Effect of DDS on Phytohemagglutinin-Induced Lymphocyte Transformation[1, 2]

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It is well known that the small lymphocytes of peripheral blood are capable of being transformed into blast-like cells under stimulation by phytohemagglutinin (PHA). Since this reactivity is considered to be an indirect measure of the immune capacity, several authors studied the blastogenicity incited by PHA in lymphocytes of leprosy patients either on morphologic grounds or by evaluation of DNA synthesis ([1-8, 10-13]).

While some investigators have observed a severe depression of PHA-induced lymphocyte transformation in leukocyte cultures from lepromatous patients ([1-4, 7, 8, 13]), as well as a less marked reduction in those from tuberculoid cases ([1, 2, 4, 13]), others have not been able to confirm these findings ([5, 6, 10, 12]). Thus, Potier ([6]) claimed that a severe impairment of the blastogenic PHA stimulus was only observed in cultures from patients affected with tuberculoid leprosy but not among those from lepromatous cases. Contrarily, Sheagren *et al* ([10, 11]) could not find a significant difference between the means of transformed cells in controls and the cultures from lepromatous patients, in spite of observing that some cases tended to show an impairment of PHA-induced lymphocyte transformation. More recently, Ulrich *et al* ([12]) were also unable to detect a depression of transformed lymphocytes in PHA stimulated cultures of lepromatous, tuberculoid and borderline cases as compared to controls.

The studies of Nelson *et al* ([5]) on leprosy patients and controls matched for race has further contributed to the heterogeneity of the data on this matter. Thus, the lymphocytes of lepromatous Chinese transformed significantly more extensively than those of normal Chinese, whether cultured in normal (reference) or in autologous serum. The same was not true for Malay and Indian lepromatous patients whose leukocyte cultures showed a decreased transformation rate when the lymphocytes were incubated in autologous serum. Nevertheless, the proportion of blast-like cells of those patients has not differed significantly from the controls when their lymphocytes were cultured in normal serum. With respect to the tuberculoid cases in this study, only the Malays showed a depressed lymphocyte transformation in cultures made in autologous serum.

With the exception of Bullock and Fasal ([1]) who touched briefly on the possibility of plasma levels of sulfones in leprosy patients being a cause of depressed lymphocyte transformation, this question has not been raised by other authors as an explanation of their controversial results. The present paper is concerned with the investigation of the influence of DDS (4, 4'-diaminodiphenylsulfone) on PHA-induced lymphocyte transformation, since DDS is the most widely used drug for leprosy therapy.

MATERIALS AND METHODS

The frequency of small lymphocytes and of blast-like cells was evaluated in leukocyte cultures of two samples of ten healthy Caucasoid individuals, who were not taking any drug at the time of the investigation.

The plasma drawn from 10 ml venous heparinized blood of each individual of Sample 1 was distributed in two sterile 100 ml prescription bottles, while that drawn from individuals of Sample 2 was distributed in four bottles. In both samples one bottle was used as control and contained 6 ml of tissue culture medium plus two drops of PHA prepared in the authors' laboratory. The second bottle of Sample 1 contained 0.4 μg/ml DDS, while the second, third and fourth bottles of Sample 2 contained, respectively, 4 μg, 8 μg and 16 μg of DDS per ml of tissue culture medium. This medium consisted of 60% Hanks balanced salt solution enriched with 0.5% lactalbumin hydrolysate, 20% fe-

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TABLE 1. *Percentage of blast-like cells in the controls and DDS-treated leukocyte cultures from the individuals of Samples 1 and 2.*

Sample 1					Sample 2				
Individual			Control 1	DDS 0.4 μg/ml	Individual		Control 2	DDS	
No.	Sex	Age			Sex	Age		4 μg/ml	8 μg/ml	16 μg/ml
1	M	21	72.1	71.7	M	33	70.9	76.4	74.4	70.6
2	M	20	71.3	69.1	M	22	85.6	75.2	75.6	74.2
3	F	25	77.6	71.9	M	24	77.0	71.8	73.9	68.3
4	F	19	78.9	77.2	M	29	82.1	75.7	73.7	65.1
5	M	20	79.3	81.1	M	26	64.5	77.0	69.5	55.7
6	M	24	80.5	81.9	M	16	91.4	67.6	80.8	66.2
7	M	19	73.3	67.8	M	20	80.3	72.8	66.3	64.3
8	M	16	85.2	83.1	M	40	76.6	64.7	68.9	70.6
9	M	22	83.4	78.8	F	39	78.5	63.5	65.2	59.8
10	F	20	74.5	71.2	F	21	70.4	68.5	71.8	64.7

tal bovine serum, 20% ascitic fluid, 100 I.U./ml penicillin and 100 μg/ml streptomycin.

The cultures were incubated for 72 hours at 37°C, after which the contents of the bottles were transferred to conical tubes and centrifuged for five minutes at 1,000 rpm. Drops of the sedimented cells were spread on slides and air-dried. Fixation with methanol and staining with May-Grünwald and Giemsa's reagent were performed according to Rosenfeld's technic (9). At least 600 cells from each bottle have been examined for calculating the percentage of transformed lymphocytes.

