Inhibition of p38 mitogen-activated protein kinase attenuates experimental autoimmune hepatitis: Involvement of nuclear factor kappa B

Xiong Ma, Yi-Tao Jia, De-Kai Qiu

Xiong Ma, Yi-Tao Jia, De-Kai Qiu, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai Institute of Digestive Disease, Shanghai 200001, China

Correspondence to: De-Kai Qiu, Shanghai Renji Hospital, Shanghai Institute of Digestive Disease, Shanghai Jiaotong University School of Medicine, 145 Shandong Middle Road, Shanghai 200001, China. dekaiqiu@sh163.net

© 2007 WJG. All rights reserved.

Abstract

AIM: To investigate the role of p38 mitogen-activated protein kinase (p38MAPK) in murine experimental autoimmune hepatitis (EAH).

METHODS: To induce EAH, the syngeneic S-100 antigen emulsified in complete Freund's adjuvant was injected intraperitoneally into adult male C57Bl/6 mice. Liver injury was assessed by serum ALT and liver histology. The expression and activity of p38 MAPK were measured by Western blot and kinase activity assays. In addition, DNA binding activities of nuclear factor kappa B (NF-κB) were analyzed by electrophoretic mobility shift assay. The effects of SB203580, a specific p38 MAPK inhibitor, on liver injuries and expression of proinflammatory cytokines (interferon-γ, IL-12, IL-1β and TNF-α) were observed.

RESULTS: The activity of p38 MAPK and NF-κB was increased and reached its peak 14 or 21 d after the first syngeneic S-100 administration. Inhibition of p38 MAPK activation by SB203580 decreased the activation of NF-κB and the expression of proinflammatory cytokines. Moreover, hepatic injuries were improved significantly after SB203580 administration.

CONCLUSION: p38 MAPK and NF-κB play an important role in an animal model of autoimmune hepatitis (AIH) induced by autoantigens.

Key words: Autoimmune hepatitis; p38 mitogen-activated protein kinase; Nuclear factor kappa B; Proinflammatory cytokines.

INTRODUCTION

Autoimmune hepatitis (AIH) is a predominant periportal hepatitis with hypergammaglobulinemia and tissue autoantibodies, which is responsive to immunosuppressive therapy in most cases[1]. Experimental autoimmune hepatitis (EAH) shares several features with human AIH periportal hepatitis[2], such as lymphocytic infiltrates[3,4], T cell reactivity to liver antigens[5], autoantibody production[6] and response to immunosuppressive therapy[7]. Experiments on EAH in inbred mice could give valuable information on effector cells and regulatory phenomenon in liver-specific immune reactions, while they could not induce a chronic relapsing AIH[8].

p38 MAPK, a stress-activated serine/threonine protein kinase that belongs to the the mitogen-activated protein kinase (MAPK) superfamily, is expressed ubiquitously, with high levels in the liver, spleen, thyroid, placenta, bone marrow, and leukocytes[9]. p38 MAPK activation is involved in the pathogenesis of human autoimmune diseases, including sialoadenitis of Sjögren syndrome[10], rheumatoid arthritis[11], inflammatory bowel disease[11] and autoimmune renal injury in systemic lupus erythematosus[12]. Activation of p38 MAPK may contribute to the pathogenesis of autoimmune diseases via the activation of the signal transduction and expression of cytokines and chemokines[13]. In liver, there is evidence that highlights a central role of MAPK family in several effects of ethanol[14] and hepatitis viruses such as hepatitis B, C and E viruses[15]. Recently, Tsutsumi et al[16] showed that HCV core protein activates ERK and p38 MAPK cooperatively with ethanol and modulates the expression of several genes related to cell transformation, cell cycle, and antioxidants in a mouse model of HCV-associated hepatocyte carcinoma. Interestingly, Gilbert et al[17] reported that trichloroacetaldehyde, one of the major metabolites of trichloroethylene, promotes T-cell activation via stimulation of MAPK pathway. It has been shown previously by the same group that MRL+/-
mice exposed to trichloroethylene in their drinking water develop lupus-like symptoms and AIH\textsuperscript{[1]}. In the present study, we investigated the activation of p38 MAPK signaling pathway as well as nuclear factor-κB in the liver of a murine experimental model of autoimmune hepatitis induced by hepatic S100 antigen in complete Freund’s adjuvant. We further examined the protective effects of SB203580, a specific inhibitor of p38 MAPK, on the expression of proinflammatory cytokines, such as IFN-γ, IL-12, IL-1β, TNF-α in this model.

