Lymphopenia and lymphocyte transformation in alcoholics[1]

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Summary. The basis of peripheral blood lymphopenia observed in patients with chronic alcoholism and liver disease was investigated by examining the effect of sera of these patients on in vitro transformation of normal human peripheral blood lymphocytes. A positive correlation was demonstrated between the serum inhibition of phytohaemagglutinin- and pokeweed mitogen-induced transformation and the degree of lymphopenia. Thus serum factors may contribute to the observed lymphopenia by inhibiting lymphocyte production in vivo.

Reduced numbers of circulating T-lymphocytes have been reported in patients with alcoholic liver disease[2] but it is not generally recognized that peripheral blood lymphopenia may accompany this disorder[3,4]. During an investigation of the immune status of chronic alcoholics we were impressed by the frequency of lymphopenia in these patients, particularly those with alcoholic hepatitis or cirrhosis. In the majority, causes for the observed lymphopenia, such as uraemia, severe folic acid deficiency and viral infection were not present. In agreement with previous reports we found that sera from these patients could suppress in vitro lymphocyte transformation[5-7]. In view of the possible in vivo significance of this suppression we have examined the correlation between the observed lymphopenia and corresponding inhibitory effect of serum in a large pool of patients.

Subjects. A total of 32 hospitalized chronic alcoholics were studied. Chronic alcoholism was defined as a daily alcohol consumption of greater than 100 g for 10 years. Criteria for exclusion were: uraemia, severe infection, neoplasia and treatment with immunosuppressive drugs. On the basis of clinical, biochemical and liver histological findings patients were arbitrarily divided into 2 groups: a) 9 with normal or fatty livers and normal liver function b) 23 with alcoholic cirrhosis and/or hepatitis[4].

Materials and methods. At the time that peripheral venous blood was obtained from each patient by venepuncture, the peripheral blood lymphocyte count was determined by standard methods. Lymphocyte transformation was carried out by the microculture technique[8] using lymphocytes isolated by Ficoll-Hypaque gradient centrifugation from healthy, non-alcoholic controls. Cells were cultured at 37 °C in a 5% CO_2 humidified air mixture in the presence of 4 µg/ml pokeweed mitogen (Grand Island Biological Co., N.Y.) or 46 µg/ml purified phytohaemagglutinin (CSL, Melbourne) in either 20% patient serum or pooled human serum. 4 h prior to harvesting at 72 h 0.5 µCi of ^{3}H-thymidine was added to each culture. Radioactivity was measured in trichloroacetic acid precipitates in a Packard liquid scintillation counter. The correlation coefficient (r) was determined by standard statistical methods and significance determined by Student's t-test[9].

Results. In 24 normal controls the mean absolute peripheral blood lymphocyte count was 2,570±120 μl^{-1} (±SE). In alcoholics with normal or fatty livers the observed mean was 1,560±261 lymphocytes μl^{-1} ($p < 0.005$) while that observed in patients with alcoholic hepatitis and/or cirrhosis was 1,262±141 cells μl^{-1} ($p < 0.001$). Figures 1 and 2 show the relationship between absolute lymphocyte counts of individual patients and the corresponding pokeweed mitogen- and phytohaemagglutinin-stimulated transformation of normal human peripheral blood lymphocytes in each patient's serum. With either mitogen a significant positive correlation was observed between the degree of lymphopenia and observed lymphocyte transformation.

Discussion. These results indicate that a significant relationship exists between these 2 parameters, i.e. alcoholics with the most marked lymphopenia tended to have the sera most inhibitory to lymphocyte transformation. This suggests that the serum inhibitory factors (SIF) responsible may have an important in vivo effect, giving rise to lymphopenia by suppressing lymphocyte DNA-synthesis and hence production. At the present time the nature of SIF is not yet elucidated but as lymphopenia is more marked in alcohol-

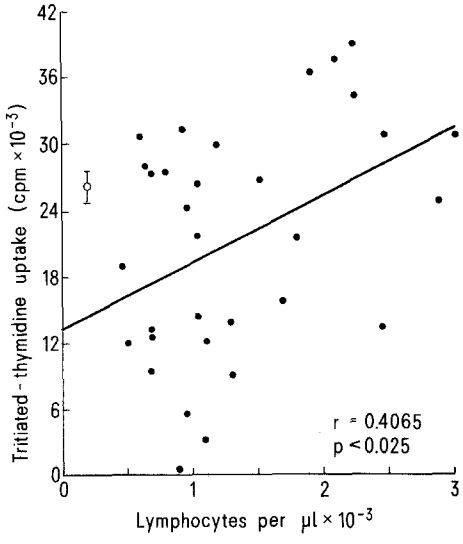

Fig. 1. Relationship between the peripheral blood lymphocyte count and phytohaemagglutinin-P-induced normal lymphocyte transformation (cpm/1.25 × 10^5 cells) cultured in 20% patient serum. Mean transformation±SE of lymphocytes in normal pooled human serum is indicated thus, ⊢○⊣.

