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Lymphocyte Cytotoxicity to Autologous Liver Cells in Chronic Active Hepatitis*

(prednisone)

JACK R. WANDS AND KURT J. ISSELBACHER

Department of Medicine, Harvard Medical School and the Gastrointestinal Unit, Massachusetts General Hospital, Boston, Mass. 02114

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ABSTRACT **Lymphocyte function *in vitro* was evaluated in patients with chronic active hepatitis and compared to normal controls. Circulating lymphocytes of patients were spontaneously cytotoxic to ^{51}Cr-labeled human Chang liver cells and to suspensions of autologous liver cells obtained at the time of liver biopsy when tested at a lymphocyte target cell ratio of 200:1. Prednisone treatment of patients with chronic active hepatitis inhibited both spontaneous and concanavalin A-stimulated lymphocyte cytotoxicity to human Chang liver cells. Similarly, chronic prednisone administration substantially reduced lymphocyte cytotoxicity towards the patients' own liver cells *in vitro*, which correlated with a clinical, biochemical, and histological response to such therapy. Thus, patients with chronic hepatitis have circulating lymphocytes that are capable of causing destruction of their own liver cells *in vitro*. The beneficial effect of prednisone therapy in such patients may be related to this inhibition of lymphocyte cytotoxicity.**

Previous studies in our laboratory have demonstrated that peripheral lymphocytes obtained from patients with acute and chronic hepatitis are cytotoxic to human Chang liver cells (1). We have extended these studies to examine lymphocyte cytotoxicity in patients with chronic active hepatitis (CAH) toward their own liver cells in an attempt to understand better the pathogenesis of this disorder. The effect of prednisone therapy *in vivo* on lymphocyte cytotoxicity toward Chang and autologous liver cells *in vitro* was investigated because of the frequent clinical response of patients to such therapy.

MATERIALS AND METHODS

Patients. Nine patients with clinical and liver biopsy findings consistent with CAH and three patients with chronic persistent hepatitis were studied. Seven of the nine patients with CAH were male and six of these seven patients were positive for the hepatitis-B surface antigen. Hepatitis-B surface antibody was undetectable in all nine patients with CAH. The other three male patients with chronic persistent hepatitis were asymptomatic carriers of hepatitis-B surface antigen. An additional 13 healthy volunteers served as control subjects. All control subjects were negative for hepatitis-B surface antibody and hepatitis-B surface antigen by tests of hemagglutination and hemagglutination inhibition (2).

Isolation of Lymphocytes. Thirty to 50 ml of heparinized venous blood from patients and controls were collected in plastic syringes and allowed to sediment at 37° for 90 min. The leukocyte-rich plasma was pipetted off and placed over sterile glass wool packed in 30-ml glass syringes. The column was then washed with 100 ml of Hank's balanced salt solution. The resultant cell suspension was centrifuged, washed three times, and resuspended in 10 ml of RPMI medium supplemented with 10% heat-inactivated fetal calf serum, 40 mM glutamine, plus 100 U of penicillin, and 100 μg of streptomycin (complete medium). The cell suspensions usually contained 95–98% small lymphocytes, 2–5% monocytes, and numerous erythrocytes. Lymphocyte viability was determined by trypan blue exclusion and was approximately 95%. The final lymphocyte concentration was adjusted to 1×10^6 cells per ml.

Preparation of Target Cells. Chang cells, a human derived epitheloid liver cell line (Microbiological Associates, Bethesda, Md.), was continuously cultivated in plastic petri dishes containing complete RPMI medium at 37° and in an atmosphere of 95% air and 5% carbon dioxide. Cells were harvested by trypsinization, yielding single cell suspensions which were concentrated by centrifugation at 1000 rpm. Chang liver cells (1×10^6) were suspended in 5 ml of complete RPMI medium and incubated with 300 μCi of radioactive sodium chromate (^{51}Cr) for 45 min at 37°. The cells were then washed three times with 30 ml of complete medium, counted, and rechecked for viability with trypan blue. The final concentration of target cells was adjusted to 1×10^4 cells per ml.

