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Influences of Kupffer cell stimulation and suppression on immunological liver injury in mice[1]

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KEY WORDS Kupffer cells; liver; lipopolysaccharides; BCG vaccine; nitric oxide

AIM: To study the possible involvement of Kupffer cells (KC) in immunological liver injury in mice. **METHODS:** Liver injury was induced by iv injection of *Bacillus* Calmette-Guerin (BCG) 5×10^7 viable bacilli followed by iv injection of lipopolysaccharides (LPS) 7.5 μg to each mouse. Indian ink and silica were iv injected to suppress KC and retinol was given *po* to stimulate KC in these mice. Plasma alanine aminotransferase (AlaAT), aspatate aminotransferase (AspAT), nitric oxide (NO), and liver tissue were examined. **RESULTS:** Injection of LPS following BCG injection resulted in a remarkable elevation of plasma NO, AlaAT, and AspAT levels, and severe liver damage. The damages were enhanced by the activation of KC with retinol and reduced by suppression of KC with silica and Indian ink. **CONCLUSION:** The degree of liver injury induced by BCG + LPS is closely correlated with the status of KC, and NO from KC plays an important role in the pathogenesis of the liver damage in mice.

Severe liver injury may be induced by injection of a small dose of bacterial lipopolysaccharides (LPS) into mice primed with *Bacillus* Calmette-Guerin (BCG) or *Corynebacterium parvum*[1-3]. Injection of BCG causes an infiltration of mononuclear cells into the liver, which is a prerequisite for LPS-induced liver damage. An explanation for the pathogenesis of this kind of liver injury is that macrophages are recruited into the liver under the stimulation of infections agent, and then these macrophages sited contiguous to the hepatocytes and released hepatotoxic soluble mediators upon the second challenging agents such as LPS[4].

Kupffer cells (KC) represent the most numerous phagocytic cell type of the reticuloendothelial system[5] and a unique population of macrophages by virtue of their fixed position next to hepatocytes[6]. The close proximity of these two cell populations makes it likely that they communicate both in normal and pathological conditions. KC like macrophages, when exposed to a septic stimulus, can release a number of cytokines, which could alter hepatocyte function[7]. Among them, Nitric oxide (NO) has been deserved much attention recently and thought to be an important factor in macrophage-mediated cytotoxicity[7-11].

To investigate the involvement of KC in the pathogenesis of BCG + LPS-induced liver damage, influence of stimulation of KC by retinol, a known KC stimulator[12], and suppression of KC by silica and Indian ink, two commom KC suppressors[13], on BCG + LPS-induced liver injury were studied.

MATERIALS AND METHODS

Mice Kunming strain ♀ mice ($n = 260$, aged 4 - 6 wk) weighing 24 ± s 3 g were obtained from the Animal Center of Chinese Academy of Medical Sciences. The mice were allowed food and tap water *ad lib* until 12 h of fasting before they were killed.

Reagents Viable BCG suspension, grown in a glycerol-free culture medium for 14 d, was purchased from the National Vaccine & Serum Institute, Beijing, China. LPS from *E coli* was obtained from Difco, USA. Retinol (all *trans*) and *N*-1-naphthylethylenediamine dihydrochloride were purchased from Sigma, USA. Other reagents were all of AR.

BCG and LPS injections Each mouse was injected iv with 0.2 mL of the BCG suspension (5×10^7 viable bacilli). Twelve days later, they received an iv injection of LPS 7.5 μg

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dissolved in 0.2 mL of saline The mice were decapitated 10 h after LPS injection.

Stimulation and suppression of KC Retinol was prepared as an emulsion in sesame oil, which (0.2 mL) was ig given to each mouse. Silica (< 5 μm) and Indian ink were prepared as a suspension in sterilized saline and mixed ultrasonically just before use. A dose of 5 mg of the particles was injected iv to each mouse.

Blood AlaAT and AspAT determinations Plasma AlaAT and AspAT activities were determined using the kits produced by the Beijing Chemical Factory.

Measurement of plasma NO[14] NO in heparinized blood samples was analyzed. Briefly, 0.1 mL plasma was deproteinized in $ZnSO_4$-NaOH. The supernatant was diazotized on ice with 0.1 mL of 3 % sulfanilic acid in 20 % glacial HAc and 0.1 mL of *N*-1-naphthyetylenediamine dihydrochloride (6 mmol·L^{-1}). Absorbance was read at 545 nm. $NaNO_2$ was used as a standard.

Histological examination Histological examination was done by a separate reviewer in a blind fashion. The liver was fixed in 10 % neutral formalin, embedded in paraffin wax, and stained with HE. The necrosis of liver was scored: 0 = no necrosis; 1 = mild necrosis; 2 = moderate necrosis; and 3 = severe necrosis. The index of necrosis in each group was deduced by dividing the sum of the scores by the number of mice in each group.

Statistical analysis All values are expressed as $\bar{x} \pm s$ and analyzed with t-test.