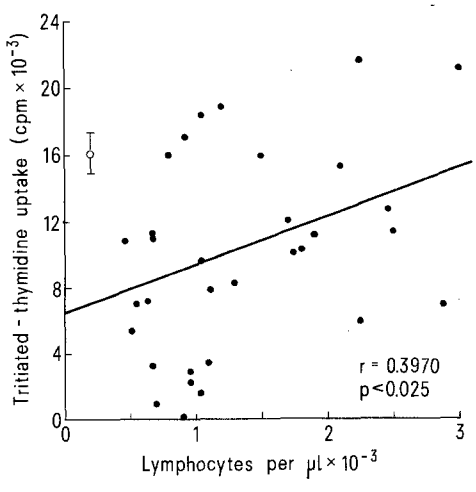

Fig. 2. Relationship between the peripheral blood lymphocyte count and pokeweed mitogen-induced normal lymphocyte transformation (cpm/1.25 × 10^5 cells) cultured in 20% patient serum. Mean transformation±SE of lymphocytes in normal pooled human serum is indicated thus, ⊢○⊣.

ics with hepatitis and/or cirrhosis, the liver dysfunction may be responsible for the presence of SIF in the peripheral circulation rather than the alcoholic state per se.

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Alkaline phosphatase in human lymphocyte subpopulations

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Summary. The levels of membrane alkaline phosphatase have been measured on different lymphocyte fractions from human peripheral blood separated on bovine serum albumin discontinuous gradients. The peak in enzyme activity was observed in a non-T-, non-B-cell fraction, rich in 'null' lymphocytes; the lowest values were found in the fraction with the highest proportion of T-cells.

Alkaline phosphatase (E.C. 3.1.3.1.) has been demonstrated on the surface of normal and leukaemic lymphocytes[1-3]. No correlation has been established so far between enzyme activity and any particular lymphocyte subpopulation. Ruuskanen et al.[1] suggested that it is present in immature T-cells, based on their findings in guinea-pig thymocytes. High values of alkaline phosphatase were recently demonstrated in the mononuclear cells of human cord blood[3], which contains a low proportion of T-lymphocytes and a high proportion of cells negative for T- and B-cell markers ('null')[4,5].

In the present study we have investigated the levels of enzyme activity in the different lymphocyte fractions from human blood separated by bovine serum albumin (BSA) discontinuous gradient.

Materials and methods. Blood samples collected in CPD (citrate-phosphate-dextrose) were obtained from 4 normal adult donors, washed once and diluted 1:1 with sodium chloride. The mononuclear cell population was separated with 'Lymphoprep' (Nyegaard & Co., AS., Oslo, Norway), washed twice with TC 199 medium, resuspended in a small volume (3 ml) and then layered on a BSA discontinuous gradient as modified from Dicke et al.[6]. Briefly, decreasing concentrations of BSA solutions (from 35% to 11%, in 4% decrements) were prepared and 3 ml of each fraction was carefully laid on top of the fraction with the next highest concentration of BSA. The interface between 11% and 15% BSA was considered as fraction 1, and so on, up to fraction 6, between 31% and 35% BSA. The gradients with the lymphocytes (between 19×10^7 and 50×10^7) were centrifuged at 10 °C for 30 min at $900\times g$. Cells from each interface were collected and washed 3 times with TC 199 medium. On each fraction and on a sample of cells before BSA separation the following tests were performed:

1. T-lymphocyte percent was assessed by the capacity for forming rosettes with papain-treated sheep RBC (E-rosettes)[7] (blood lymphocytes of normal adults 62% ± 8 SD).
2. Surface immunoglobulins were assessed by direct immunofluorescence with a polyvalent $F(ab)_2$ antiserum to human Ig; samples were incubated at 37 °C in serum-free medium for 1–2 h before adding the antiserum (blood lymphocytes of normal adults 14.1 ± 4.8 SD).

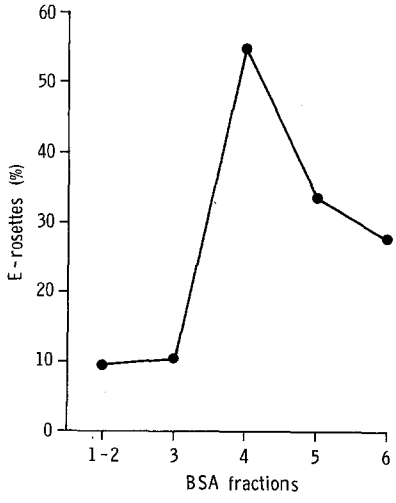

Fig. 1. Normal human blood separated on a BSA discontinuous gradient: distribution of T-lymphocytes as shown by percentage of E-rosette forming cells in each fraction.

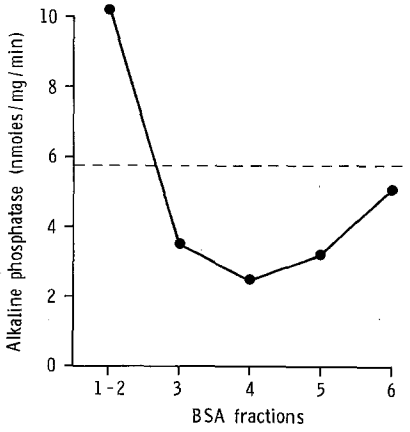

Fig. 2. Normal human blood separated on a BSA discontinuous gradient: levels of alkaline phosphatase activity (the broken line shows the mean value for unseparated normal blood lymphocytes). Results are expressed as nmoles of activity per mg of protein per min.