunity *in vivo*.

Our findings are in agreement with that of Hayakawa *et al.* (2) that the lymphocyte response (tritiated thymidine uptake) to ordinary concentrations of PHA in DS patients was similar to that of control patients. Our results do not accord with those of Agarnal *et al.* (7) who found a hypoblastic response to PHA stimulation (measured by ^{3}H-thymidine incorporation or by DNA-polymerase activity) on the part of lymphocytes of DS patients compared with those of mentally retarded control patients. The latter authors did state, however, that when the response to PHA stimulation was measured by morphological transformation into blast cells, the lymphocytes of DS patients did not differ from those of controls. Since the lymphocyte response to PHA is highly variable even among normal individuals, a difference in response between relatively small groups in different hospitals is not unexpected, although it cannot be readily explained at present.

Lymphocyte transformation *in vitro* following specific antigenic stimulation has been shown to correlate with *in vivo* tests for delayed hypersensitivity in guinea pigs and man (8). In our study, stimulation of lymphocytes from the different groups failed to differ in response to specific antigenic stimulation. This observation is in agreement with the unpublished findings of one of us (D.H.) that delayed hypersensitivity as measured by skin tests in 60 DS patients and 60 age–sex matched mentally retarded controls showed no difference in response to PPD, histoplasmin, dermatophytin "O", or Schick antigen.

Summary. An attempt has been made to assess cellular immunity in patients with Down's syndrome (DS) in comparison with controls by evaluating the response of peripheral lymphocytes to specific antigenic stimulation with tuberculin (PPD) as well as to phytohemagglutinin (PHA) stimulation *in vitro.* Incorporation of ^{3}H-thymidine was measured as the parameter of response to stimulation. The results support the view that, in their response to stimulation by the specific antigen (PPD) or by PHA, the lymphocytes of DS patients are not significantly different from those of mentally retarded (MR) individuals without DS or from those of normal control (NC) individuals.

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