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TRANSFORMATION OF LYMPHOCYTES FROM PATIENTS WITH RHEUMATIC FEVER BY STREPTOLYSIN S

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SUMMARY

Lymphocytes from peripheral blood were tested for blast transformation reaction by streptolysin S (SLS), phytohaemagglutinin (PHA) and staphylococcal filtrate (SF). The subjects comprised twenty-eight patients with acute rheumatic fever, twenty-seven with other diseases and eight normal donors. The degree of transformation stimulated by SLS was markedly lower in most patients with acute rheumatic fever than in the other groups. On the other hand, all subjects reacted similarly to PHA and SF. The degree of blast formation induced by SLS was changed in many cases when the calf serum in the test medium was substituted by autologous plasma.

The value of the reaction to SLS as a possible diagnostic test for acute rheumatic fever is limited as some patients with acute rheumatic fever react normally while some patients with other diseases show a diminished response.

INTRODUCTION

The *in vitro* transformation of lymphocytes by various stimulants has attracted much interest in recent years. Specific antigens as well as non-specific stimulants have been found to induce this reaction, which consists of increased metabolic activity of the cell, blast formation and mitoses (Ling & Husband, 1964; Robbins, 1964).

Hirschhorn *et al.* (1964) found that streptolysin S (SLS) induces a 'non-specific' stimulation of lymphocytes from normal human donors, with formation of 40–90% blasts, while lymphocytes from patients with acute rheumatic fever showed blast values similar to those of the unstimulated control cultures (3–15%). Normal reactions were found, however, in rheumatic fever patients on penicillin treatment and in most patients with non-rheumatic diseases. In view of the clear differences between the groups, it was suggested that this reaction would be a useful test for the diagnosis of rheumatic fever.

We have been unable to trace any confirmation of these observations in the literature and

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report here the results of our own study which modify and extend those of Hirschhorn *et al.* (1964).

MATERIALS AND METHODS

Subjects

Fifty-five children, admitted to Bikur Cholim Hospital, Jerusalem, between September 1966 and April 1967, twenty-eight of them with acute rheumatic fever, were tested together with eight normal adults (Table 1). Diagnosis of rheumatic fever was based on the Jones criteria, as revised by Markowitz & Kuttner (1965).

Lymphocyte cultures

The technique was based on that of Hirschhorn *et al.* (1964), with some modifications. Lymphocytes were separated out of 5–15 ml heparinized venous blood. The cell cultures, in rubber stoppered glass tubes, consisted of $1{\cdot}2–1{\cdot}5 \times 10^6$ white blood cells (with 50–95% small lymphocytes) in a 2-ml volume containing medium 199 culture medium with 100 units penicillin and 100 μg streptomycin/ml, 20% calf serum or autologous plasma and stimulant. Two kinds of calf serum were used and gave similar effects, newborn calf serum purchased from Microbiological Associates (Bethesda, Maryland) and calf serum prepared in our laboratory.

All cultures were incubated at 37°C and harvested after 5 days. Smears were stained with orcein and the extent of response expressed as the percentage transformed out of 500–1000 cells examined. The reaction to each stimulant was calculated by subtracting the per cent of blasts found in the donor's control culture, to which no stimulant was added. The control values ranged between 0·0 and 7·3%, with the exception of one case that reached 16·3%.

Stimulants

Phytohaemagglutinin P (PHA, Difco) was used for stimulation of all tested lymphocytes, 100 μg being added to each culture.

Staphylococcal filtrate (SF), prepared according to Ling & Husband (1964) was used in some of the lymphocyte suspensions, 0·5 ml being added to each tested culture.

Streptolysin S (SLS) was prepared with RNA-core by the technique of Bernheimer, as modified by Ginsburg & Harris (1963). Titrations of the SLS preparation showed a lympholytic effect at doses above 100 units/culture and optimal transformation inducing concentrations between 20 and 100 units/ml, with variations among different tested cultures. Some cell suspensions were examined with a single concentration of SLS (50 units/culture). Most cases, however, were tested against two different doses (20 and 80 units/culture), the highest response of each subject being that recorded. A culture of a healthy donor was included in each experiment, in order to check the activity of the SLS preparation. Some normal donors were tested repeatedly (up to seven times) during the period covered by the present study: the data obtained at the first bleeding in each case are used for this report.

RESULTS

The degrees of blast formation stimulated by streptolysin S (SLS), phytohaemagglutinin (PHA) and staphylococcal filtrate (SF) in cultures of lymphocytes from different groups of

subjects are summarized in Table 1. A high percentage of transformation was induced in all cultures by both PHA and SF, with only small variations between individuals of each group or between group averages. On the other hand, the degree of reaction to SLS differed

