

HLA-DR Positive Epidermal Langerhans' Cells in Liver Cirrhosis and Immunosuppressed Liver Transplanted Patients

AMOS GILHAR¹, RAFAEL ENAT² and YAACOV BARUCH²

¹Skin Research Laboratory, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, and ²Department of Medicine B, Rambam Medical Center, Haifa, Israel

The aim of the present study was to evaluate the number of Langerhans' cells (LC) in immunosuppressed liver transplanted patients, compared to patients with liver cirrhosis and healthy volunteers. The detection of LC was performed in the epidermal sheet of each patient by using indirect immunoperoxidase and ATPase staining. A significant reduction in the number of LC was found in the liver transplanted patients as compared to patients with liver cirrhosis and healthy volunteers. This reduction may be related to prolonged treatment with corticosteroids and azathioprine. Key words: Cyclosporine; Corticosteroids; Azathioprine.

(Accepted August 2, 1993.)

Acta Derm Venereol (Stockh) 1994; 74: 93–94.

A. Gilhar, Skin Research Laboratory, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, POB 9649, Haifa 31096, Israel.

The dermatological literature deals widely with the side effects of topical steroids but to a lesser extent with the systemic treatment (1, 2). We found it important to evaluate the number of epidermal LC in liver transplanted patients during and after prolonged treatment with systemic steroids.

MATERIALS AND METHODS

Subjects

Sixteen patients (6 women and 10 men) with liver disease of various etiologies were included in this study (Table I). The patients were divided into two groups. One group consisted of cyclosporine (CyA)/prednisone and azathioprine immunosuppressed liver transplant patients (mean age 46 ± 12 years) who were treated for at least 1 year. The second group consisted of 8 patients (mean age 49 ± 7.5 years) with clinical and laboratory signs of liver cirrhosis. The dosage of steroids ranged from 10 to 20 mg/day; that of cyclosporine was 5 mg/kg/day, and azathioprine was given at a low dose of 50 mg/day. Two punch biopsies (3 mm) were obtained from each subject to stain for ATPase activity and HLA-DR. The biopsies were taken from neighbouring unexposed skin of the thigh. Local anesthesia with 1% lidocaine was used. Control skin biopsies were obtained from 8 healthy volunteers of the same age. Informed consent was obtained from all subjects participating in the study.

Immunohistochemical staining

Identification of HLA-DR positive cells in the epidermis was performed as described previously (3). Epidermal sheets were obtained by immersing the skin in 2N sodium bromide for 45 min at 37°C. They were fixed in cold acetone, rehydrated with phosphate-buffered saline (PBS), incubated for 1 h with the test antibody, and washed with PBS. A mouse monoclonal antibody against human DR antigen which binds to the Ia-like antigens of human LC was used in this study (Becton-Dickinson, Palo Alto, CA).

The presence of tissue-bound antibody to target antigen was assessed by the indirect immunoperoxidase technique (Vectastain ABC immuno-

peroxidase-staining procedure and reagents, mouse IgG PK 4002 kit, Vector Laboratories Inc., Burlingame, CA, USA). Incubation with the secondary reagents was for 30 min at room temperature, followed by washing with PBS and incubation for 30 min with ABC reagent. After washing with PBS the sheets were incubated for 5 to 7 min in 3-amino- α -ethyl-carbazol and washed in tap water before being mounted in glycerol.

The number of ATPase positive cells were determined as described previously (4). The mean number of HLA-DR and ATPase positive cells per unit was determined by random counts in 15 fields at $\times 400$ magnification using an ocular grid of known area. Results were expressed as number of LC/mm².

Statistical analysis was carried out using the Student's *t*-test.

RESULTS

The mean number of LC/mm² in the transplanted group was lower than in the control group (288 ± 111 and 476 ± 75 , respectively, $p < 0.0001$) and the liver cirrhosis group (509 ± 105 , $p < 0.0001$). Table I shows the number of HLA-DR positive LC in each subject.

The lowest number of LC was observed in the transplanted group. In patient 6 (Table I) only 60 HLA-DR positive cells/mm² were detected. This patient had signs of graft rejection with ongoing active hepatitis B. No significant difference was noted between the mean number of positive ATPase and HLA-DR dendritic cells in all groups.

DISCUSSION

The LC is a dendritic cell capable of binding various allergens and migration to the lymph nodes for antigen presentation to T-cells (5, 6). This study demonstrates that the number of LC is reduced in skin biopsied from immunosuppressed liver transplanted patients. However, no reduction was noted in the liver cirrhosis group. The significant reduction of ATPase positive dendritic cells may reflect a marked decrease in epidermal LC population (1). Shaieb et al. (7) described a similar decrease in number of HLA-DR and ATPase LC in patients treated systemically with steroids. In the present study, however, the immunosuppressed patients were also treated with azathioprine and thus it cannot be determined which immunosuppressive drug reduced the number of LC. Servitje et al. (8) found a significant decrease in the LC numbers in patients treated with azathioprine and prednisolone, as compared to patients treated with CyA and prednisolone. All our transplanted patients were treated for at least 1 year with a combination of prednisone/CyA/azathioprine. Urabe et al. (9) and our group (4) have previously shown that systemic administration of CyA had no effect on the density and distribution of human LC. There is, however, increasing evidence to show that topical and systemic steroids can cause a

Table I. Epidermal LC/mm² in 8 immunosuppressed liver transplanted patients versus 8 cirrhotic and 8 volunteer subjects

PBC: primary biliary cirrhosis, HBV: hepatitis B virus, HVB PNC: hepatitis B related post necrotic liver cirrhosis.

Transplanted patients			Liver cirrhosis patients			Control subjects	
Disease	HLA-DR	ATPase	Disease	HLA-DR	ATPase	HLA-DR	ATPase
PBC	216	198	PBC	483	468	640	581
PBC	218	209	PBC	425	422	400	406
HBV PNC	384	385	HBV PNC	681	593	416	389
HBV PNC	332	324	HBV PNC	547	501	506	486
HBV PNC	310	296	HBV PNC	429	380	514	467
Ongoing HBC	60	51	Idiopathic PNC	637	596	483	435
Wilson's Dis.	440	343	Idiopathic PNC	528	518	393	349
Wilson's Dis.	332	288	Idiopathic PNC	346	326	457	438
Mean	288	262		509	475	476	444

decrease in the number of HLA-DR positive LC and inhibit T-cell activation by LC (2, 10, 11).

In the present study the decrease in LC does not seem to be related to the primary disorder, since no alteration was found in patients with liver cirrhosis not subjected to liver transplantation and immunosuppressive treatment.

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