RECOMBINANT HUMAN INTERLEUKIN 1β AND TUMOR NECROSIS FACTOR AFFECT GLYCOSYLATION OF SERUM α1-ACID GLYCOPROTEIN IN RATS

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Abstract—Serum concentration and glycosylation of rat α1-acid glycoprotein (α1-AGP) were evaluated after the in vivo administration of recombinant human interleukin-1β (rhIL-1β) and tumor necrosis factor α (rhTNF-α), alone or associated. The effect of LPS and turpentine was also studied. In all models, serum α1-AGP concentrations were increased and glycosylation was altered. The α1-AGP levels reached 1.8 g/liter with cytokines alone, 2.1 g/liter with cytokines associated or LPS, and 3.4 g/liter with turpentine. Analysis by concanavalin A (Con A) affinityimmunoelectrophoresis (CAIE) revealed that the relative proportion of Con A unreactive form always decreased whatever the inducing agent. On the other hand, the resulting effect on the concentrations of Con A unreactive α1-AGP concentrations was an increase with cytokines alone or LPS and a decrease with cytokines associated or turpentine. These results suggest a dissociation between the alteration in the level of α1-AGP synthesis and in the pattern of its glycosylation in the various inflammatory models.

INTRODUCTION

The acute-phase response involves a series of events including major changes in liver functions. The hepatic biosynthesis of some proteins quickly rises with
a subsequent increase in their plasma concentrations. It has been previously shown that the increase in serum levels of different glycoproteins during experimental inflammation such as turpentine injection or laparotomy is associated with a modification of their glycosylation (1). It is now known that at least a part of the acute-phase response is mediated by inflammatory cytokines (2–5) and glucocorticoids (6). The main cytokines involved are interleukin-1 (IL-1), tumor necrosis factor (TNF), and interleukin-6 (IL-6). Among the acute-phase proteins (APPs), α1-acid glycoprotein (α1-AGP), which possesses an important glycan moiety, is well suited to study some aspects of the posttranslational maturation of glycoproteins. It was previously shown that α1-AGP is one of the APPs that are more sensitive to IL-1 and TNF than to IL-6 in vitro (7) and that IL-6 induces only a weak increase in serum α1-AGP concentrations in vivo (8). However, there are no data available concerning the effects of IL-1 and TNF on the glycosylation of rat α1-AGP in vivo.

The aim of this study was to determine the effect of IL-1 and TNF on serum α1-AGP glycosylation by comparison with the effects of turpentine and lipopolysaccharide (LPS) after their administration to rats.

**MATERIALS AND METHODS**

rhIL-1β and rhTNF-α were generous gifts from Laboratoires Roussel Uclaf (Romainville, France) and Knoll AG/BASF (Ludwigshafen, Germany), respectively; their contamination with bacterial endotoxin was less than 0.625 pg/μg protein. RPMI 1640 medium supplemented with 2 mM glutamine was purchased from Gibco. *Escherichia coli* LPS (055:B5) was from Wellcome Research Laboratories (Beckenham, Kent, England). Con A was obtained from l’Industrie Biologique Française (Villeneuve-la-Garenne, France).

**Treatment of Animals.** Male Fisher 344 rats (8–10 weeks old, 190–220 g) (CNRS, Villejuif, France) were used in all experiments. The cytokines and LPS were diluted in RPMI medium. The 1-ml intravenous injections were performed in the caudal vein 24 h prior to sacrifice by decapitation except for the highest dose of LPS (2.5 mg/kg), which was injected intraperitoneally. This time corresponds to the approximate peak of serum α1-AGP (9). Turpentine was injected subcutaneously (5 ml/kg), and blood was collected 48 h later. Serum samples were kept at −80°C until assay.

**α1-AGP Concentration Measurement.** Serum α1-AGP was quantified by rocket immunoelectrophoresis according to Laurell (10) in a 1% (w/v) agarose, 4% (w/v) polyethylene glycol 6000 gel containing 0.3% (v/v) of rabbit anti-rat α1-AGP antiserum (11).

**Con A CAIE Patterns of Serum α1-AGP.** Con A-CAIE of rat serum was performed according to Monnet et al. (12) using 1.25 mg/ml of Con A in the first dimension and 1.2% of anti-rat α1-AGP antiserum in the second dimension. Peaks were integrated using a planimeter (A. Ott Kemplien Bayern). The peak areas were expressed in two ways: (1) as the ratio of Con A-unreactive α1-AGP (the fraction of Con A-unreactive α1-AGP was divided by the sum of the four fractions), and (2) as the absolute concentration of serum α1-AGP corresponding to each peak.

**Statistics.** All the results are expressed as the mean ± SE of four rats per group. Comparisons were performed using Student’s t test.