Clin. exp. Immunol. (1978) 33, 211–216.

Antibody dependent cellular cytotoxicity (ADCC) in acute hepatitis B and in chronic active hepatitis

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(*Received* 13 *March* 1978)

SUMMARY

Antibody-dependent cellular cytotoxicity (ADCC) of peripheral blood lymphocytes against chicken red blood cells (ChRBC) in the presence of specific antiserum has been studied in normal subjects and in patients with acute hepatitis B and with chronic active hepatitis (CAH).

ADCC was significantly reduced in patients with acute hepatitis B studied three weeks after the onset of jaundice and in patients with CAH showing clinical, biochemical and histological features of activity. On the other hand, lymphocytes from patients with CAH in histological remission or in clinical and biochemical resolution, showed a significantly increased cytotoxicity.

The effect of serum factors on ADCC of normal lymphocytes was investigated using serial serum samples from five patients with acute hepatitis B and five with CAH. Our data suggest that serum factors may be responsible for the impairment of ADCC in our patients, although other mechanisms may also be implied. Sera obtained at the time when ADCC of patients' lymphocytes was reduced, significantly inhibited ADCC of normal lymphocytes when compared with sera obtained at the time when ADCC of patients' lymphocytes was normal or increased. In all cases with CAH, the disappearance or reduction of inhibiting activity correlated with histological remission.

In patients with CAH the study of serum factors inhibiting ADCC of normal lymphocytes may be a useful parameter in assessing disease activity.

INTRODUCTION

Recently, lymphocyte cytotoxicity has been employed as a measure of cell-mediated immune reactions to examine mechanisms of liver cell injury in acute hepatitis and in chronic active hepatitis. Reports using different target cells support the hypothesis that both cell-mediated and antibody-dependent cellular cytotoxicity may be implied in the pathogenesis of liver damage (Cochrane *et al.*, 1976a,b; Alberti *et al.*, 1977).

In this study we investigated ADCC of peripheral blood lymphocytes against ChRBC in the presence of anti-ChRBC serum in normal subjects and in patients with acute hepatitis B and with chronic active hepatitis.

MATERIALS AND METHODS

Patients. Four groups of subjects were examined: (1) sixteen healthy controls with normal liver function tests and without a history of liver disease; (2) ten patients with acute hepatitis B studied at weekly intervals during the first six weeks of illness from the onset of jaundice; (3) fifteen patients affected by either HBsAg positive (nine cases) or negative (six cases) CAH with

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0099-9104/78/0800-0211$02.00

clinical, biochemical and histological features of activity (Soloway *et al.*, 1972). Liver histology was consistent with severe disease in five cases and with moderate disease activity in ten cases (De Groote *et al.*, 1968). Five cases had already progressed to cirrhosis. All but two patients were untreated. (4)Thirteen patients with either HBsAg positive (seven cases) or negative (six cases) CAH in clinical and biochemical resolution and eight patients with either HBsAg positive (six cases) or negative (two cases) CAH in remission (Soloway *et al.*, 1972). Cirrhosis was present in nine cases. All patients had received immunosuppressive therapy. All liver biopsies were interpreted by the same pathologist.

Test System. The method used was that described by Perlmann & Perlmann (1971), modified. Target cells were fresh ChRBC labelled with radioactive sodium chromate (^{51}Cr, Amersham), washed in normal saline and adjusted to a concentration of 10^5/ml in Eagle's medium (Serva).

The effector-cell population was obtained from peripheral blood by centrifugation on a Ficoll-Triosil gradient after removal of macrophages by carbonyl iron incubation (30 min at 37°C). Since it has been demonstrated that macrophages and granulocytes are much more efficient than lymphocytes in lysing antibody-coated ChBRC (Greenberg, Shen & Roitt, 1973), the final population employed in each test should contain more than 97% lymphocytes.

Rabbit anti-ChRBC serum, IgG fraction (Cappel Laboratories), was heat-inactivated and used at a final dilution of 10^{-6} in Eagle's medium. Cultures were set up in triplicate.

