

## STUDY ON AUTOIMMUNE PHENOMENON IN LIVER DISEASES

### —Blastoid Cell Transformation of Lymphocytes Cultured With Autologous Liver Homogenate—

BY

Hideo SHISHIDO, Hidetoshi ENOMOTO  
and Masaharu OKUDA\*<sup>1</sup>

#### ABSTRACT

Correlation between self-perpetuation of hepatitis and autoimmune phenomenon still remains obscure. Recently, attention has been drawn to the fact that blastoid cell transformation occurs with the addition of phytohemagglutinin when culturing human lymphocytes.

Using lymphocytes taken from 55 patients with chronic liver diseases, which were cultured with a medium containing autologous liver homogenate obtained by needle biopsy, the occurrence of blastoid cell transformation was studied.

Blastoid cell transformation was demonstrated in about one-third of the patients. The transformation rate, ranging from 0.2% to 7.4%, was lower than that of lymphocyte cultures to which phytohemagglutinin was added. But no evidence of blastoid cells was found in the patients without liver diseases.

As compared to those with a negative response, the values of GOT, GPT and BSP were significantly elevated in the patients with a positive response and, histologically, marked infiltration of small lymphocytes and necrosis of liver cells were observed. Furthermore, a tendency toward poor prognosis was shown by many patients with a positive response.

In patients with a positive blastoid cell response, positive rheumatoid serologic findings were also present significantly, indicating a close correlation between the two.

The above observations suggest that the lymphocytes, sensitized by the patient's own liver, play a certain part in the mechanism of self-perpetuation of hepatitis.

#### INTRODUCTION

Correlation between the self-perpetuation of hepatitis and autoimmune phenomenon has been discussed by many investigators and the presence of specific antibodies against the liver cells in the blood, rheumatoid factor,

\*<sup>1</sup> 穴戸英雄, 榎本英寿, 奥田正治: 1st Department of Internal Medicine (Chief: Prof. M. KOMIYA), School of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).

Received for publication, January 5, 1970.

antinuclear factor, AICF, etc. in the patients with chronic hepatitis and liver cirrhosis has been demonstrated<sup>1-3</sup>). In view of the fact that the fundamental questions have been left unanswered in regard to the investigations of humoral antibodies, it is obvious that a thorough study on the cellular antibodies has become essential.

It has recently been shown that the blastoid cell transformation occurs in a certain part of the lymphocyte following the addition of phytohemagglutinin (PHA) when the human peripheral lymphocytes are being cultured, which has proven to be a phenomenon of cellular antibody against PHA<sup>4,5</sup>).

Speculating that the blastoid cell transformation might be seen when the autologous liver homogenate was added to the cultured blood lymphocytes of patients with hepatitis if the autoimmune phenomenon were concerned with the self-perpetuation of hepatitis through the cellular antibodies, the occurrence of blastoid cell transformation and its relation with the clinical picture were investigated.

#### MATERIALS AND METHODS

A total of 55 patients with chronic liver diseases (27 with chronic hepatitis, 28 with liver cirrhosis), diagnosed on the basis of liver function tests and liver biopsies, were selected. Ten patients without liver diseases (chronic gastroenteritis) and 20 healthy individuals served as controls.

Lymphocytes, obtained according to the method of Bach<sup>6</sup>), were added in a concentration of  $5-7 \times 10^5$  cells per 1 ml of the medium TC 199 (Difco) and then rotation-cultured at 37°C for five days. The cells were then collected by centrifugation (at 1,000 rpm for 5 minutes), and the smears were prepared and stained with Giemsa stain. The number of blastoid cells per 1,000 lymphocytes was counted.

As shown in Fig. 1, the transformed lymphocytes were taken as cells about the size of a large lymphocyte or a little larger (15-30  $\mu$  in diameter), with deep basophilic cytoplasm, fine nuclear chromatin forming a coarse network and with distinct nucleoli.

Lymphocytes were cultured in the following three series: a) saline added to the culture medium as control; b) addition of autologous liver homogenate obtained by needle biopsy of the liver; c) addition of phytohemagglutinin-P (Difco). In some cases, cultures were repeated to observe any variation during the course of the disease.