Autologous human liver cells were obtained and prepared at the time of liver biopsy (performed as part of the patients' medical management). Tissue not needed for routine pathology studies (usually 20–35 mg of liver core) was immediately placed on ice in complete medium. The liver core was then subjected to mechanical disruption with fine needles, followed by a 30-min incubation with 0.1% collagenase solution at 37°. This preparation yielded a single cell suspension of hepatocytes. The liver cells were subsequently washed three times in complete medium, concentrated by centrifugation at 1000 rpm, resuspended in 5 ml of complete medium, and incubated with 300 μCi of ^{51}Cr for 45 min at 37°. The cells were washed three times with 10 ml of complete medium and counted; 80–85% of the hepatocytes were viable by trypan blue exclusion. The final concentration of target cells was adjusted to 5×10^4 cells per ml.

Cytotoxicity Studies. Two milliliters of 1×10^6 lymphocytes per ml were added to 1 ml of 1×10^4 labeled Chang liver cells, and 2 ml of 5×10^6 lymphocytes per ml were similarly added to 1 ml of 5×10^4 human liver cells to yield a ratio of lymphocyte to target cell of 200 to 1. These suspensions were

Abbreviations: CAH, chronic active hepatitis; Con A, concanavalin A.

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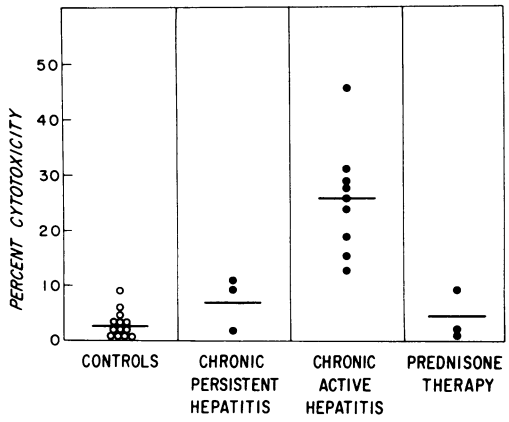

FIG. 1. Percent lymphocyte-mediated cytotoxicity against human liver cells in controls, patients with chronic persistent hepatitis, and patients with chronic active hepatitis before and after prednisone therapy.

incubated on a rocker platform for 8 hr at 37° in 95% air and 5% CO_2. At the end of the incubation, the cell suspensions were transferred from 35 × 35 mm plastic petri dishes to small test tubes and centrifuged at 1000 rpm for 15 min. The supernatant was carefully decanted into counting vials, and radioactivity was measured in triplicate in a liquid scintillation counter. The mean counts released per 5 min from 1×10^4 Chang cells and 5×10^4 human liver cells and standard error of the mean were calculated. Cytotoxicity was measured by ^{51}Cr release into the medium from ^{51}Cr-labeled liver cells after incubation with peripheral lymphocytes. The percent cytotoxicity was expressed as the mean number of counts released from cells in the presence of lymphocytes, minus spontaneous ^{51}Cr cell release, divided by the total number of releasable counts determined by freeze-thawing of the cells.

Concanavalin A (Con A) Stimulation Studies. Con A (Miles Laboratory, Inc., Kankakee, Ill.) was added to 2 ml of 1×10^6 lymphocytes per ml obtained from patients and controls at a previously determined optimal concentration of 10 μg/ml. After a 72-hr incubation with this lectin, lymphocytes were added to 1×10^4/ml of ^{51}Cr-labeled Chang cells and incubated 8 hr on a rocker platform as described above. Patients with CAH were studied before and after prednisone therapy.

Statistical Analyses. The geometric mean of percent lymphocyte cytotoxicity at 8 and 72 hr for patient and control groups were compared by applying Student's *t* test.

RESULTS

Lymphocyte Cytotoxicity in Chronic Active Hepatitis. Peripheral lymphocytes from patients with CAH were spontaneously cytotoxic to human Chang liver cells when tested at a ratio of lymphocyte to target cell of 200:1. The mean spontaneous lymphocyte cytotoxicity in patients with CAH was 9.6% ± 1.7, compared to 3.6% ± 0.7 ($P < 0.001$) in normal controls (Table 1). There was no difference in Con A maximally stimulated cytotoxicity between patient and control lymphocytes. We then determined if lymphocytes obtained from patients with CAH were cytotoxic toward their own liver cells. As shown in Fig. 1, lymphocytes were markedly cytotoxic to autologous liver cells (26.1% ± 3.0) in contrast to the action of control lymphocytes on these same cells (2.7% ± 0.6, $P < 0.001$). Furthermore, patients' lymphocytes were more cytotoxic towards autologous liver cells (26.1% ± 3.0) than to the Chang liver cell line (9.6% ± 1.7), suggesting some target cell specificity ($P < 0.001$).

