Effects of Concanavalin A on Cytokine mRNA Expression in Mouse Liver

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ABSTRACT—The effects of concanavalin A (Con A) on liver cytokine gene expression was studied in mice. The CD4 mRNA expression in normal liver suggests the presence of CD4⁺ T cells. The administration of Con A induced interleukin (IL)-1 β , IL-2 and IL-2 receptor mRNAs, which implies lymphocyte activation in the liver. Interferon- γ and tumor necrosis factor- α mRNA expressions were increased gradually. The present results showed that Con A induced liver cytokine genes. This cytokine gene induction might have been the result of lymphocyte activation in the liver.

Keywords: Concanavalin A, Liver, Cytokine mRNA

Concanavalin A (Con A) is a plant lectin from jack beans known to mitogenically activate T lymphocytes in vitro (1). Intravenous administration of Con A to mice induces T lymphocyte-dependent hepatic injury (1). Treatment with Con A releases cytokines into the serum of animals (1, 2). However, there has been no study on the effect of Con A on liver cytokine expression. Since cytokines act locally in many cases, the cytokine levels in the serum do not reflect the real activity in the liver. In the present study, we examined the effect of Con A on cytokine expression in the liver. We used the polymerase chain reaction (PCR) because this method is sensitive enough for identification of cytokines that are expressed at low levels.

Female BALB/c mice obtained from Charles River Japan, Inc. (Atsugi) were used at 7–10 weeks of age. The animals were kept in an air-conditioned room and given chow and water ad libitum. Con A was administered to mice (0.2 mg/mouse) via a tail vein. The sources of Con A and dexamethasone (DEX) were Seikagaku Kogyo (Tokyo) and Wako Pure Chemical Industries, Ltd. (Osaka), respectively. Mice were anesthetized with ether before sampling. Blood was sampled from an abdominal vein for plasma alanine aminotransferase (ALT) measurement (3) at 8.5 hr after Con A treatment. The liver was sampled without perfusing it. Data were analyzed according to the test for equal variance and Welch's correction.

RNA isolation (4) and PCR analysis were performed as described previously (5). First strand cDNA was amplified by 30 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec and extension at 72°C for 1.5 min. The

previously reported (5) specific primers for CD4, interleukin (IL)-1 β , IL-2, interleukin-2 receptor (IL-2R), interferon (IFN)- γ , tumor necrosis factor (TNF)- α , IL-6, transforming growth factor (TGF)- β 1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used.

Intravenous injection of Con A induced liver injury, as assessed by the serum transaminase level (ALT (U/L): 2681 $\pm$ 1171, compared with the non-treated level, 39 $\pm$ 9, mean $\pm$ S.E., n=6, P<0.01). CD4 is a glycoprotein expressed on the helper T cell surface (6). The expression of CD4 mRNA in normal liver suggests the presence of CD4⁺ T cells in the liver (Fig. 1). IL-1 β facilitates the activation of T cells (7), and IL-2 and IL-2R are markers of T cell activation (7, 8). At 60 and 120 min after Con A treatment, IL-1 β mRNA was elevated, and IL-2 and IL-2R mRNAs were induced (Fig. 2A). Since IL-1 β primers amplified several bands, the presence of IL-1 β mRNA was ascertained by observing the size of the amplified product. The induction of those mRNAs in the liver suggests the activation of T cells at 60 and 120 min after Con A

Fig. 1. PCR analysis of CD4 mRNA expression in the liver. Livers were removed at 15, 30, 60 and 120 min after Con A administration. RNA was isolated and its expression was measured by means of PCR.

Fig. 2. PCR analysis of cytokine gene expression in the liver. Livers were removed at 15, 30, 60 and 120 min after Con A administration. RNA was isolated and subjected to PCR amplification. A: Expression of IL-1 β , IL-2 and IL-2R mRNAs. B: Expression of IFN- γ and TNF- α mRNAs. C: Expression of IL-6, TGF- β 1 and GAPDH mRNAs.

treatment. IFN- γ and TNF- α are important mediators of liver injury in this model (1, 2). Con A induced IFN- γ and TNF- α mRNAs gradually (Fig. 2B). Initial slight induction of IFN- γ and TNF- α mRNAs at 15 and 30 min after Con A injection was followed by late strong induction of these mRNAs at 60 and 120 min (Fig. 2B). IFN- γ and TNF- α are also produced upon T cell activation (8, 9). Furthermore, the late strong expressions of IFN- γ and TNF- α mRNAs were induced along with those of IL-2 and IL-2R mRNAs. Thus, it is plausible that the late strong induction of IFN- γ and TNF- α mRNAs might be a result of T cell activation. CD4 mRNA expression and the faint band of CD8 mRNA (not shown) suggest the contribution of CD4⁺ T cells to induce these cytokine

Fig. 3. Effects of DEX on cytokine gene expression. Livers were removed 60 min after Con A administration. RNA was isolated and subjected to PCR amplification. Pretreatment with DEX (2.5 mg/kg, i.p.) was performed at 1 hr before Con A injection. The liver samples in the left two lanes were from two Con A-treated mice and those in the right two lanes from two Con A + DEX-treated mice. Top: TNF- α mRNA expression. Bottom: IL-1 β mRNA expression.

mRNAs. However, further studies are needed to elucidate this possibility. The IL-6 mRNA expression shown here (Fig. 2C) indicates the contribution of this cytokine within the liver. TGF- β 1 mRNA, which has been reported to enhance liver extracellular matrix components (10), was constitutively expressed (Fig. 2C). The constant GAPDH mRNA expression showed that there was no marked tube to tube variation. Glucocorticoids suppress immune and inflammatory reactions by inhibiting cytokine expression (11). Pretreatment with DEX (2.5 mg/kg, i.p.) at 1 hr before Con A treatment prevented Con A-induced hepatitis (ALT (U/L): Con A 960 ± 109 , Con A + DEX 37 ± 3 , $n=4$, $P<0.01$). Moreover, the Con A-induced cytokine gene expression was inhibited by DEX (Fig. 3). Thus, cytokine induction may be related to the development of hepatitis in this model.

Taking all available results into account, we conclude that Con A administration induces cytokines in the liver, which might be the result of lymphocyte activation. The cytokine expression in the liver is closely related to the induction of hepatitis by Con A.

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