Autologous liver homogenate was prepared from the liver tissue obtained by needle biopsy which was homogenized under sterile condition, dissolved in 0.5% saline solution, centrifuged and the sediment discarded. Then, 0.1 ml of the homogenate per 1 ml medium was added to each

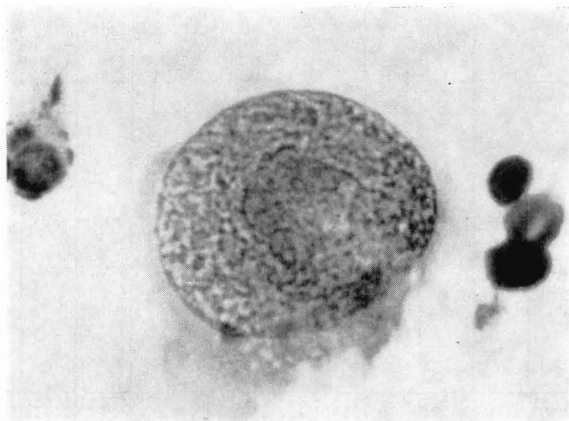


Fig. 1. Appearance of a cell when lymphocytes in patients with chronic hepatitis were cultured with autologous liver homogenate. ( $\times 1,500$ )

culture tube.

One vial of phytohemagglutinin-P was dissolved in 5 ml of sterile saline, and 0.01 ml PHA-P was added per 1 ml medium.

#### RESULTS

Fig. 2 summarizes the results of the three culture series.

When saline was added to the culture medium, 0.2% blastoid cell transformation was observed in 1 of the 27 patients with chronic hepatitis, but the specimens of the 28 patients with liver cirrhosis and the 10 patients without liver diseases were all negative.

Following the addition of autologous liver homogenate to the culture medium, blastoid cell transformation was observed in 9 of 27 patients with chronic hepatitis and in 10 of 28 patients with liver cirrhosis. All ten cases without liver diseases showed a negative response. Blastoid cell transformation in the positive cases ranged from 0.2% to 5.8% in the patients with chronic hepatitis, average 2.4%, and from 0.2% to 7.4% in the patients with liver cirrhosis, average 2.5%. More than 5% blastoid cell transformation was shown in three cases each of chronic hepatitis and liver cirrhosis.

The addition of PHA to the culture medium produced a blastoid cell transformation in every instance: the rates were  $41.9 \pm 12.2\%$  in the patients with chronic hepatitis,  $35.6 \pm 12.0\%$  in the patients with liver cirrhosis,  $49.2 \pm 10.2\%$  in the patients without liver diseases and  $54.3 \pm 13.7\%$  in the healthy individuals. With the aggravation of the liver disease, the rate of blastoid cell transformation tended to be lower. However, the different rates among the three groups were not found to be statistically significant

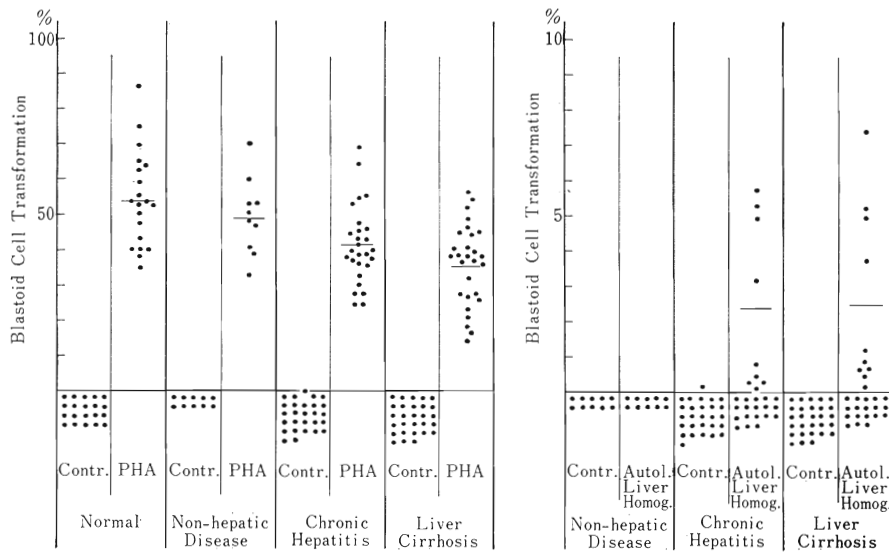


Fig. 2. Blastoid cell transformation when cultured with PHA or autologous liver homogenate.

Table 1. Blastoid Cell Transformation and Sex

	Blastoid Cell Transformation	Male	Female	Total
Chronic Hepatitis	(+)	7 (41.2%)	2 (20.0%)	9 (33.3%)
	(-)	10	8	18
Liver Cirrhosis	(+)	7 (31.8%)	3 (50.0%)	10 (35.7%)
	(-)	15	3	18
Total		39 (35.9%)	16 (31.3%)	55 (34.5%)

Figures show the number of cases.