RESULTS

The frequency of transformed lymphocytes observed in the controls and in the DDS treated cultures of both samples is presented in Table 1. The paired data permits comparison of the effect of the different concentrations of DDS on the percentage of lymphocyte transformation by testing the null hypothesis that the mean difference of the pairs is zero (Table 2). This test was performed by calculating $t = \bar{d} / s(\bar{d})$ with $n - 1$ degrees of freedom (DF), where $\bar{d}$ is the mean difference, $s(\bar{d})$ is the standard error of $\bar{d}$ and n is the number of paired data.

DISCUSSION

The data presented in Table 2 show clearly that the frequency of lymphocyte transformation induced by PHA was significantly reduced by DDS in all concentrations used. The higher the concentration of dapsone the higher the reduction in the cultures, which was substantial in those to which DDS was added to a final concentration of 16 μg/ml. No significant differences could be observed when the effects of 4 μg/ml and 8 μg/ml were compared.

The present data is not sufficient to explain the controversial results concerning the PHA-induced lymphocyte transformation among leprosy patients, since Fliess *et al* (3) have observed a marked impairment of the

TABLE 2. *Comparison of the effects of different concentrations of DDS on the frequency of PHA-induced lymphocyte transformation.*

Comparison	$\bar{d}$	$s(\bar{d})$	t; 9 D.F.	p
Control 1 — 0.4 μg/ml DDS	2.23	0.83	2.686	<0.025
Control 2 — 4 μg/ml DDS	6.41	3.24	1.978	<0.05
Control 2 — 8 μg/ml DDS	5.42	2.11	2.569	<0.025
Control 2 — 16 μg/ml DDS	11.78	2.34	5.034	<0.001
4 μg/ml DDS — 8 μg/ml DDS	-0.69	1.86	-0.371	>0.05
4 μg/ml DDS — 16 μg/ml DDS	5.37	2.26	2.376	<0.025
8 μg/ml DDS — 16 μg/ml DDS	6.06	1.65	3.673	<0.01

blastogenic stimulus of PHA in leukocyte cultures from both untreated lepromatous cases and their healthy lepromin negative consanguineous relatives. Nevertheless, the findings here presented are a strong indication that the plasma levels of DDS may contribute to the depression of the blastogenic capacity of the lymphocytes, which is revealed by the stimulus of PHA. Therefore, investigations taking into account the plasma concentrations of DDS may throw more light on the problem of PHA-induced lymphocyte transformation in leprosy.

SUMMARY

The influence of DDS on PHA-induced lymphocyte transformation was investigated in leukocyte cultures from two samples of healthy Caucasoid individuals. In one sample the sulfone-treated cultures differed from the controls in that they contained 0.4 μg/ml of tissue culture medium plus PHA. In the other sample, the treated cultures contained DDS in concentrations of 4 μg/ml, 8 μg/ml and 16 μg/ml.

The frequency of lymphocyte transformation induced by PHA was significantly reduced by DDS in all concentrations used. The data obtained are a strong indication that the plasma levels of dapsone among leprosy patients may contribute to the depression of the blastogenic capacity of their lymphocytes when stimulated by PHA.

RESUMEN

Se estudió la influencia del DDS en la transformación linfocitaria inducida por fitohemaglutinina en cultivos de linfocitos de dos muestras de individuos caucasóides sanos. En una muestra los cultivos tratados con sulfona diferían de los controles en que contenían 0,4 μg/ml de medio para cultivo de tejido más PHA. En la otra muestra, los cultivos tratados contenían DDS en concentraciones de 4 μg/ml, 8 μg/ml y 16 μg/ml.

La frecuencia de la transformación linfocitaria inducida por la PHA fué significativamente reducida por el DDS en todas las concentraciones utilizadas. Los datos obtenidos presentan fuerte evidencia que los niveles plasmáticos de dapsona en los pacientes lepromatosos pueden contribuir a la depresión de la capacidad blastogénica de sus linfocitos, cuando éstos son estimulados con PHA.

RÉSUMÉ

On a étudié l'influence de la DDS sur la transformation lymphocytaire induite par la phyto-hémagglutinine sur des cultures de leucocytes provenant de deux échantillons d'individus caucasiens en bonne santé. Dans un échantillon, les cultures traitées par les sulfones se sont distinguées des cultures témoins par le fait qu'elles contenaient 0,4 μg/ml de milieu de culture tissulaire additionnée de phyto-agglutinine. Dans l'autre échantillon, les cultures traitées contenaient de la DDS aux concentrations de 4 μg/ml, 8 μg/ml, et 16 μg/ml.

A toutes les concentrations utilisées, la fréquence de la transformation lymphocytaire induite par la phyto-hémagglutinine était significativement réduite par la DDS. Les données obtenues constituent une indication très nette que les niveaux de dapsone dans le plasma des malades de la lèpre peut contribuer à réduire la capacité plastogène de leurs lymphocytes, lorsque ceux-ci sont stimulés par la phyto-hémagglutinine.

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