A lymphocyte-target cell ratio of 25:1, which resulted in a mean cytotoxic index of 45·8% in the control population, was employed in the cytotoxic test. 5×10^4 target cells were mixed with $1{\cdot}25 \times 10^6$ effector cells in Eagle's medium supplemented with 5% heat-inactivated foetal calf serum. In one group of samples the target cells were sensitized by the addition of 0·5 ml of diluted antiserum, while medium was added in place of antibody in the other group. The final volume was 1·5 ml per culture tube. Control cultures consisted of target cells incubated with unlabelled ChRBC in place of the effector lymphocytes (spontaneous release); maximum isotope release was estimated by lysing erythrocytes with distilled water. Cultures were incubated at 37°C for 18 hr in air and 5% CO_2 and then centrifuged at 200 *g* for 10 min.

Counts per minute in the supernatant and in the pellet were determined in a well-type gamma counter (Packard). The results are expressed as a cytotoxic index:

$$\frac{\text{experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}}{\text{maximum } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}} \times 100.$$

Inhibition of normal ADCC by serum. Sera were obtained from five patients with acute hepatitis B during the first and the third week of illness, and from five patients with CAH before and after treatment.

$1{\cdot}25 \times 10^6$ effector cells obtained from six normal subjects were pre-incubated with 0·5 ml of Eagle's medium, containing 5% heat-inactivated serum from each of the patients studied at 37°C for 2 hr. The culture and conditions for terminating the cultures were the same as described previously. Control cultures consisted of effector cells pre-incubated with medium alone and with normal serum obtained from a pool of five AB sera.

The percentage of inhibition determined by sera from patients was calculated using normal serum as the 0% inhibition base-line.

Serological tests for HBsAg. All sera were tested for the presence of HBsAg by radioimmunoassay (Ausria II, Abbott). HBsAg titres were determined in acute hepatitis patients by a direct passive haemagglutination test (Hepatest, Wellcome).

Statistical significance. Statistical significance of the results was assessed by the Student's *t*-test and by the paired-data test.

RESULTS

No significant cytotoxicity occurred when effector cells were incubated with target cells in the absence of antibody. The mean spontaneous release of isotope was 3%; maximum isotope release ranged from 80% to 85%.

Fig. 1 shows the mean cytotoxic index when lymphocytes from control subjects and from patients with acute hepatitis B were tested against ChRBC in the presence of anti-ChRBC serum. In the latter group, ADCC was reduced during the second, third and fourth week from the onset of jaundice, although only reaching statistical significance ($P < 0{\cdot}05$) in the third week. After the fourth week ADCC returned to normal values. As expected, HBsAg titres fell throughout the observation period (Fig. 1) and HBsAg was no longer detectable by passive haemagglutination in all cases within 9 weeks. The maximum drop of HBsAg titre (geometric mean) was observed between the second and the third week.

A significantly reduced ADCC was detected in CAH with clinical, biochemical and histological features of activity, while a significantly increased cytotoxic index was observed in CAH patients either in remission or in clinical and biochemical resolution. No difference was observed between HBsAg positive and negative cases with the exception of the patients in resolution in whom cytotoxicity was higher in the presence of HBsAg when compared with negative cases (Fig. 2).

