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STUDIES ON RAT CYTOKINES AS MEDIATORS OF THE ACUTE PHASE RESPONSE

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ABSTRACT: A cytokine preparation from rat peritoneal exudate cells was studied. The preparation was pronase sensitive and heat labile, but was insensitive to trypsin treatment. Administration to rats resulted in elevated serum levels of α_1-acid glycoprotein, sialyltransferase activities and cortisol, but depressed serum albumin levels; in addition, hepatic sialyltransferase activities were increased and hepatic β-galactosidase and β-N-acetylhexosaminidase activities were depressed.

Tissue injury caused by chemical inflammatory agents, bacterial infections, rheumatoid arthritis, tissue infarction and neoplasia results in biochemical and physiological changes known as the acute phase response (1-3). Examples of these changes include elevated levels of certain serum glycoproteins known as acute phase reactants, altered serum and hepatic enzyme activities, altered hormone levels and depressed albumin levels (1-3). Altered rates of synthesis and secretion by the liver are responsible for the changes in plasma protein levels during the acute phase response (1-5). Leukocytes, presumably stimulated at the site of tissue injury, are responsible for producing cytokines which are involved in mediating many aspects of the acute phase response including elevated hepatic synthesis of the acute phase reactants (6-9). While a great deal is known about these cytokines in humans, rabbits and mice (6-12), much less is known about the factors in the rat (6,13).

The present study describes the *in vivo* effects of rat cytokines in the rat. Administration of cytokines resulted in elevated α_1-acid glycoprotein and depressed albumin levels and affected other parameters that have been shown to be part of the acute phase response (2,14,15), these are: elevated serum

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sialyltransferase activities and cortisol levels and depressed hepatic activities of β-galactosidase and β-N-acetylhexosaminidase.

MATERIALS AND METHODS: Materials were obtained as follows: CMP-[^{14}C]-NeuAc (247 mCi/mmole), NEN Corp., Lachine, P.Q.; Cortisol radioimmunoassay kit, Clinical Assays, Travenol Laboratories Inc. Cambridge, Mass.; Pronase, Calbiochem, La Jolla, Ca., E Toxate assay kit, Slipper limpets type VIII glycogen, phenol extracted E. coli lipopolysaccharide, trypsin, heparin, streptomycin sulfate, penicillin G, imidazole, Sigma Chemical Co., St. Louis, Mo. Sterile, pyrogen-free water was prepared using a Milli-RQ System (Millipore Corp., Bedford, Mo.). Protein was assayed as described by Lowry et al. (16) but with modified reagents and volumes as described by Miller (17).

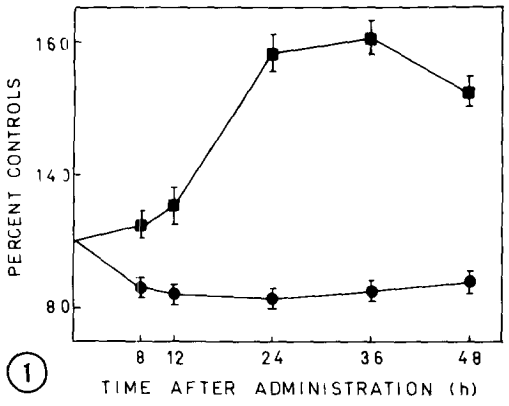
Male Long Evans Hooded rats were used in all experiments and were treated as previously described (18). Cytokines were prepared from peritoneal exudate cells by a modification of the procedure of Kampschmidt et al. (19). Rats were injected i.p. with 20 ml 0.2% glycogen, 10 μg/ml lipopolysaccharide, 0.15 M NaCl 0.5 mg/ml streptomycin sulfate and 100 U/ml penicillin. Rats were sacrificed by etherization 18 h after infusion, the abdomens were swabbed with 70% ethanol, and 20 ml 0.15 M NaCl, 0.5 mg/ml streptomycin sulfate, 100 U/ml penicillin injected i.p. The peritoneum was massaged gently, peritoneal exudate was removed with a syringe and heparin added to a concentration of 10 U/ml. Exudates containing large numbers of erythrocytes were discarded. Cells were collected by centrifugation at 800 g_{av} for 10 min at 4°C and then suspended in 0.168 M NH_4Cl to lyse erythrocytes (20), pelleted and washed three times with 0.15 M NaCl. Cell density was determined with a hemocytometer, cells were suspended at 1 x 10^8 cells/ml in 0.15 M NaCl and incubated for 2 h at 37°C with gentle shaking. Cells were then removed by centrifugation and the supernatant taken as the cytokine preparation. Unless otherwise noted, cytokines were administered within hours of preparation. All solutions injected into animals were sterilized by filtration through Millex-GV disposable filters (Millipore Corp.), cytokine preparations were tested for endotoxin contamination using the E Toxate assay kit. Pronase and trypsin digestions were at a concentration of 0.1 mg/ml for 3 h at 37°C and were stopped by heating at 100°C for 1 min. Dialysis was with Spectropor 1 tubing (Fisher Scientific, Pittsburg, Pa.) which was sterilized by heating in a boiling water bath for 2 min. Concentration was with an Amicon Ultrafiltration Cell and UM-2 membranes (Amicon Corp., Lexington, Mass.).