Percentage of positive response shown in ( ).

( $p > 0.05$ ).

Dividing the cases into two groups according to whether or not the blastoid cells were demonstrated in the specimens to which an autologous liver homogenate was added, various aspects of the clinical picture in the positive and negative groups were compared.

Table 1 shows the sex distribution of the two groups. No particular difference according to sex is seen.

Table 2 compares the degree of liver function impairment. The degree of functional impairment of the liver is divided into four grades according to the results of the liver function tests, as shown in Table 3. GOT and BSP values in the positive group were significantly elevated in the patients with chronic hepatitis ( $p < 0.05$ ). GOT, GPT and  $\gamma$ -globulin values in the posi-

Table 2. Blastoid Cell Transformation and Disorder of Liver Function

Liver Function Test		Degree of Disorder				Total
		0	I	II	III	
Icterus Index	CH	4/14	4/10	0/1	1/2	9/27
	LC	4/17	3/7	1/1	2/3	10/28
Total Protein	CH	8/24	1/3			9/27
	LC	8/23	1/1			9/24
$\gamma$ -Globulin	CH	2/10	6/14			8/24
	LC	2/8	5/13	2/2	1/1	10/24
GOT	CH*	0/16	4/6		5/5	9/27
	LC	4/16	5/9	1/1		10/26
GPT	CH	6/20	2/5	1/1		9/26
	LC*	1/18	4/5	5/5		10/28
TTT	CH	2/14	6/12	1/1		9/27
	LC	5/13	4/10	1/3		10/26
ZnTT	CH	3/14	3/9	3/4		9/27
	LC	2/8	3/9	1/3	1/1	7/21
Al-ph	CH	3/10	4/10	1/1	1/5	9/26
	LC	3/8	2/8	3/4	2/5	10/25
BSP	CH*	0/5	3/6		6/6	9/17
	LC	1/3	1/4	2/4	2/3	6/14

CH=Chronic hepatitis

\*  $p < 0.05$ 

LC=Liver cirrhosis

Denominators show total number of cases in each category and numerators show the positive cases.

Table 3. Classification of the Disorder of Liver Function Tests

Liver Function Test	Normal		Abnormal	
	0	I	II	III
Icterus Index	-9	10-19	20-29	30-
Total Protein (g/dl)	7-9	9.1- -6.9		
$\gamma$ -Globulin (g/dl)	-1.9	2.0-2.9	3.0-3.9	4.0-
GOT (u.)	-40	41-199	200-349	350-
GPT (u.)	-40	41-199	200-349	350-
TTT (u.)	-4	5-9	10-19	20-
ZnTT (u.)	-9	10-19	20-29	30-
Al-ph (KA)	-9	10-19	20-29	30-
BSP	-9	10-19	20-29	30-

tive group tended to be high in the patients with liver cirrhosis; in particular, the GPT value was significantly higher than that of the negative group ( $p < 0.05$ ).

Concerning the results of liver needle biopsies (Table 4), the histological findings of infiltration of small lymphocytes in Glisson's sheaths and

Table 4. Blastoid Cell Transformation and Histological Findings of the Liver

	Blastoid Cell Transformation	Number of Cases	Cell Infiltration						Necrosis of Liver Cells			Fibrosis			Biliary Stasis		
			Glisson's Sheath			Sinusoid			-	+	++	-	+	++	-	+	++
			-	+	++	-	+	++									
Chronic Hepatitis	(+)	9	1	1	7	2	2	5	2	7	1	5	3	5	3	1	
	(-)	18	3	11	4	4	12	2	3	12	3	4	14	15	2	1	
Liver Cirrhosis	(+)	10		3	7		3	7	2	8		4	6	5	2	3	
	(-)	18	2	9	7	3	14	1	1	15	2	11	7	14	4		

Figures show number of cases.

Table 5. Blastoid Cell Transformation and Serological Findings

Blastoid Cell Transformation	Liver-antigen-like Substance		Anti-liver Antibody		Rheumatoid Factor*	
	(+)	(-)	(+)	(-)	(+)	(-)
(+)	2	12	7	7	14	4
(-)	5	8	4	7	14	17

\*  $p < 0.05$

sinusoids, necrosis of liver cells, fibrosis, etc. were relatively more pronounced in the positive group.