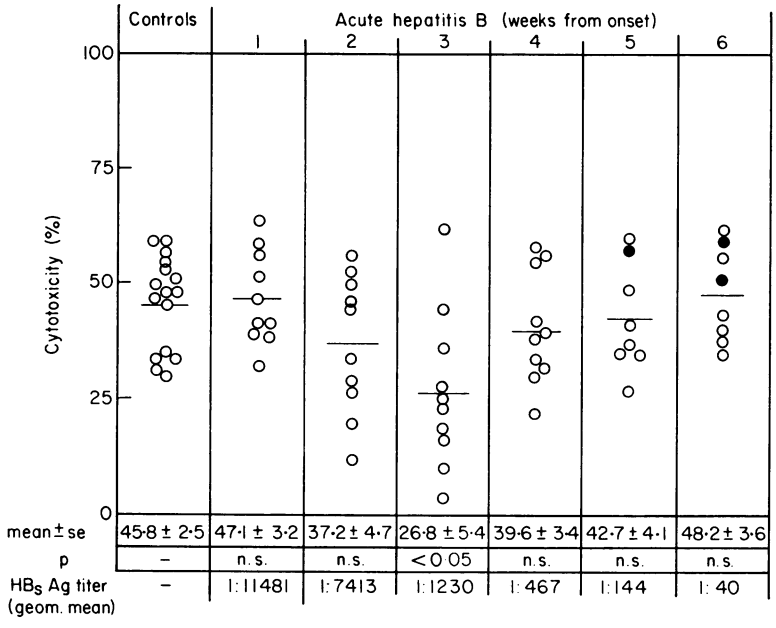

FIG. 1. ADCC in controls and in acute hepatitis B patients examined during the first six weeks from the onset of jaundice. Two subjects could not be studied in the fifth and sixth week. The solid line represents the mean in each group. (●) Subjects with undetectable HBsAg.

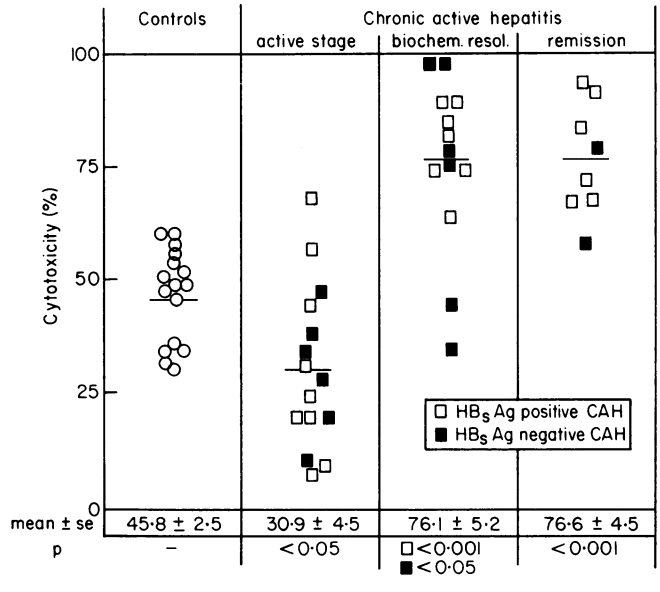

FIG. 2. ADCC in controls and in CAH patients in different stages of the disease. Solid line represents mean in each group. (□) HBsAg positive CAH; (■) HBsAg negative CAH.

Table 1 shows percentages of inhibition of ADCC of normal lymphocytes by sera obtained from five patients with acute hepatitis B during the first and the third week and from five patients with CAH before and after treatment. When normal serum was studied, less than 10% inhibition was detected. Using this serum as the 0% inhibition base line, the percentage inhibition induced by sera from patients ranged from 0 to 59.

TABLE 1. Inhibition of ADCC activity of normal lymphocytes by sera from acute and chronic active hepatitis patients

Patients		Inhibition of normal ADCC by sera (%)*		
		1st week		*3rd week*
	1)	10		26
	2)	12		31
Acute	3)	19		48
Hepatitis B	4)	14		59
	5)	0		32
			$P < 0{\cdot}01$ †	
		active stage		*remission*
	1)	46		23
Chronic	2)	31		16
active	3)	52		0
hepatitis	4)	29		12
	5)	44		9
			$P < 0{\cdot}01$ †	

* Percentage inhibition calculated using normal serum as 0% inhibition base line.

† Statistical significance calculated by paired data test.

Sera from patients whose lymphocytes exhibited a reduced cytotoxicity significantly inhibited ADCC of normal lymphocytes when compared with sera obtained from the same patients at the time when ADCC was normal or increased.

DISCUSSION

Our results show a significant impairment of ADCC during the third week of acute hepatitis B and in CAH with clinical, biochemical and histological features of activity. A similar reduction in cytotoxic activity has been reported in other disorders including the active stage of systemic lupus erythematosus (Feldmann *et al.*, 1976; Scheinberg & Cathcart, 1976), rheumatoid arthritis (McGill & Twinn, 1977) and HBsAg-positive periarteritis nodosa (Fye *et al.*, 1977).