TABLE 1. Lymphocyte transformation by different stimulants

Diagnosis:	Streptolysin S				Phytohaemagglutinin		Staph. filtrate	
	Calf serum		Autologous plasma					
	No. tested	Mean* (±SE†)	No. tested	Mean (±SE)	No. tested	Mean (±SE)	No. tested	Mean (±SE)
Normals	8	20·1 (±3·3)	6	19·9 (±2·6)	8	84·7 (±4·4)	6	40·7 (±3·1)
Acute RF‡, 1st attack	10	2·9 (±0·7)	4	6·7 (±4·7)	10	86·1 (±1·9)	2	47·1
Acute RF, 2nd attack	13	8·1 (±2·3)	8	9·5 (±2·4)	13	87·5 (±1·8)	4	47·4 (±12·4)
Acute RF, ⩾3rd attack	4	6·6 (±4·1)	4	12·4 (±7·6)	5	90·1 (±1·8)	3	50·5 (±2·8)
Inactive RF	5	16·7 (±2·7)	5	16·9 (±5·1)	5	89·5 (±4·1)	2	69·8
FMF§	5	19·1 (±2·1)	3	25·0 (±2·7)	5	88·4 (±3·3)	1	54·0
Glomerulonephritis	4	14·3 (±4·9)	2	11·5	4	85·3 (±2·2)	2	59·6
Other diseases¶	11	18·9 (±3·6)	8	16·0 (±3·3)	13	89·0 (±1·8)	7	55·3 (±3·9)

* Arithmetic mean of per cent transformed cells.
† Standard error.
‡ Rheumatic fever.
§ Familial Mediterranean fever.
¶ Included: pneumonia; diabetes mellitus; Henoch-Schonlein purpura; erysipelas; streptococcal pharyngitis; psychosomatic syndrome; urinary tract infection; abdominal pain; fever of unknown origin.

markedly between the different diagnostic groups as well as between the members of each group. The individual reactions to SLS are summarized in Fig. 1. Transformation of more than 7% of the cells was considered as positive reaction.

Lymphocytes of seven out of eight normal subjects reacted to SLS with values above 7% and gave a mean of 20·1% when cultured with calf serum. The lymphocytes of the two subjects that gave the low values of 6·3 and 7·2% in presence of calf serum gave a markedly higher response (15·1 and 16·8%, respectively) when cultured with autologous plasma.

Lowest reaction to SLS was shown by lymphocytes from patients with the first attack of rheumatic fever. All ten members of this group showed less than 7% transformed cells in the presence of calf serum and gave a mean of 2·9%. Lymphocytes from four patients of this group were tested in the presence of autologous plasma and in one of them, a high response (20·6%) was obtained with SLS. Higher mean percentages and a greater scatter were found in groups of patients with the second or subsequent attacks of rheumatic fever. Six of thirteen patients with the second attack of the disease and one of four with the third or more attacks reacted with more than 7% transformed cells in the presence of calf serum

while with autologous plasma, reactions above 7% were found in four out of eight and two out of four, respectively. All five patients with inactive rheumatic fever, however, reacted to the same degree as normal donors.

Reactions to SLS with more than 7% blasts were found in eighteen out of twenty patients with non-rheumatic diseases, the mean percentages being similar to those of normal controls. The two patients with abnormally low reactions were cases of glomerulonephritis and fever of an unknown origin.

Some donors were tested repeatedly and the results obtained from seven tests of a normal

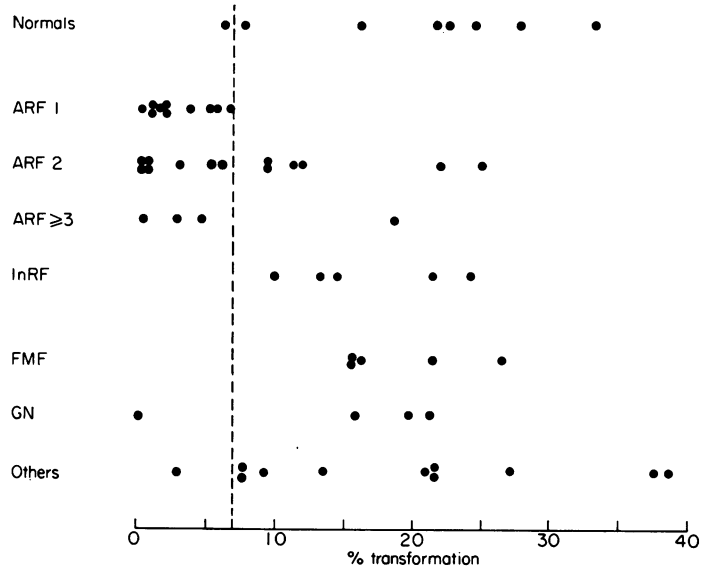

FIG. 1. Percentage of transformation stimulated by streptolysin S in lymphocytes from subjects of the different groups. Calf serum was added to all cultures. ARF, Acute rheumatic fever; arabic numerals express number of attack. InRF, inactive rheumatic fever. FMF, Familial Mediterranean fever; GN, glomerulonephritis; Others, see legend of Table 1 for specification of other diseases.

donor are shown in Table 2. The reactions to SLS varied considerably in the repeated tests, while those to PHA remained constant.

