

# BLAST-TRANSFORMATION OF CIRCULATING LYMPHOCYTES OF PATIENTS WITH TYPHOID FEVER AFTER STIMULATION BY SPECIFIC ANTIGENS

N. I. Vylegzhanin and D. K. Bashirova

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The blast-transformation reaction of blood cells was investigated in patients with typhoid fever by the short-term culture method in vivo in response to stimulation by the following specific antigens: complex Ty<sub>2</sub> typhoid antigen and Vi-antigen. Altogether 600 cultures were studied. No blast-transformation reactions of the blood lymphocytes to typhoid antigens were found in healthy donors. In typhoid patients stimulation by the antigens caused a significantly higher blast-transformation reaction of the blood lymphocytes. As a rule recovery of the patients was accompanied by a marked specific blast-transformation reaction of the blood lymphocytes.

The blood lymphocytes of man and animals immunized by the pathogenic microorganisms of various species give a blast-transformation reaction if they are cultivated with specific antigens in vitro. Under these conditions the lymphocytes of unsensitized organisms do not give this reaction [1, 3, 5]. There are reports in the literature of a positive blast-transformation reaction of the blood lymphocytes of persons vaccinated with typhoid-paratyphoid vaccines [1, 4].

The blast-transformation reaction in typhoid fever patients was investigated.

TABLE 1. Blast-Transformation Reaction of Lymphocytes of Typhoid Fever Patients during Stimulation by CTA and Vi-Antigen ( $M \pm m\%$ ) at Different Periods of the Disease (6th day of cultivation)

Subjects tested	CTA		Vi-antigen		Cultures without antigen
	5 $\mu$ g	15 $\mu$ g	4 $\mu$ g	12 $\mu$ g	
Healthy donors (control) . . . . .	2,03 $\pm$ 0,46	2,5 $\pm$ 0,62	1,72 $\pm$ 0,49	2,8 $\pm$ 0,54	3,12 $\pm$ 1,2
Patients with typhoid fever:					
febrile period . . .	19,75 $\pm$ 3,5	16,49 $\pm$ 3,06	15,42 $\pm$ 2,8	13,65 $\pm$ 2,8	7,16 $\pm$ 1,3
1st-5th day of convalescence	16,2 $\pm$ 4,6	18,6 $\pm$ 0,2	22,05 $\pm$ 4,5	14,25 $\pm$ 2,9	9,32 $\pm$ 1,8
6th-15th day of convalescence	28,0 $\pm$ 5,8	29,1 $\pm$ 5,1	27,8 $\pm$ 5,6	31,2 $\pm$ 4,5	9,3 $\pm$ 1,6
16th-25th day of convalescence	21,61 $\pm$ 2,7	21,13 $\pm$ 2,5	24,81 $\pm$ 3,4	21,7 $\pm$ 3,0	8,5 $\pm$ 1,3

Note: The difference between indices for cultures of leukocytes from healthy persons and patients with typhoid fever was significant both in the case of stimulation ( $P < 0.001$ ) and without stimulation ( $P < 0.04$ ).

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## EXPERIMENTAL METHOD

Altogether 600 cultures of blood cells from typhoid fever patients and 126 cultures of cells from healthy donors were investigated. Blood was taken at different times of the disease, twice or three times from some patients. The method of cultivation was that described by Braude and Gol'dman [1] with slight modifications.

The specific antigenic stimulators used were complex typhoid antigen (CTA) of strain Ty<sub>2</sub> and Vi-antigen.

To cultures of blood cells with a density of  $1 \times 10^6$  cells/ml from 15 to 25% autologous serum was added. Stimulation by CTA was brought about by the addition of 5  $\mu$ g in one series and 15  $\mu$ g in another, and by means of Vi-antigen by the addition of 4  $\mu$ g in one series and 12  $\mu$ g in the other.