Sialyltransferase (EC 2.4.99.1) assays were performed as described by Baxter and Durham (21) using asialo-α_1-acid glycoprotein as acceptor prepared by hydrolysis of α_1-acid glycoprotein with 0.05 M H_2SO_4 at 80°C for 1 h (22). Hepatic β-galactosidase (EC 3.2.1.51) and β-N-acetylhexosaminidase (EC 3.2.1.30) were determined in liver homogenates as previously described (14). Rat serum albumin and α_1-acid glycoprotein were determined immunologically (23). Serum cortisol levels were assayed by radioimmunoassay, data was analyzed by an interative curve fitting for competitive binding assays (24).

RESULTS AND DISCUSSION: In studies on the effect of turpentine inflammation, it was found that at 48 h after inflammation, serum α_1-acid glycoprotein was elevated four-fold and serum sialyltransferase activity was elevated about five-fold (2,23). These parameters were chosen as bioassays for cytokine activity during initial experiments on the stability of the cytokine acting as a mediator of the acute phase response. It was found that the rat cytokine was retained

by dialysis tubing and was stable to trypsin, freeze-drying and storage in freeze-dried form at -20°C for up to one month. The cytokine lost activity on heating at 80°C for 1 h, on incubation with pronase and when concentrated with Amicon UM-2 membranes. These observations suggest the active cytokine is a protein with a molecular weight greater than 8,000. The above properties are shared by cytokines mediating the acute phase response in other species. Stability to trypsin is found with the human, but not the rabbit factor, while both are sensitive to pronase digestion (6,9). Also, loss of activity during attempts at concentration is consistent with properties of the human factor which binds irreversibly to many ultrafiltration membranes (11). These observations suggest that the rat cytokine more closely resembles the human rather than the rabbit cytokine.

In addition to the elevated sialyltransferase activity and α_1-acid glycoprotein levels mentioned above, turpentine-induced inflammation results in a decline in serum albumin levels to about 80% of controls at 48 h (23) and a reduction in hepatic β-galactosidase and β-N-acetylhexosaminidase activities to about 60% of controls at 24 h after inflammation (14). Serum cortisol was elevated with the maximum increase at 12 h after inflammation (15). All the above parameters were examined following the administration of cytokines to normal rats; rats given heat inactivated or pronase treated cytokine served as controls. Figure 1 shows that the serum albumin response was similar to that found after turpentine inflammation (23), but serum α_1-acid glycoprotein levels increased only 1.6-fold following cytokine administration. Hepatic and serum sialyltransferase activities peaked at 36 hr after cytokine (Fig. 2) which is slightly earlier than that found after turpentine (see above). Although cytokine caused a liver sialyltransferase response of similar magnitude to that found after turpentine, the serum sialyltransferase response to cytokine was only half that found after turpentine (see above). Figure 3 shows that hepatic β-galactosidase and β-N-acetylhexosaminidase were also reduced after cytokine, but the reduction in activities was only half that found after turpentine. While some parameters of the acute phase response, including acute phase



