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Cytotoxicity of Lymphocytes in Experimental Alcoholic Liver Injury in the Baboon[1] (39576)

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Although immunologic reactions and a cytotoxic activity of lymphocytes against the patient's own liver cells have been considered an important factor in some chronic liver diseases (e.g., chronic active hepatitis, primary biliary cirrhosis) (1–5), little is known whether they play any role in the induction and perpetuation of alcoholic liver disease. An altered cell mediated immunity to liver antigens (6, 7) and a pronounced decrease in peripheral blood T lymphocytes in patients with alcoholic hepatitis (8) have suggested a basic impairment in cell-mediated immunity in alcoholic liver diseases and have called for further investigations directed at determining the role of immunologic factors in the devleopment and progression of alcoholic liver diseases.

The experimental model of baboons undergoing prolonged feeding with alcohol and developing the entire spectrum of alcoholic liver disease (9) provides a valuable system to test the hypothesis of the importance of immunological reaction in the development of alcoholic liver diseases.

Materials and Methods. Twenty-four baboons (12 alcohol treated and 12 pair-fed controls) were studied. Alcohol was administered as previously described (10) for a period ranging from 16 to 38 months.

Lymphocyte-mediated cytotoxicity was utilized as the *in vitro* correlate of cellular immunity. The cytotoxic activity of lymphocytes was investigated against liver cells derived from the animal's own liver, thus avoiding a reaction due to histocompatibility difference. Lymphocytes were isolated through a Ficoll-Hypaque gradient (11), then incubated for 16 hr in plastic containers at 37° in 5% CO_2 saturated with water vapor and further purified through nylon column (12). Cytotoxicity was measured first by the microcytotoxic test of Takasugi and Klein (13) based on the destruction or detachment of adherent target cells by purified lymphocytes and evaluated by counting the target cells that remain attached. In later experiments, the method of Bean *et al.* (14) was employed. This method measures cytotoxicity by counting residual [^{3}H]proline label in cultured liver cells after 2 days of incubation with purified lymphocytes. After liver biopsy, liver cells were cultured according to the method of Demoise *et al.* (15).

Results. Liver cells from all 12 control pair-fed animals formed a monolayer within 4–5 weeks and could be easily trypsinized and subcultured. Liver cells from alcohol-treated animals were much more difficult to establish; only 6 of 12 cell lines could be subcultured. Histologic examination of these livers revealed marked steatosis and portal fibrosis. One liver exhibited moderate accumulation of lymphocytes in the portal tract and in the parenchyma. No alcoholic hepatitis or cirrhosis was observed in these animals. The cells of three animals with incomplete cirrhosis and/or hepatitis did not grow. Lymphocytes of alcohol-fed baboons exhibited cytotoxicity against autochthonous liver cells, as detected by the morphologic (Table I), and the radioisotopic (Table II) methods. Cytotoxicity was observed only if a lymphocyte to liver cell ratio of 100:1 was utilized. When the number of lymphocytes was decreased, no cytotoxicity was observed (Table I). Lymphocytes of control pair-fed animals did not display cytotoxicity.

Discussion. Lymphocytes of alcohol-treated baboons acquire cytotoxicity against

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TABLE I. Cytotoxicity of Baboon Lymphocytes against Autochthonous Liver Cells Grown in Tissue Culture.[a]

Treatment	Length of treatment (months)	Lymphocyte cytotoxicity	
		L:T[b] = 10:1	L:T[b] = 100:1
Alcohol	16	12	70[c]
Alcohol	20	11	48[c]
Control		7	5
Control		10	7

[a] Monolayer was incubated for 2 days with lymphocytes. Cytotoxicity measured as percentage reduction of number of hepatocytes incubated with lymphocytes in relation to control hepatocytes (2).
[b] Lymphocyte to target cell ratio.
[c] $P < 0.001$.

TABLE II. Cytotoxicity of Baboon Lymphocytes against Autochthonous Liver Cells Grown in Tissue Culture

Treatment	Length of treatment (months)	Lymphocyte cytotoxicity[a]
Alcohol	21	1.16[b]
Alcohol	34	2.20[b]
Alcohol	32	1.10[b]
Alcohol	38	1.40[b]
Control		0.97
Control		0.98
Control		1.00
Control		1.04

[a] Monolayers of [^{3}H]proline-labeled hepatocytes were incubated for 2 days with lymphocytes. (Ratio of lymphocyte to target cells, 100:1.) Cytotoxicity is expressed as counts per minute (cpm) control hepatocytes/cpm hepatocytes with lymphocytes. Method according to Bean *et al.* (14)
[b] Changes statistically significant: $P < 0.001$.

autochthonous liver cells before alcoholic hepatitis and cirrhosis ensues. It appears important in these experiments to utilize cells derived from the animal's own liver.

Pilot experiments attempting to use allogeneic liver cells revealed that lymphocytes of both control and alcoholic animals are cytotoxic. Most likely lymphocytes recognize histocompatibility differences still present in the cultured cells. In the present investigation, no attempts were made to establish the organ specificity of the cytotoxic reaction, why liver cells of alcoholic animals are established with difficulty, and whether they are more susceptible to injury.

Little is known about the acute or chronic effect of alcohol on lymphocyte activity. It has been claimed that alcohol induces lymphocyte stimulation in patients with alcoholic hepatitis and chronic active hepatitis (6) and blocks lymphocyte stimulation by phytohemagglutinin (16). The present demonstration of cytotoxicity of lymphocytes against autochthonous liver cells before the development of alcoholic hepatitis or cirrhosis may have an important pathogenetic significance and warrants further investigations on the role of altered lymphocytic reactivity as a mechanism for the development of alcoholic liver disease.

Summary. To study the role of immunologic reactions in the development of alcoholic liver disease, cytotoxic activity of lymphocytes against autologous liver grown in tissue culture was investigated. The experimental model was that of ethanol-fed baboons which develop the entire spectrum of alcoholic liver disease. Liver tissue was obtained at laparatomy in 12 animals given alcohol for 16–38 months and in 12 pair-fed controls. Lymphocytes of all alcohol-treated animals (but not of the controls) exhibited cytotoxicity against autochthonous liver cells: there was a marked decrease in the number of surviving cells and a significant reduction in their [^{3}H]proline label.

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