## EXPERIMENTAL RESULTS

Characteristic findings with 24-h cultures of lymphocytes from the patients and donors, regardless of stimulation, were lysis of the leukocytes and the appearance of reticulum cells (macrophages). The clearest changes in the cells were found on the 6th day of cultivation. The first discovery was that blast-formation of the lymphocytes was low in the control cultures of cells of healthy donors in the presence of the antigens, and it was actually higher without antigenic stimulation although the difference was not statistically significant.

The picture was completely different at this period in the cultures of patients' cells. Antibiotic therapy evidently had no adverse effect on the reaction of the lymphocytes. To examine the development of the reaction and to compare it with the course of the disease, two periods were distinguished in the usual way: febrile and afebrile. The results of assessment of the blast-transformation reaction to specific antigens during the febrile period and during the first 25 days of the recovery period are given in Table 1. In Table 2 the indices of the blast-transformation reaction are compared with the course of the disease: severe, moderately severe, and mild forms, distinguished by means of the usual clinical criteria.

The first point to mention is that marked blast-transformation of the lymphocytes was observed in the cultures of the patients' cells even without stimulation, and this presumably was a spontaneous reaction to the components of the medium. It was significantly higher than the control group ( $P < 0.05$ ) and it persisted throughout the disease. Since autologous serum of the patients themselves was added to the cultivation medium, the possibility cannot be ruled out that it contained specific antigenic stimulators in threshold concentrations.

It follows from Table 1 that at all periods of the disease a definite blast-transformation reaction of the lymphocytes significantly different from the control, occurred to antigenic stimulation in cultures of the patients' cell. The reaction was at its highest on the 6th-15 day of recovery.

A high level of blast-transformation reaction to both antigens persisted in the patients throughout the disease. However, significant differences were found in its intensity depending on the severity of the disease (Table 2).

During the first 2 weeks the blast-transformation reaction in cultures obtained from patients with a severe form of the disease showed no significant difference whether stimulated or not stimulated by antigens. The reaction was significantly increased in stimulated cultures from these patients compared with unstimulated in the subsequent weeks of recovery. It can be

TABLE 2. Blast-Transformation Reaction in Patients with Typhoid Fever Depending on Severity of Disease ( $M \pm m$ ) (6th day of cultivation)

Week of disease	Severe			Moderately severe			Mild		
	with CTA		without antigens	with CTA		without antigens	with CTA		without antigens
	5 $\mu$ g	15 $\mu$ g		5 $\mu$ g	15 $\mu$ g		5 $\mu$ g	15 $\mu$ g	
1st-2nd	18.9 $\pm$ 4.46	13.41 $\pm$ 3.9	9.88 $\pm$ 3.5	18.9 $\pm$ 4.01	16.0 $\pm$ 3.61	7.15 $\pm$ 1.38	40.91 $\pm$ 11.13	37.58 $\pm$ 8.93	7.52 $\pm$ 1.67
5th-6th	25.8 $\pm$ 5.4	31.95 $\pm$ 6.35	11.21 $\pm$ 4.0	19.9 $\pm$ 2.98	16.64 $\pm$ 2.82	6.84 $\pm$ 0.99	21.95 $\pm$ 7.34	21.52 $\pm$ 5.95	8.27 $\pm$ 3.2

emphasized that the reaction to large and small doses of antigens in these patients during the first weeks of the disease was identical. In the subsequent weeks there was a distinctly stronger reaction to larger doses of the antigens.

In patients with a moderately severe or mild form of the disease blast-transformation remained at a high level in the stimulated cultures throughout the illness, and it differed significantly from the level of the reaction in unstimulated cultures.

A common feature to all forms of the disease, regardless of their severity, was a high level of specific blast-transformation of the blood lymphocytes during recovery. No significant difference at this stage could be found between the reactions to CTA and Vi-antigen. This is perhaps because the patient was exposed throughout the period of the disease to the action of typhoid bacteria, on the surface of which both these antigens are located together [2].

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