Interpretation of the cause and significance of impaired ADCC is limited as the effector cells represent a heterogeneous population and their possible role *in vivo* is not yet clarified. First, the impaired cytotoxic activity observed in our study could be due to a reduction in the number of effector cells. Since the defect is transient in acute hepatitis and limited to the active stage of CAH, it might be related to a sequestration of effector cells within the liver. Recent studies on the specific cytotoxicity of peripheral blood lymphocytes in AH and in CAH have shown that both cell-dependent cytotoxicity and ADCC may be involved in the pathogenesis of liver damage. In a previous report (Alberti *et al.*, 1977) we were able to demonstrate a significant T-cell-mediated cytotoxicity to HBsAg-coated ChRBC in acute hepatitis B and in 33% of HBsAg-positive CAH patients. On the other hand, Cochrane *et al.*, (1976a,b) using rabbit hepatocytes as target cells and T-depleted lymphocytes as effector cells, have shown a significant cytotoxicity in both acute and chronic active hepatitis; the reaction could be blocked by the addition of aggregated IgG, suggesting an antibody-dependent K-cell-mediated reaction.

The hypothesis of a sequestration is also supported by MacSween *et al.* (1976) who found a reduced number of circulating K cells and an impaired ADCC to Chang liver cells in some cases of CAH. However the characterization of lymphocyte subpopulations in liver infiltrates of biopsies from patients with inflammatory liver diseases has provided controversial results (Chen *et al.*, 1976; Miller, Dwer & Klatskin, 1977; Sanchez-Tapias, Thomas & Sherlock, 1977).

Studies from different laboratories now suggest that surface receptors for immune complexes also play a significant role in ADCC (MacLennan, 1972; Wisloff, Michaelson & Froland, 1974; Fye *et al.*, 1977). We have therefore examined the possibility that the reduced cytotoxic activity in acute and chronic hepatitis might be due to blocking of Fc receptors by serum inhibitory factors.

Our data show that sera from patients with reduced ADCC significantly suppress the cytotoxicity of normal lymphocytes when compared with sera from patients with normal or increased ADCC. The nature of these serum factors is unknown but soluble immune complexes might be involved, as well as anti-lymphocyte-antibodies or rheumatoid factors. It is noteworthy that Fye *et al.* (1977) found a close correlation between decreased ADCC and high concentrations of circulating immune complexes in HBsAg positive periarteritis nodosa.

In acute hepatitis patients the behaviour of the HBsAg titre, showing maximum drop between the second and third weeks of illness, supports the hypothesis that complexes involving HBsAg may be implied. Moreover, Brzosko *et al.* (1971) reported the highest frequence of detectable HBsAg-HBsAb complexes during the third week of acute hepatitis.

Finally, a significant increase of cytotoxic activity has been observed in CAH in remission and in clinical and biochemical resolution. An increased ADCC of peripheral blood lymphocytes has been also reported by Calder *et al.* (1976) in some cases of newly diagnosed or short term treated autoimmune thyroiditis with no detectable serum thyroid autoantibodies. These authors suggest that the elevated cytotoxic activity may reflect an increase in the number of effector cells rather than a stimulation of their functional capacity, although they found no correlation between ADCC and the percentage of circulating null cells. Since it has been shown that a proportion of human peripheral blood cells can be stimulated into DNA synthesis by immune complexes (Möller, 1969), it is possible that the continuous presence of circulating complexes may stimulate the expansion of Fc receptor-bearing cell population by the same mechanism, as suggested by Ghaffar, Clader & Irvine (1976) in tumour-bearing animals.

So far no significant change in the number of peripheral null cells has been reported during the active stage and remission of chronic liver disease. On the other hand, none of the five sera obtained from patients with CAH in remission had a stimulatory effect on ADCC of normal lymphocytes. It seems therefore unlikely that serum factors enhance ADCC of peripheral blood lymphocytes in these patients.

It can be speculated that the fluctuation of cytotoxic activity in CAH, as detected by our *in vitro* method, might be related to variations in the size and amount of circulating immune complexes at different stages of the disease. The impairment of ADCC during the active stage cannot be attributed to immunosuppressive therapy since only two patients were treated at the time of study.

Our results also show that in CAH patients the reduction or disappearance of serum inhibitory activity on ADCC normal lymphocytes, correlates with histological remission. In these patients then, the study of serum factors may be an useful parameter to assess disease activity.

This work was supported in part by grant No. 76.01843.04 from Consiglio Nazionale delle Richerche, Roma, Italy.

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