TABLE 1. *Effect of prednisone therapy on lymphocyte cytotoxicity in vitro to ^{51}Cr-labeled Chang cells in chronic active hepatitis*

Incubation conditions	% Lymphocytoxicity		
	Controls	Patients before prednisone	Patients after prednisone
RPMI*	3.6 ± 0.7(10)	9.6 ± 1.7(8)	2.8 ± 1.7(5)
Con A† + RPMI	34.3 ± 7.0(10)	36.0 ± 7.4(5)	7.4 ± 3.6(5)

Numbers in parentheses represent number of subjects or patients.

* Lymphocyte cytotoxicity was studied after an 8-hr incubation with ^{51}Cr-labeled Chang liver cells in complete RPMI medium. Ratio of lymphocyte to target cell, 200:1.

† Lymphocyte cytotoxicity after a 72-hr incubation with 10 μg/ml of Con A followed by an 8-hr incubation with ^{51}Cr-labeled Chang liver cells in complete RPMI medium. Ratio of lymphocyte to target cell, 200:1.

Effect of Prednisone Therapy on Lymphocyte Cytotoxicity. Five patients with CAH in clinical remission, who had been taking between 30 and 60 mg of prednisone daily for a mean period of 2.5 months, were studied. Eight patients were studied prior to the administration of prednisone. Chronic prednisone administration markedly inhibited both spontaneous and Con A-stimulated lymphocyte-mediated cytotoxicity to Chang liver cells. As shown in Table 1, spontaneous lymphocyte cytotoxicity decreased from 9.6% ± 1.7 to 2.8% ± 1.7, and Con A-stimulated lymphocyte cytotoxicity decreased from 36.0% ± 7.4 to 7.4% ± 3.6 with prednisone therapy ($P < 0.005$). More importantly, as shown in Fig. 1, prednisone therapy *in vivo* substantially diminished lymphocyte cytotoxicity *in vitro* toward the patients' own liver cells. These three patients had been on a tapering prednisone dosage for 6–10 months and had a complete clinical, biochemical (normal serum transaminase, globulins, alkaline phosphatase, and bilirubin determinations), and histological response to therapy.

DISCUSSION

Lymphocyte-mediated cytotoxicity is a property of thymus-dependent lymphocytes (T-cells). The present findings demonstrate that circulating lymphocytes from patients with CAH, in the absence of human serum, were spontaneously cytotoxic to human Chang liver target cells. More importantly, patient lymphocytes exhibited substantial cytotoxicity toward their own liver cells *in vitro* when compared to the effect of control lymphocytes on these same cells. Human serum was replaced by heat-inactivated fetal calf serum (56° for 45 min) in these experiments; therefore, target cells were never exposed to human cytotoxic antibody (3). This observed spontaneous lymphocyte cytotoxicity in CAH suggests T-cell activation *in vivo*. It is possible, however, that B cells could participate in target cell destruction when autologous liver cells are used as the target cells (4). Lymphocyte cytotoxicity toward autologous liver cells may be important in the pathogenesis of CAH. Furthermore, the demonstration of circulat-

ing lymphocytes mediating cell damage against two types of target cells supports the concept that CAH is an autoimmune disease.

Corticosteroids are widely used in clinical diseases in which immunological factors appear to play a pathogenetic role (5). Studies with human lymphocytes *in vitro* suggest that corticosteroids abolish the effector cytotoxic phase of cell-mediated immunity and enhance the induction phase (6). Short-term administration of corticosteroids transiently reduces the absolute number of circulating T-lymphocytes in normal individuals (7, 8). The present study shows that prednisone treatment of patients with CAH results in a marked inhibition of the effector cytolytic phase of cell-mediated immunity. Depression of spontaneous lymphocyte cytotoxicity toward the patients' own liver cells thus offers one explanation for the often observed beneficial effect of corticosteroids on the clinical, biochemical, and histological features of chronic active hepatitis (9).

It should be noted that since the completion of these studies and during the preparation of this report, Thompson *et al.* (10) have also provided evidence of lymphocyte-mediated cytotoxicity in chronic active hepatitis.

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