SHORT REPORTS

Natural and Chang Liver Cell Cytotoxic Studies of Methotrexate-treated Psoriatic Patients with Liver Cirrhosis

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Forty patients with psoriasis were investigated for unspecific and specific lymphocyte cytotoxicity in order to evaluate the significance for liver cell damage in methotrexate-treated (MTX) patients. We found no difference between psoriasis patients with regard to cytotoxicity. We also observed a normal proliferation of lymphocytes after stimulation with tuberculin and phytohemagglutinin. *Key words: Chang liver cells; Natural killer cell; Lymphocytes.* (Received March 2, 1987.)

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Methotrexate (MTX) therapy of psoriasis may lead to a mild cirrhosis in more than 25% of the patients treated for more than five years (1). We have previously reported that patients developing cirrhosis have an increased percentage of HLA-A8 (2), a tissue type associated with autoimmune chronic active hepatitis (3). Therefore, we studied the lymphocyte reactivity in psoriatic patients in order to see, if the development of cirrhosis was accompanied by an increased lymphocyte cytotoxicity towards liver cells.

PATIENTS AND METHODS

Four groups of psoriasis patients were studied: Group A included seven women and twelve men with a median age of 38 years, range 16–80 years. All suffered from psoriasis vulgaris with a duration ranging between six months and 42 years. None had received MTX previously and none had arthritis. All had normal serum levels of liver transaminases. In only one patient there was a liver biopsy performed and its histology was normal.

Group B included six women with an age range between 41 and 68 years with psoriasis vulgaris of 6–50 years duration. All were previously or presently receiving MTX for their disease. Four had arthritis. Liver histology was examined in four patients and found normal. Liver transaminases were normal.

A group with minor liver changes (Group C) included three women and four men, age range 29 to 69 years. All were presently or previously treated with MTX for psoriasis vulgaris. One had psoriasis arthritis. Liver biopsy showed fibrosis (four) or steatosis (three).

A group with cirrhosis (Group D) included two women and six men, age range 51 to 77 years, with psoriasis of 21 to 50 years duration. Seven had psoriasis vulgaris, one pustular psoriasis of the acral form. Seven had received MTX between six months and 13 years, one patient had not received MTX, but was included because he had an active alcoholic cirrhosis. Three patients suffered from arthritis. All had liver biopsies, which showed cirrhosis, probably alcoholic cirrhosis in two patients.

Control persons were twelve persons without psoriasis or liver disease.

Liver histology

MTX induced liver changes (MTX cirrhosis) are characterized by multiple, small areas of fibrosis with slight infiltration of lymphocytes. These areas of slight fibrosis are scattered through the liver tissue without zonal localization contrasting to the piece-meal necrosis seen in chronic active hepatitis and its ensuing liver cirrhosis.

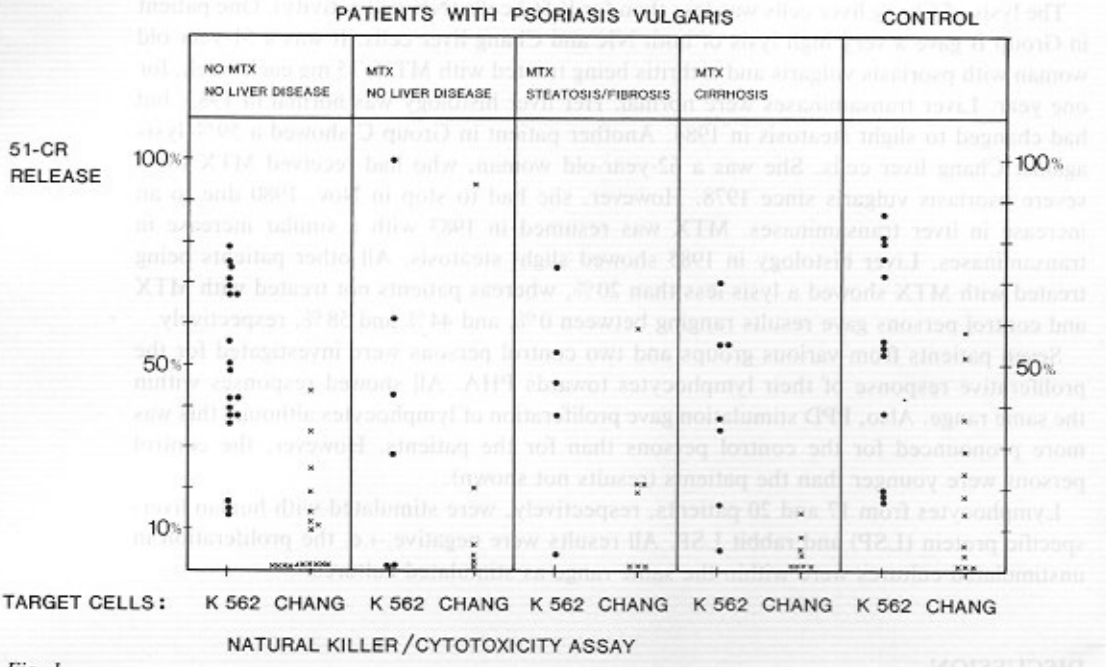

Fig. 1.

Lymphocyte studies

Mononuclear cells were isolated from peripheral blood. Technical details have been described elsewhere (4).

