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In vitro synthesis of antibody to specific bacterial lipopolysaccharide by peripheral blood mononuclear cells from patients with alcoholic cirrhosis

M. G. MUTCHNICK & D. F. KEREN *Departments of Medicine and Pathology, University of Michigan Medical Center and Research Service, Veterans Administration Medical Center, Ann Arbor, Michigan, U.S.A.*

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Summary. An enzyme-linked immunosorbent assay was used to detect antibody to specific bacterial lipopolysaccharide (LPS) in serum and in pokeweed mitogen (PWM) stimulated culture supernatants of peripheral blood mononuclear cells from four patients with alcoholic cirrhosis (AC). Antibody to LPS (derived from a single strain of *Escherichia coli* isolated from each patient's stool), was detected in the sera of each patient to a 10^{-4} dilution. Only one of four control sera was positive at the 10^{-4} dilution, with the others positive at a 10^{-3} dilution. Antibody to LPS was detected in the culture supernatants in three of the four patients and in none of the control subjects. Supernatants from patient cultures pretreated with mitomycin C or harvested after 1 day of incubation did not have detectable antibody. These results indicate that we can expand, *in vitro*, the population of peripheral blood B lymphocytes obtained from patients with AC and cause them to synthesize antibody against specific LPS from their own gut flora.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; KLH, keyhole limpet haemocyanin; LPS, lipopolysaccharide; PS-LPS, patient specific lipopolysaccharide; PA-LPS, Pseudomonas aeruginosa lipopolysaccharide; AP, alkaline phosphatase; Hepes, N-2-hydroxyethyl-piperazine-N-2-ethane sulphonic acid; PWM, pokeweed mitogen; PBM, peripheral blood mononuclear cells; PTA, phosphate-buffered saline with Tween 20 and sodium azide; OD, optical density; AC, alcoholic cirrhosis; Ig, immunoglobulin.

Correspondence: Dr M. G. Mutchnick, Research Service, VA Medical Center, 2215 Fuller Road, Ann Arbor, Michigan 48105, U.S.A.

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INTRODUCTION

Polyclonal hypergammaglobulinaemia is frequently observed in patients with chronic liver disease. Previous studies have reported that unstimulated cultures of peripheral blood mononuclear cells (PBM) isolated from patients with alcoholic cirrhosis (AC) synthesize larger quantities of immunoglobulin (Ig) than comparable cultures of PBM from normal, healthy controls (Mutchnick, Lederman, Missirian & Johnson, 1981). To date, the specificity of the newly synthesized Ig has not been determined in PBM cultures of patients with AC. This is due to the difficulty encountered in developing an assay of sufficient sensitivity to measure small amounts of specific Ig.

The enzyme-linked immunosorbent assay (ELISA) has seen increased use for the serological detection of specific antibodies to a variety of antigens derived from micro-organisms (Carlsson, Hurvell & Lindberg, 1976; Yardley, Keren, Hamilton & Brown, 1978).

The ELISA technique is particularly sensitive and has been shown to be capable of detecting as little as 1·3 ng of isotypically-specific antibody against lipopolysaccharide (LPS) per ml of immune serum (Keren,