No particular relationship was found between the liver-antigen-like substance in the blood and the positive blastoid cell transformation (Table 5). However, the blastoid cell transformation rates were more than 3% in each of the two patients who showed positive reactions to the liver-antigen-like substance, and the lymphocyte culture with saline-added medium of one of these patients also showed a small number of blastoid cells (Fig. 3).

Although the production of anti-liver antibodies and blastoid cell transformation might be mutually related, no statistically significant correlation between the two could be found (Table 5).

The serum rheumatoid reaction (RA-test) was positive in 14 of 18 patients with a positive blastoid cell response and in 14 of 31 patients with a negative response; the high rate of positive RA-test results in those with a positive blastoid cell response was statistically significant (Table 5). At the same time, the RA-tests were positive in all those who showed higher than 3% blastoid cell transformation (Fig. 3).

Fig. 4 describes the fluctuation in the blastoid cell transformation during the course of the disease in one patient with chronic hepatitis. The elevated  $\gamma$ -globulin and GPT levels gradually returned to the normal limits with treatment and the presence of the liver-antigen-like substance and anti-liver antibody in the blood, both of which were observed in the early

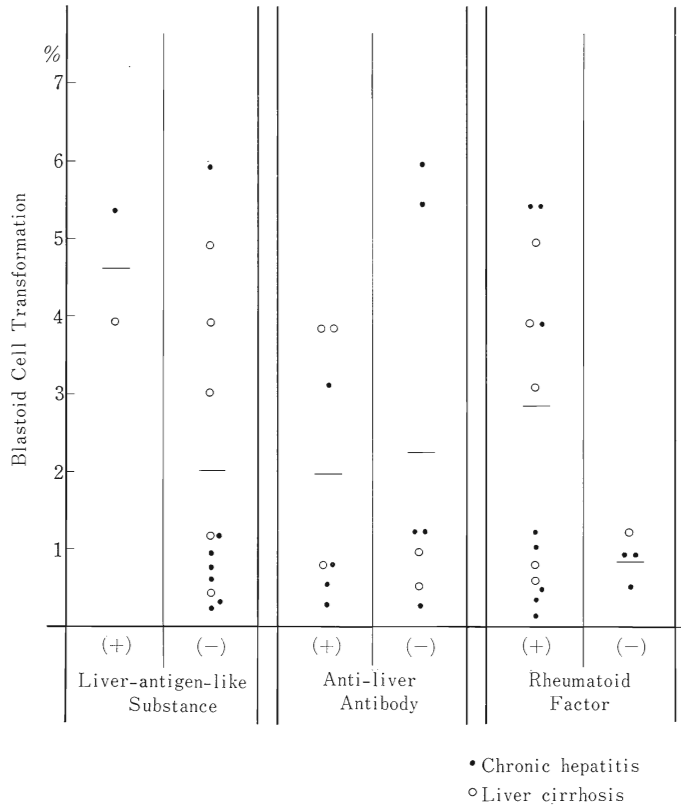


Fig. 3. Blastoid cell transformation and serological findings.

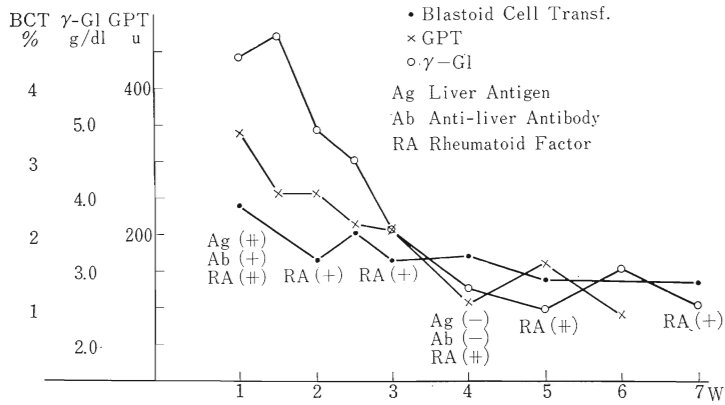


Fig. 4. Variation of blastoid cell transformation during the course of disease in one patient with chronic active hepatitis.

Table 6. Blastoid Cell Transformation and Prognosis of Diseases

	Blastoid Cell Transformation	Number of Cases	Prognosis				
			Recovered	Improved	Unchanged	Worsened	Died
Chronic Hepatitis	(+)	9	3	3	3		
	(-)	18	13	4	1		
Liver Cirrhosis	(+)	10	2	3	4	1	
	(-)	18	10	6	1	1	

Figures show number of cases.

stage of hospitalization, was no longer demonstrable. But the rate of blastoid cell transformation was hardly affected and the RA-test remained positive.