Cytotoxicity studies

Natural killer cell activity (NK) was studied using the erythroleukaemic cell line K-562 as target cells (kindly provided by Keld Kaltoft and Susanne Bisballe, Institute of Human Genetics, Univ. of Aarhus). The lymphocyte : target cell ratio was 10 : 1, 25 : 1 and 100 : 1. Following a 4-hour incubation specific Cr-51 release was measured and expressed as the percentage of releasable radioactivity (for technical details, see ref. 5). Cytotoxicity was expressed as the highest percentage of lysis found.

Chang liver cell cytotoxicity

The Chang liver cells were purchased from Gibco (no. 051-4004). They could be labelled with Cr-51 and the spontaneous Cr-51 release was less than 2% of the maximal radioactive incorporation. The cytotoxicity assay was performed as for NK with three identical lymphocyte : target cell ratios and a similar incubation period. The degree of cytotoxicity was calculated as above.

Lymphocyte transformation tests

Lymphocytes were stimulated in flat-bottomed 96-well microculture plates for 72 or 96 hours at a concentration of 2×10^5 cells per well. The mitogens were phytohaemagglutinin (0.3, 1, and 3 μg PHA/ml) which was known to give suboptimal to maximal proliferative responses, purified protein derivative of tuberculin (PPD; 3.3 and 10 μg/ml), and human and rabbit liver specific protein (LSP) kindly produced and provided by Dr MacFarlane, Univ. of London (6). This antigen was used in three concentrations (0.29, 0.88 and 2.65 μg/ml). LSP cultures were kept for both 5 and 7 days. Harvesting and counting was done as described elsewhere (4).

RESULTS

The cytotoxicity studies are depicted in Fig. 1. It is seen that all control persons had NK cell activity of their peripheral blood lymphocytes. This was also found in all patients with psoriasis, independent of MTX therapy or presence of liver changes.

The lysis of Chang liver cells was less than for K 562 cells (NK cell activity). One patient in Group B gave a very high lysis of both NK and Chang liver cells. It was a 51-year-old woman with psoriasis vulgaris and arthritis being treated with MTX, 15 mg each week, for one year. Liver transaminases were normal. Her liver histology was normal in 1983, but had changed to slight steatosis in 1984. Another patient in Group C showed a 59% lysis against Chang liver cells. She was a 62-year-old woman, who had received MTX for a severe psoriasis vulgaris since 1978. However, she had to stop in Nov. 1980 due to an increase in liver transaminases. MTX was resumed in 1983 with a similar increase in transaminases. Liver histology in 1985 showed slight steatosis. All other patients being treated with MTX showed a lysis less than 20%, whereas patients not treated with MTX and control persons gave results ranging between 0%, and 44% and 58%, respectively.

Seven patients from various groups and two control persons were investigated for the proliferative response of their lymphocytes towards PHA. All showed responses within the same range. Also, PPD stimulation gave proliferation of lymphocytes although this was more pronounced for the control persons than for the patients. However, the control persons were younger than the patients (results not shown).

Lymphocytes from 17 and 20 patients, respectively, were stimulated with human liver-specific protein (LSP) and rabbit LSP. All results were negative, i.e. the proliferation in unstimulated cultures were within the same range as stimulated cultures.

DISCUSSION

Our results do not indicate an increased lymphocyte reactivity towards LSP or Chang liver cells in MTX-treated psoriatics with liver cirrhosis. We found that the NK cell activity and Chang liver cell cytotoxicity was within the normal range, and lymphocytes from the patients did not respond to stimulation with LSP as has been found in patients with chronic active hepatitis (CAH) (7).

Other investigations have presented indirect evidence for an increased immune reactivity in psoriasis patients. Veien et al. (8) found an increased occurrence of Ig-bearing lymphocytes in liver biopsies with steatosis or cirrhosis during MTX treatment, and a decreased non-specific suppressor cell actitivy has also been observed (9).

One should also consider the possibility that certain susceptible individuals (e.g. those with HLA-B8) may react abnormally to a drug-related neoantigen on hepatocyte surfaces in a manner similar to that which has been suggested for halothane hepatitis, alcoholic liver disease and methyl-DOPA-induced chronic active hepatitis. The histology of MTX-induced cirrhosis is however not identical with that of chronic active hepatitis. Although an increased immune reactivity cannot be totally excluded, it was not apparent in our present investigations.

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HLA-DR and DQ Antigens in Lichen planus

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A study on HLA-DR and DQ typing in 40 patients with lichen planus (26 males and 14 females) and in 92 normal blood donors of both sexes was performed. Twenty-seven patients had cutaneous lesions, 11 cutaneous and mucosal involvement and 2 patients had oral erosive lesions. Serological typing revealed a highly significant increase of HLA-DR1 antigen in the patient group. No difference has been observed on the prevalence of DR1 antigen among the different clinical status of the disease. (Received May 8, 1987.)

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Lichen planus (LP) is a chronic papulosquamous disease that can affect skin and mucous membrane. The current literature about the pathogenesis of LP reflects a growing acceptance of a T-cell mediated immune mechanism (1–8). LP has been reported in patients with lupus erythematosus, myastenia gravis, alopecia areata and vitiligo, bullous pemphigoid and primary biliary cirrhosis. The detection of immune reactans at the basement membrane zone using direct immunofluorescence technique (9) and the recent demonstration of circulating antibodies reactive with epidermal antigens in patients with LP (10) are consistent with a proposed autoimmune pathogenesis.

Familial LP has been described (11–12) and an early HLA-study of LP patients showed HLA-A3 antigen to be significantly increased (13), but subsequent studies did not demonstrate statistically significant differences from normal controls (14–15).