MODULATION OF CHOLESTATIC FACTOR PRODUCTION BY SERUM COMPONENTS

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Summary

When lymph node cells from sensitized guinea pigs were stimulated in vitro with a specific antigen and their culture supernatant was injected into the mesenteric vein of rats, a marked decrease in bile flow was demonstrated. The treatment of activated lymphocytes with a higher molecular weight fraction of normal human serum Fr-1 and Fr-2 was shown to decrease the reduction of bile flow. Conversely, a lower molecular weight fraction of serum (Fr-3) was found to augment the reduction of bile flow. These findings suggest that the serum components may regulate the production of a factor (or factors) causing the decrease in bile flow from the activated lymphocytes.

Key Words: cholestatic factor, serum components.

Introduction

In recent years considerable efforts have been made to elucidate the physiological mechanism of bile excretion and change leading to intrahepatic cholestasis. Although much is still unknown, we previously reported that a reduction in bile flow and bile acid excretion could be induced by some kind of lymphokine which is produced from sensitized lymphocytes stimulated by specific antigens1-5): When the culture supernatant of peripheral blood lymphocytes from patients with drug-induced allergic cholestatic hepatitis stimulated in vitro with a specific drug in the presence of a liver soluble fraction was injected into the mesenteric vein of rats, a marked decrease in bile flow and bile acid excretion was demonstrated. This was also the case in some cases of acute viral cholestatic hepatitis in which the peripheral blood lymphocytes from patients were stimulated with a liver soluble fraction containing liver specific lipoprotein (LSP)6). After injection of the culture fluid of activated lymphocytes into the mesenteric vein, histological changes such as dilated bile canaliculi and diminution of surrounding microvilli were observed by electron microscopy. Similar histological changes are demonstrable in biopsy specimens from patients with intrahepatic cholestasis. Furthermore since no such decrease
in bile or histological changes was induced in rats by injection with lymphocyte culture supernatant prepared from normal individuals in an identical manner, it was thought that one or more factors causing intrahepatic cholestasis were produced by the sensitized lymphocytes by specific stimulation. We have designated this active material as the cholestatic factor. This cholestatic factor was also produced from the lymph node cells of sensitized guinea pigs by stimulation with a specific antigen. In a previous report\(^1\), we showed that activated macrophages had cytotoxic effect on isolated hepatocytes and this macrophage-mediated hepatocytotoxicity was modulated by serum components. A higher molecular weight component of normal human serum (Fr-1) was shown to reduce the macrophage-mediated hepatocytotoxicity and enhance the secretion of plasminogen activators from the activated macrophages. Conversely, a lower molecular weight component of serum (Fr-3) was found to enhance the hepatocytotoxic potential of activated macrophages and reduce their production of plasminogen activator. In the present work, we investigated whether the production of cholestatic factor by activated lymphocytes is modulated by serum components.

**Materials and Methods**

1. Sensitization of guinea pigs.

Ten outbred Hartley strain guinea pigs, weighing from 400 g to 500 g, were immunized by injecting with a mixed suspension containing 1.5 ml of Arlocel, 8.5 ml of Bayol F and 5 mg of heat-killed Mycobacterium tuberculosis (H37Ra). Each footpad was injected with 0.1 ml of the suspension and 0.6 ml was injected into nuchal muscle. More than four weeks after the injection delayed hypersensitivity to PPD was induced in all animals.

2. Preparation of lymph node cell suspension.

Sensitized guinea pigs were sacrificed by exsanguination, and the cervical, axillary and inguinal lymph nodes were extirpated aseptically. They were placed in cold Hanks' solution, the adipose tissue was removed and cut into small pieces. Subsequently they were squeezed gently between two glass plates in cold Hanks' solution to free the lymphocytes. The resulting cell suspensions were mixed by pipetting and filtered through two sheets of aseptic cheese cloth followed by centrifugation at 150xg for 10 min at a cold temperature. The pellets were resuspended in cold Eagle MEM containing 10% fetal calf serum, 100 units/ml of penicillin and 100 \(\mu g/ml\) of streptomycin to make a cell suspension of 1 \(\times\) 10\(^7\) cells/ml. The cells were mainly composed of lymphocytes.

3. Preparation of culture supernatant of the activated lymphocytes.

The lymph node cells prepared from the sensitized guinea pigs were stimulated in vitro with PPD (10 \(\mu g/ml\)) at 37°C for 24 hours in a humidified cell-incubator with aeration by 5% \(CO_2\) in air. After incubation, the cells were washed twice with Eagle MEM solution and the serum fraction was added as described below, followed by incubation at 37°C for 2 hours. The cells were then washed twice with Eagle MEM solution and incubated in fetal calf serum-free medium at 37°C for 48 hours. The culture supernatant was then separated by centrifugation for assay of cholestatic factor activity.


One ml of culture supernatant was injected into the mesenteric vein of six male Wistar rats weighing 250 to 350 g. We collected the bile excreted 30 min before and in the three subsequent one-hour periods after injection of the culture supernatant. The volume of bile was measured in each sample. The reduction rate of bile flow was calculated by comparison with the quantity of bile flow before injection.

5. Fractionation of serum.