RESULTS

Plasma NO, AlaAT, and AspAT levels When the mice were injected with BCG and challenged with LPS, plasma NO_2^-, AlaAT, and AspAT levels were elevated. The preliminary experiment showed that the plasma level of NO_2^- was parallel to that of NO_3^-. Therefore, in the regular experiments, only plasma NO_2^- was measured to indicate NO levels. The transaminase level reached its peak 12 h after LPS injection and then decreased, whereas the NO_2^- level increased later and fluctuated during the 4-d experiment (Fig 1).

The elevations in plasma NO and AlaAT levels depended upon the dose of LPS (Fig 2).

Effects of KC activation by retinol BCG or LPS alone induced minor increases of AlaAT, AspAT, and NO_2^- levels in mice as compared with the normal group. However, iv BCG + LPS (7.5 μg) resulted in a remarkable (3-fold) elevation of

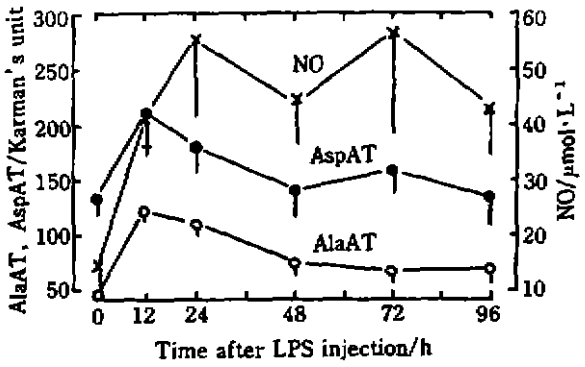

Fig 1. Plasma NO_2^-, AlaAT, and AspAT levels in mice challenged with BCG + LPS. $n = 8 - 10$. $\bar{x} \pm s$.

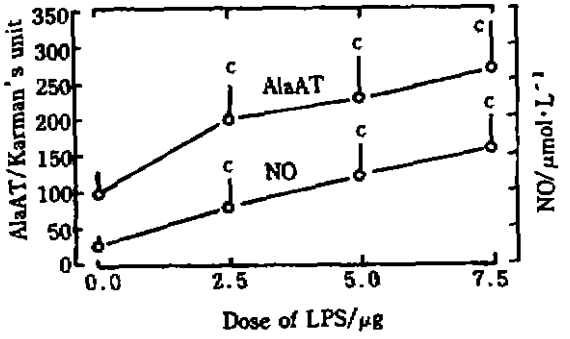

Fig 2. Plasma NO_2^- level and AlaAT activity in mice treated with BCG + LPS. $n = 10 - 12$, $\bar{x} \pm s$. $^cP < 0.01$ *vs* LPS 0 μg.

plasma AlaAT and AspAT activities, and NO_2^- levels. Administration of retinol to the mice previously primed with BCG 24 h before iv LPS induced further increases of plasma NO_2^-, AlaAT, and AspAT levels in a dose-dependent manner as compared with the BCG + LPS group (Tab 1).

Effects of KC suppression by Indian ink and silica When Indian ink and silica were iv injected into mice 24 h before iv LPS, AlaAT, AspAT, and NO_2^- levels were markedly decreased in comparison with the BCG + LPS group (Tab 1).

Histopathological examination of liver The liver from mice injected with BCG alone contained a number of monocyte infiltrations in the portal region and a large number of macrophages in form of small granulomas in the sinusoids. Marked hypertrophy of the KC was seen. Administration of LPS to the BCG-primed mice induced necrosis and thrombi

Tab 1. Influence of KC activation by retinol and suppression by India ink and silica on BCG + LPS-induced elevation of plasma NO_2^-, AlaAT, and AspAT levels in mice. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* group A; $^fP < 0.01$ *vs* group B; $^iP < 0.01$ *vs* group C. u = Karman's unit.

Group	*n*	AlaAT /u	AspAT /u	NO_2^- /μmol·L^{-1}
Activation of KC by retinol				
Normal	8	28 ± 8	97 ± 18	7.7 ± 2.6
LPS	8	35 ± 13	74 ± 20	7.2 ± 1.2
BCG	8	50 ± 23	83 ± 24	9.3 ± 2.1
BCG + LPS (A)	8	134 ± 11	247 ± 18	40 ± 5
BCG + LPS + retinol				
75 μg·kg^{-1}	8	177 ± 14^c	255 ± 21^a	39 ± 11^a
150 μg·kg^{-1}	8	190 ± 11^c	312 ± 34^c	69 ± 7^c
300 μg·kg^{-1}	8	222 ± 24^c	326 ± 10^c	87 ± 12^c
Suppression of KC by Indian ink				
Normal	10	25 ± 2	100 ± 8	7.9 ± 2.3
LPS	10	35 ± 8	102 ± 10	9.3 ± 2.4
BCG	10	48 ± 9	103 ± 12	12.3 ± 2.6
BCG + LPS (B)	10	167 ± 18	221 ± 35	27 ± 7
BCG + LPS + Indian ink	11	98 ± 23^f	165 ± 35^f	2.2 ± 1.3^f
Suppression of KC by silica				
Normal	5	45 ± 3	97 ± 19	8.3 ± 2.1
LPS	6	73 ± 8	84 ± 6	19 ± 3
BCG	7	71 ± 7	113 ± 16	24 ± 5
BCS + LPS (C)	16	157 ± 23	196 ± 28	70 ± 12
BCG + LPS + silica	8	96 ± 35^i	161 ± 18^i	22 ± 4^i

