MOUSE HEPATOCYTE SYNTHESIS AND INDUCTION OF THE ACUTE PHASE REACTANT: SERUM AMYLOID P-COMPONENT

PHONG T. LE AND RICHARD F. MORTENSEN

Department of Microbiology, Ohio State University, Columbus, Ohio 43210

(Received 28 November 1983; accepted 2 March 1984)

SUMMARY

A methodology for obtaining reproducible in vitro induction of the synthesis of the acute phase reactant serum amyloid P-component (SAP) by purified mouse hepatocytes was established. Optimal hepatocyte culture conditions for the induction and synthesis of SAP required certain hormones, a substratum for cell attachment, and activated macrophages. Leibowitz L15 medium had to be supplemented with dexamethasone, indomethacin, insulin, glucose, and fetal bovine serum. Purified mouse IL 1 could substitute for activated macrophages in the induction of SAP. Hepatocytes were allowed to adhere to a collagen matrix to enhance both cell viability and SAP synthesis induced by IL 1. Elicited macrophages cultured with hepatocytes were capable of augmenting SAP synthesis in the presence of IL 1.

Key words: mouse hepatocytes; acute phase reactants; serum amyloid P-component.

INTRODUCTION

Inflammation is the primary nonspecific host defense response to microbial infections or tissue damage. An inflammatory response is often initiated locally but is rapidly translated into a systemic response characterized in part by fever, leukocytosis, and an increase in the synthesis of a group of plasma proteins collectively termed acute phase reactants (APRs). We have developed a mouse system for studying the increase in the level of serum amyloid P-component (SAP), which is a glycosylated acute phase protein of mice (1) and a structural homologue of the prototypical APR of humans, C-reactive protein (CRP) (2). Serum amyloid P-component exhibits Ca"" dependent binding to agarose (3), heparin (4), and amyloid fibrils (5). It appears by electron microscopy as two stacked pentamers (2), each composed of five 31.0 kd identical subunits (6). Serum amyloid P-component is structurally and antigenically distinct from the precursor of the major fibril component of amyloid, serum amyloid A protein (SAA), which is also an acute phase reactant of mice (7).

The liver has been shown to be the only site of synthesis of APRs (8). Kushner and Feldmann (9) have shown that rabbit hepatocytes staining for cytoplasmic CRP are adjacent to the portal circulation and are recruited sequentially by a circulating mediator. Hepatocyte SAA synthesis in mice has been shown to be inducible both in vivo (10) and in vitro (11) by macrophage culture supernatant fluids containing IL 1. Haptoglobin is an alpha 2-plasma glycoprotein acute phase reactant in the rat and has also been shown to be induced in vitro with a partially purified human leukocytic pyrogen (12). A role for a circulating mediator as an inducer of APRs is thus widely appreciated. Our earlier work with mice demonstrated that increased SAP synthesis could be induced by a latent phase, early acute phase serum, a crude macrophage supernatant fluid and a partially purified human IL 1 preparation (6). The extent of the SAP response among inbred mouse strains was associated with the ability of macrophages from the different strains to elaborate IL 1 (13). We wished to extend these findings to examine the interaction of IL 1 with hepatocytes under controlled in vitro culture conditions that would permit the detection of induction of SAP synthesis. Several recent successful attempts to achieve functional mouse hepatocyte cultures led us to develop the method described herein for SAP synthesis and induction. The methodology should be applicable to the
study of the cellular and molecular events responsible for both induction and suppression of the synthesis of plasma proteins, especially acute phase reactants.

**MATERIALS AND METHODS**

**Animals.** Inbred C57BL/10ScN mice were purchased from Harlan-Sprague Dawley (Indianapolis, IN), kept in a Bio-Clean air chamber (Hazelton Labs), and fed ad libitum.

**Reagents.** The following reagents were purchased from Sigma Chemical Company (St. Louis, MO): collagenase Type IV, rat tail collagen, bovine serum albumin (BSA), alpha-D (+) glucose, dexamethasone (DXM), insulin, and indomethacin (IDM).