Substituting the calf serum in the medium by autologous plasma changed the degree of response to SLS, in many cases, to either increase or decrease. The reactions of the six subjects with the greatest discrepancies are shown in Table 3. In three of these cases, transformation was higher in the presence of calf serum while the other three reacted more strongly with autologous plasma. Repeated examinations of lymphocytes from normal donor I.G. were carried out with both calf serum and autologous plasma, as shown in Table 2. The values found with autologous plasma were in all tests higher than those with calf serum by a fairly constant factor of 2–3. The plasma of patient K.C., a girl suffering from abdominal pain, low prothrombin values and deficiency of coagulation factors VII and X, that gave repeated low values with her own lymphocytes did not reduce the blast transformation when added to lymphocyte cultures of two other donors. In both cases, the

reaction to SLS was similar to that obtained in the presence of calf serum. Blood lymphocytes from a sister of K.C. were also examined against SLS, in the presence of both calf serum and autologous plasma. The reaction with calf serum was 26·5% transformed cells, but with autologous plasma, a very high value of 68·9% was found.

TABLE 2. Variability in response to streptolysin S of lymphocytes collected from a normal donor (I.G.) at repeated bleedings

Date of bleeding	Streptolysin S		Phytohaemagglutinin
	Calf serum	Autologous plasma	
20 December	6·3	15·1	90·9
28 December	13·3	28·6	92·2
8 January	5·0	18·5	91·9
11 Januray	5·8	19·4	92·1
15 January	10·2	20·3	—
20 January	—	21·4	89·6
2 February	9·1	34·3	83·7

Numbers represent per cent transformed cells in total cell count.

TABLE 3. Variations in response to streptolysin S due to the use of calf serum or autologous plasma

Donor	Diagnosis	% cells transformed with	
		Calf serum	Autologous plasma
I.G.	Normal	6·3	15·1
R.C.	Rheumatic fever, 1st attack	6·8	20·6
Y.M.	Streptococcal infection	7·8	18·3
E.H.	Inactive rheumatic fever	14·5	0·8
A.B.	Inactive rheumatic fever	24·2	13·9
K.C.	Abdominal pain, coagulation defects	27·0	3·6

DISCUSSION

The degree of transformation induced in normal blood lymphocytes by SLS in the present studies was 10–30%, a figure lower than the 40–90% reported by Hirschhorn *et al.* (1964). On the other hand, a high degree of transformation was reported by these authors to occur in unstimulated control cultures, whereas in our control cultures the values were lower. These differences may be due to variations in techniques as well as in evaluation of the transformation reaction, although similar degrees of reaction to PHA were found in both studies. Low transformation values (10–35%) were reported also by Keiser & Kushner (1967) in lymphocytes from normal subjects after stimulation with SLS.

The variation found between repeated cultures of one donor to SLS (Table 2) is not understood. The reactions to PHA, on the other hand, were similar in all of these cultures. No data in regard to the consistency of the reactions to SLS are reported by Hirschhorn *et al.* (1964). It may be assumed, however, that individual variations will not affect, within limits, the group means.

The transformation stimulated by SLS in patients with rheumatic fever was in most cases significantly lower than the normal response, the lowest response being found in those with the first attack. The higher values of response among patients with recurrent attacks of rheumatic fever may be due to the effects of the prophylactic penicillin treatment given to most of these children. This possibility is in line with the findings of Hirschhorn *et al.* (1964), that penicillin treatment restores the reaction to SLS to normal in rheumatic patients. On the other hand, very low responses were found in our study in other patients with rheumatic fever history, known to be on penicillin prophylaxis.

The low response to SLS in patients with acute rheumatic fever is emphasized in view of the 'normal' reactions found in most patients with other diseases that included five with inactive rheumatic fever, five with familial Mediterranean fever (FMF) and three out of four with glomerulonephritis.

The poor reaction to SLS in the patients with rheumatic fever contrasts with their strong normal reaction to other 'non-specific' stimulants, namely, phytohaemagglutinin and staphylococcal filtrate. This difference, as well as the mechanism of action of these stimulants are not yet clear. An hypothetical explanation to the low response against SLS by some lymphocyte cultures, suggesting a defect in the cell surface that affects the binding of SLS, is under investigation.

The SLS preparation used in the present study was not purified and it is not known whether the blastogenic factor is identical with the lysin.

Another factor that affects the culture response is the type of serum added to the medium. Different degrees of reaction were obtained when the calf serum was substituted by autologous plasma, with either increase or decrease in the rate of transformation in many cases (Table 3). The stimulating effect of SLS on lymphocytes is considered to be independent of immunological response (Hirschhorn *et al.*, 1964). One cannot exclude, however, the possibility that some components of the crude SLS preparation may provoke antibody formation which affect the response to SLS. This possibility should be examined in any further study aimed at identification of the plasma factors involved in the reaction to SLS.

The abnormally low response of most patients with rheumatic fever to SLS is in accord with the findings of Hirschhorn *et al.* (1964). Some of our patients, however, reacted normally to this stimulant while low reponses were found in patients with non-rheumatic diseases. More examination of this *in vitro* test are needed therefore in order to evaluate its use in the diagnosis of rheumatic fever. The introduction of tritiated thymidine uptake for quantitative determination of cell transformation (Caron *et al.*, 1965; Benezra, Gery & Davies, 1967), promises to be a useful tool for this purpose and is under further study.

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