within the small vessels and sinusoids besides the formation of granulomas. Blockade of KC with silica and Indian ink before the iv LPS reduced liver necrosis. Activation of KC with retinol enhanced liver damage in the mice treated with BCG + LPS (Tab 2).

Tab 2. *Influence of KC activation and supression on liver necrosis induced by BCG + LPS in mice.*

Group	*n*	Necrosis score
Normal	8	0
LPS	8	0
BCG	8	0
BCG + LPS	10	0.45
BCG + retinol + LPS	8	0.65
BCG + India ink + LPS	11	0.38
BCG + silica + LPS	16	0.30

DISCUSSION

The results of present study showed that the degree of liver injury and the changes in plasma NO levels were closely related to the functional status of KC. These suggestion was based on the evidence that activation of KC by retinol increased plasma NO and liver damage. Whereas the suppression of KC by silica and Indian ink attenuated BCG + LPS-induced elevation in plasma NO and liver injury.

In a word, KC is involved in BCG + LPS-induced hepatic injury and NO from KC plays an important role in the pathogenesis of this immunological liver injury model in mice.

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173-176

激活与封闭库普弗细胞对小鼠免疫性肝损伤的影响

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关键词 库普弗细胞；肝；脂多糖；卡介苗；肝损伤；一氧化氮 维生素A

目的：研究库普弗细胞(KC)是否参与小鼠免疫性肝损伤. 方法：给小鼠静脉注射卡介苗(BCG) 5 $\times 10^7$ 活菌后再静脉注射脂多糖(LPS) 7.5 μg 以诱导免疫性肝损伤. 以维生素 A 激活 KC 和以印度墨汁或硅砂封闭 KC 后，测定血浆一氧化氮(NO)，谷丙转氨酶(AlaAT)，谷草转氨酶(AspAT)的变化并检查肝组织的病理改变. 结果：注射 BCG 后，再注射 LPS 7.5 μg，可导致小鼠血浆 NO，AlaAT，AspAT 剧烈升高及严重的肝损伤. 以维生素 A 激活 KC 后，肝损伤更为严重，而以印度墨汁或硅砂封闭 KC 后，肝损伤则显著减轻. 结论：BCG+LPS 诱导的小鼠肝损伤与 KC 的功能关系密切，来源于 KC 的 NO 在 BCG+LPS诱导的肝损伤中起重要作用.

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Effect of epidermal growth factor on cultured rat hepatocytes poisoned by CCl_4

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KEY WORDS epidermal growth factor-urogastrone; alanine aminotransferase; aspartate aminotransferase; liver; carbon tetrachloride poisoning; cultured cells

AIM: To study the effects of epidermal growth factor (EGF) on CCl_4-induced primary cultured hepatocytes injury. **METHODS:** Alanine aminotransferase (AlaAT) and aspartate aminotransferase (AspAT) activities and K^+ concentractions were determined by the Auto-biochemistry Assay System. Malondialdehyde (MDA) was determined by thiobarbituric acid method. Radioactivity was determined by liquid scintillometry. Light microscopy and electron microscopy were used. **RESULTS:** EGF 40 $\mu g \cdot L^{-1}$ decreased CCl_4 (10 $mmol \cdot L^{-1}$)-induced damages of rat primary cultured hepatocytes by decreasing AlaAT and AspAT leakage and MDA production, and promoted RNA and DNA synthesis, with a high positive correlation between intracellular K^+ leakage and DNA syntheses ($r = 0.99$, $P < 0.01$). Cytopathological study showed that EGF decreased damage of liver cells. **CONCLUSION:** EGF maintains the stability of cellular lipid membrane and promotes syntheses of RNA and DNA of hepatocytes, and intracellular K^+ transference is a promotor of the message transmission of DNA synthesis.

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CCl_4 induced the liver cell damage and necrosis by way of attacking the phospholipid fatty acid to lead to lipid peroxidation in the biomembrane[1,2]. This is a model of CCl_4-induced primary cultured hepatocytes injury[3,4].

Epidermal growth factor (EGF), found in mouse submaxillary gland in purifying nerve growth factor[5], promoted DNA synthesis of intoxicated hepatocytes[6-8]. The aim of this study was to examine the protective effects of EGF against CCl_4-induced primary cultured hepatocytes injury.

MATERIALS AND METHODS

Isolation and primary cuture of rat hepatocytes