**Liver perfusion.** Mice were lightly anesthetized with sodium pentobarbital and the abdominal cavity opened. The inferior vena cava (abdominal portion) was ligated just above the right kidney with a 4-0 silk surgical suture (Ethicon, NJ). The animal rib cage was removed to expose the heart and the inferior vena cava. Canulation of the inferior vena cava was performed via an incision in the right atrium, using a 20-G blunt-end needle, which was sutured in place. The liver was perfused immediately with 50 ml of a Ca**²⁺**-free solution (8.3 g NaCl, 0.5 g KCl, 2.4 g HEPES, pH 7.4) (14) containing 15 mM glucose and 0.5 mM EGTA at a flow rate of 10 ml/min to remove all of the blood. Perfusion was continued immediately with 50 ml of Leibowitz L15 medium (GIBCO, Grand Island, NY) containing 100 U/ml of collagenase Type IV at the same flow rate (15). Intrahepatic pressure was regulated by alternatively opening and closing the severed hepatic portal vein. The completely blanched liver was removed en bloc and suspended in 5.0 ml of the L15 collagenase solution. The perfusion solutions were maintained at 37°C. The entire perfusion procedure was completed within 15 min.

**Preparation of single hepatocyte suspension.** The liver cells were forced out of the reticulum by gentle tweezing and washing through a wire mesh with 25 ml of Hanks’ balanced salt solution (HBSS) containing 5% fetal bovine serum (FBS). The cells were washed in 25 ml HBSS twice and once with Leibowitz L15 supplemented with 8 x 10⁻⁵ M glucose, 10⁻⁷ M bovine insulin (25.6 U/mg), 1.5 x 10⁻³ M BSA, 10⁻⁸ M DXM, 10 mM HEPES, and 15% FBS (HyClone Sterile Systems, Logan, UT) (modified L15). Hepatocytes were enriched in the pellet after centrifugation at 50 xg for 5 min at room temperature (16). The cells were resuspended at 2 x 10⁶ cells/ml in modified L15 medium and plated on a collagen-coated 6-well plate (35 mm) (2 x 10⁶ cells/well) or a 24-well plate (4 x 10⁵ cells/well) (Costar, Cambridge, MA) at 2.5 x 10⁴ cells/cm². The cells were incubated at 37°C in a humidified air chamber. Viability was determined by trypan blue dye exclusion. The entire process was completed within 1 h.

**Preparation of collagen-coated plates.** Multiwell plates (6 well and 24 well) were coated with 0.6 ml or 0.15 ml, respectively, of a solution of 1 mg/ml acid soluble rat tail collagen dissolved in L15 medium containing glacial acetic acid (1/1000 dilution). The pH of the collagen solution was adjusted to 7.4 with a predetermined volume of 0.15 M NaOH in L15 medium. To allow the collagen to gel the plates were incubated at 25°C for 18 h and the excess NaOH washed off with L15 medium just before use.

**Preparation of IL 1.** The IL 1 was purified from culture supernatants of the mouse macrophage line P388D1, which was superinduced as described by Mizel (17). The IL 1 purification was taken through the phenyl-Sepharose (Pharmacia, Piscataway, NJ) chromatography step, which removes all of the other proteins detectable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. The IL 1 activity was measured by the thymocyte proliferation assay (17). The preparation of IL 1 used in these experiments had 320 U/ml.

**Measurement of SAP.** Serum amyloid P-component was measured by an indirect competitive ELISA. A 96-well plate (Immulon II; Dynatech, Cambridge, MA) was coated with 50 µg of affinity purified SAP (18) in Na₂CO₃ (15 mM) and NaHCO₃ (35 mM) buffer, containing 3 mM NaN₃ pH 9.6, by incubating at 4°C for 18 h. In a second 96-well microculture plate (Costar), precoated with 100 µg bovine serum albumin (BSA), standards and test samples (10 µl) were allowed to react with rabbit anti-mouse SAP (IgG fraction) at a 1/2000 dilution (10 µl) in a total of 50 µl of 0.01 M phosphate buffered saline containing 0.02% Tween 20, pH 7.3, for 18 h at 4°C. The specificity of the anti-SAP antibody was as described earlier (6). At the end of the incubation period, the reaction mixtures were transferred to SAP-coated wells and incubated at room temperature for 2 h. The plate was washed six times with washing buffer (0.01 M PBS, 0.05% BSA, pH 7.3). The SAP-bound IgG