

MALNUTRITION AND ACUTE PHASE-RELATED INCREASE OF α_2 -MACROGLOBULIN IN RAT LIVER. Aleksandra Uskoković*, Mirjana Mihailović, Svetlana Dinić, Nevena Grdović, Melita Vidaković, Jelena Arambašić, Desanka Bogojević, Svetlana Ivanović-Matić, Vesna Martinović, M. Petrović, G. Poznanović, and Ilijana Grigorov. *Laboratory of Molecular Biology, Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia.*

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α_2 -Macroglobulin (MG) is the major acute-phase (AP) protein in rats (Ito et al., 1989), and it which becomes considerably elevated during the AP response - a metabolic reaction of the liver against a variety of stress stimuli ranging from inflammation to tissue injury (Mackiewicz, 1997). The increase of MG concentration in a serum is the result of the transcriptional activation of its gene, mediated by proinflammatory cytokines, mainly interleukin-6 (IL-6) and the regulatory proteins that are under its control (Kunz et al., 1989; Poli, 1998). Among the regulatory proteins that participate in transcriptional regulation of the MG gene, the IL-6-activated signal transducers and activators of transcription (STAT) (Alonzi et al., 2001) and CCAAT/enhancer-binding protein (C/EBP) families of transcription factors found in nuclear extracts of rat hepatocytes (Petrović et al., 1995) assume a prominent role. As it is established that the ability of an organism to respond to and recover from various stress stimuli depends on its nutritional status, and that a poor nutritional status affects metabolic functions (Tschopp and Heimann, 2001), this study was aimed at elucidating the liver's inflammatory AP response via MG expression in relation to malnutrition. Our objective was to study the influence of chronic food restriction on MG synthesis in rats as well as on STAT3 and C/EBP β expression.

Male Wistar strain albino rats aged 1 and 2.5 months were used. One-month-old rats were fed daily for 6 weeks with an amount of chow equivalent to 50% of the normal food intake until they reached body weight about 50% of that of *ad libitum*-fed age-mates. The AP response was induced by a subcutaneous injection of turpentine oil (μ l/g per body weight) in the lumbar region of malnourished (MN) and *ad libitum*-fed rats aged 2.5 months, the latter of which are termed well-nourished (WN) (sterile tissue injury model). The animals were sacrificed after 12 h, when the maximal transcription rate of MG was established and blood and liver samples were collected. The serum level of MG was determined by rocket immunoelectrophoresis according to Baumann (1988) using monospecific antibodies to human MG (Sigma-Aldrich Inc.). Liver nuclear extracts (NE) were isolated from WN and MN untreated and turpentine-treated rats, following the procedure of Gorski et al. (1986). Western immunoblot analysis of rat liver NEs was performed according to the method of Towbin et al. (1979) with rabbit

polyspecific antibodies to rat STAT3 and C/EBP β (Santa Cruz Biotechnology). Immunoreactive bands were identified using an enhanced chemiluminescence (ECL) detection system (Santa Cruz Biotechnology) according to the manufacturer's instructions.

Comparison of the serum levels of MG between WN and MN rats (Fig. 1A) revealed an increase in the basal level of MG in MN rats compared with the WN ones. When MN rats were treated with turpentine, the relative level of serum MG was higher than after the same treatment of WN rats, suggesting that the effects of turpentine treatment were potentiated by malnutrition. The elevated MG expression in untreated and turpentine-treated MN rats could be caused by changes in the expression profile of transcription factors involved in the regulation of MG gene expression. We therefore further investigated the nuclear expression profile of STAT3 and C/EBP β proteins in liver NEs of WN and MN untreated and turpentine-treated rats by Western immunoblot analysis (Fig 1B). In NE from MN rats without turpentine treatment, STAT3 (91 and 86 kD isoforms) content was elevated compared to WN ones (where its presence was not detected). During the turpentine-induced AP response, increased STAT3 levels were observed in NEs from both WN and MN rats (WNT12h, MNT12h). The C/EBP β (38, 35, and 30 kD isoforms) level decreased in NE from MN rats compared with the WN ones. However, the AP response induced significant increase of the 35 kD C/EBP β isoform known as LAP (liver activatory protein) in MN rat NE (MNT12h).

Chronic food restriction is one of the most important causes of several metabolic and immune disfunctions (Giovambattista et al., 2000). Since the rat liver response to acute inflammation and malnutrition is characterized by increased production of MG, it would appear that chronic food restriction provokes an inflammatory status by means of elevated MG synthesis. Malnutrition was capable of further elevating MG expression in conjunction with turpentine treatment, which indicates that the capacity for response to an additional stress stimulus is preserved, suggesting adaptive preservation of acute-phase responsiveness during malnutrition. Malnutrition probably affects the hepatic synthesis of MG by changing the expression profile of active members of C/EBP and STAT transcription factors. The presented results provide evidence that malnu-

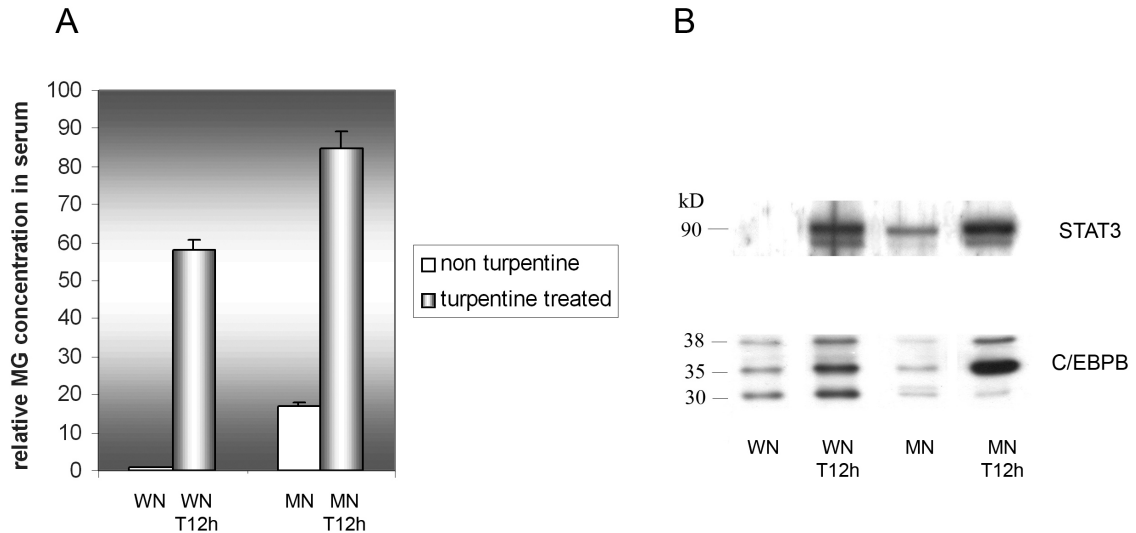


Fig. 1. A. Relative MG level in serums of well-nourished and malnourished rats in basal (WN, MN) and turpentine-induced AP conditions (WNT12h, MNT12h). B. Western immunoblot analysis of STAT3 and C/EBP β protein levels in the liver NEs from well-nourished and malnourished rats in basal conditions (WN, NM) and in response to turpentine treatment (WNT12h, MNT12h).

trition-related increase of MG synthesis could be connected with a positive regulatory role of STAT3, while the additional AP-related increased synthesis of MG in malnourished rats besides STAT3 requires enhanced activity of C/EBP β .

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