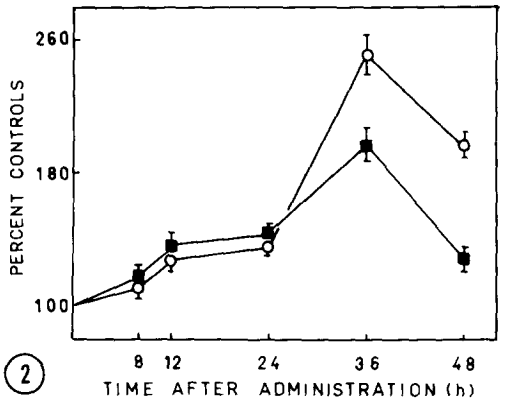


Figure 1. Effect of cytokinine administration on rat serum albumin and α_1-acid glycoprotein levels. Albumin (-●-), control level 37 mg/ml; α_1-acid glycoprotein (-■-), control level 2.3 mg/ml. Rats received i.p. injections of cytokines produced by 3 x 10^8 cells under the conditions described in the methods. Results are the means of 4-6 analyses, standard errors of the means are indicated. Values in rats receiving pronase-inactivated preparations did not deviate appreciably from untreated controls. See methods for further details.

Figure 2. Effect of cytokine administration on serum and hepatic sialyltransferase activities. Serum sialyltransferase (-■-), control 26 pmole N-acetylneuraminic acid transferred/min/ml, hepatic sialyltransferase (-○-), control levels 37 pmoles N-acetylneuraminic acid transferred/min/mg protein. See figure 1 for further details.

reactant synthesis, are known to respond to cytokines (6-13), to the authors' knowledge, the response of sialyltransferase and the hepatic glycosidases represents the first report of an effect of cytokines on enzymes involved in glycoprotein metabolism. It would appear that these enzyme activities could be controlled by the same factors responsible for elevated synthesis of acute phase reactants. As mentioned above, the cortisol response to an inflammatory stimulus is rapid; while cytokine administration significantly elevated serum

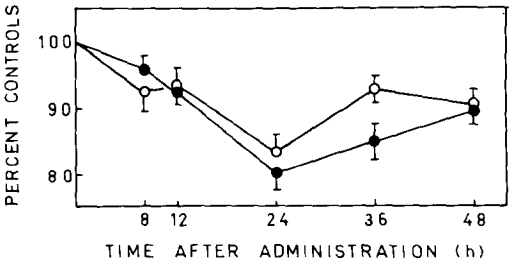


Figure 3. Effect of cytokine administration on hepatic β-galactosidase and β-N-acetylhexosaminidase activities. Galactosidase (-○-), control levels 0.51 nmoles p-nitrophenyl-β-D-galactoside hydrolyzed/min/mg protein, hexosaminidase (-●-), control level 31 nmoles p-nitrophenyl-N-acetyl-β-D-glucosaminide hydrolyzed/min/mg protein. See figure 1 for further details.

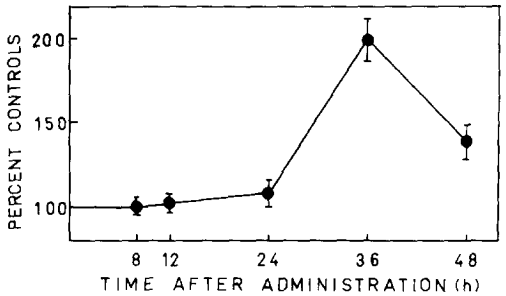


Figure 4. Effect of cytokine administration on serum cortisol levels, control level 80 ng/ml. See figure 1 for further details.

cortisol levels, the response did not occur until 36 h (Figure 4) compared with 12 h after turpentine inflammation (15).

The above results show cytokine causes changes in serum α_1-acid glycoprotein and albumin levels, sialyltransferase activities and hepatic glycosidase activities which mimic those found following turpentine inflammation although some of the responses to cytokine are of lower magnitude and occur earlier. The lower response could be due, in part, to the stability of the cytokines since it has been reported that the rabbit factor(s) has a half-life of less than 10 min when administered to rats (6). Also, hormones or neurological involvement may be needed for full expression of the acute phase response (6). Glucocorticoids have been shown to elevate α_1-acid glycoprotein mRNA levels in rat liver, but this may not reflect physiological mediation since acute phase reactants can be induced in the absence of glucocorticoids (25).

Studies are underway to investigate the direct action of rat cytokines on rat liver and possible hormonal involvement in this action. Other work is aimed at purifying and characterizing the active component(s) in the cytokine preparation described in this report.

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