The effect of prednisolone administration on the blastoid cell transformation produced by the addition of an autologous liver homogenate to the culture medium was studied in seven patients. No significant change was observed during the observation period of five to six weeks. There was also no instance of negative conversion among the positive cases.

Lastly, the prognostic significance of this phenomenon was studied. As shown in Table 6, 8 of 19 patients with a positive blastoid cell response became worse or died. This number of aggravated or fatal cases in the positive group was significantly higher than in the negative group, as only 3 of the 36 patients in the latter group became worse or died ( $p < 0.05$ ).

#### DISCUSSION

Blastoid cell transformation was demonstrated in about one-third of the patients with chronic liver diseases when their lymphocytes were cultured with autologous liver homogenate. In the individual cases the rate of blastoid cell transformation was considerably lower with the addition of autologous liver homogenate than when PHA was added to the culture medium. In the control cultures to which saline was added, no blastoid cell transformation was observed except in a single case with chronic hepatitis, while the blastoid cells were present almost constantly throughout the course of observation in each of the specimens of the positive cases. All patients without liver diseases showed a negative response. The above findings may be regarded as a phenomenon related to the liver diseases.

A liver-antigen-like substance was found in the serum of one case whose saline-added culture of lymphocytes showed a blastoid cell transformation. Thus, this substance might have played a certain role in the appearance of blastoid cells.



Results of studies with autologous liver homogenate, similar to those of the authors', have been reported at about the same time and independently by Tobias et al.<sup>7,8)</sup>. Recently, Kosaka et al.<sup>9)</sup>, also using an autologous liver homogenate, reported a rate as high as 4.5% of blastoid cell transformation. Attempts to test the individual sensitivity by adding various agents (e.g. vaccine<sup>6)</sup>, sulphonamide<sup>10)</sup>, antibiotics<sup>6)</sup>, pollen extract<sup>11)</sup>, bacterial filtrate<sup>12)</sup>, etc., to the cultured samples of lymphocytes have been reported in the literature. Similar to our observations, the positive rates obtained in these studies also indicate that the rate of blastoid cell transformation remains fairly low.

Next, the clinical data of the positive and negative cases of blastoid cell transformation when an autologous liver homogenate was added to the culture medium of the lymphocytes were compared. A statistically significant relationship was found between the values of GOT, GPT and BSP and the positive blastoid cell production. The results of liver biopsy showed a more marked tendency toward infiltration of small lymphocytes, necrosis of liver cells and fibrosis in patients with a positive response. Concerning the prognosis, a significantly greater number of seriously aggravated or fatal cases was found among the patients with a positive response. These observations would seem to indicate a tendency toward self-perpetuation and aggravation of the liver disorder in those who show a positive blastoid cell response.

Anti-liver antibody and rheumatoid factor are found in the patients with chronic hepatitis and liver cirrhosis, and also a close connection between the rheumatoid factor and self-perpetuation of these liver diseases has been reported by Nakagawa<sup>13)</sup>. In comparing these serologic reactions with the positive blastoid cell pictures, it is unlikely that there is any direct correlation with the anti-liver antibody response. However, a positive rheumatoid serological reaction is often found together with the blastoid cell transformation and both reactions rarely become negative during the course of observation. Thus, there is some correlation between the rheumatoid factor and blastoid cell transformation. From the data available at present, however, we have not yet come to the conclusion as to whether or not the presence of a rheumatoid factor is easily caused in patients with a positive response to the cellular antibody.

The rate of blastoid cell transformation with the addition of PHA tends to be lower in the patients with chronic hepatitis and liver cirrhosis than in the normal individuals. It might, therefore, be considered that the self-perpetuation of liver disorder results in the decreased activity of the cellular antibody.

The results of this study indicate a close correlation of the positive blastoid cell response to self-perpetuation and aggravation of the liver

disorder in the patients with chronic liver diseases. This may, therefore, suggest that the lymphocytes, sensitized by the patient's own liver, are associated with the pathologic processes in chronic hepatitis and liver cirrhosis.

Part of this paper was presented at the 63rd annual meeting of the Japanese Society of Internal Medicine in April, 1966, and at the 16th and 17th annual meeting of the Japanese Society of Allergology in October of 1966 and 1967.

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