**MATERIALS AND METHODS**

**Antigen preparation and induction of EAH**

Fresh liver antigens from syngeneic animals were prepared after perfusion of livers with phosphate-buffered saline (PBS) as described previously\textsuperscript{[3,6]}. Briefly, livers were homogenized on ice. After 10 min of centrifugation at 150 × g, supernatant was collected and re-centrifuged for 1 h at 100000 × g. The remaining supernatant was used for immunization (called S100).

EAH was induced as described previously\textsuperscript{[3,6]} with a minor modification. Freshly prepared syngeneic S-100 antigen at a dose of 0.5-2 mg/mL in 0.5 mL PBS emulsified in an equal volume of complete Freund’s adjuvant (CFA, Sigma-Aldrich, St. Louis, MO, USA) was injected intraperitoneally. All visible portal tracts of a liver section were evaluated by a pathologist in a blinded fashion and graded as 0: normal histomorphology, 1: minor inflammatory infiltrates with occasional liver cell necrosis, 2: moderate liver damage with inflammatory infiltrates and focal necroses, and 3: extensive infiltrates in portal tracts and lobules accompanied with diffusely distributed liver cell necroses. At least two separate sections were assessed per liver\textsuperscript{[2,3]}. Serum alanine aminotransferase (ALT) levels were measured with an autoanalyzer.

Adult (age 5-7 wk) male wild type C57BL-6 mice (5-7 wk old) were obtained from the Central Animal Experimentation Facility of Renji Hospital, Shanghai Second Medical University (SSMU). All mice were maintained in a temperature- and light-controlled facility, and had free access to water and pellet chow. We observed the expression of phosphorylated p38 MAPK and NF-κB in mouse liver taken on d 1, 3, 7, 14, 21, 28 after immunization. Three mice were sacrificed at each time point. For p38 MAPK inhibition experiments, SB 203580 (Calbiochem, Shanghai, China, 10 μmol/kg) in 3% of DMSO was injected intraperitoneally (i.p.) daily into the mice. The mice were then killed after 14 d to obtain serum and liver tissue. The control mice were injected with vehicle (3% DMSO) or PBS. All animal experiments fulfilled the SSMU criteria for humane treatment of laboratory animals.

**Western blot analysis**

Cytoplasmic and nuclear protein extracts from liver were prepared with the NE-PER\textsuperscript{®} nuclear and cytoplasmic extraction reagent kit (Pierce, Beijing, China) according to the manufacturer’s instructions. To minimize proteolysis, all buffers contained protease inhibitor cocktail (Roche, Shanghai, China). Protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA, USA). Equal amounts of proteins (30-50 μg) were separated by SDS-PAGE (10% running, 4% stacking gel) and transferred to PVDF membranes (Millipore, Bedford, MA, USA) in 192 mmol/L glycine, 25 mmol/L Tris, and 10% methanol. Non-specific sites on membranes were blocked with 5% BSA in TBST overnight at 4°C. The membrane was incubated overnight at 4°C with a rabbit polyclonal antibody to phospho-p38 (Cell Signaling Technology Inc, MA, USA) at a dilution of 1:1000 followed by horseradish peroxidase-coupled donkey anti-rabbit Ig antibody (Santa Cruz Biotechnology, Santa Cruz, California, USA) at a dilution of 1:3000. Signals were detected using ECL Western blotting detection reagents (Amersham Bioscience, Castle Hill, Australia). The membrane was then stripped using Immuno pure IgG elution buffer (Pierce, Beijing, China) and reprobed with an antibody specific for total p38 (Cell Signaling Technology, Hong Kong, China). Signal intensity was quantified using a gel documentation system (Fluor-S-MultiImager and Quantity one software version 4.1, Bio-Rad). The level of total p38 served as an internal standard for protein loading and transfer.

**Immunoprecipitate kinase activity assay for p38 MAPK**

Intracellular p38 MAPK activity was detected using p38 MAP kinase assay kit (Cell Signalling Technology). Equal amounts of cytoplasmic protein from each group were used for the immunoprecipitation procedures. Fifteen microliters of anti-phospho-p38 monoclonal antibody (Cell Signaling Technology, 1:1000). Immune complexes were collected by centrifugation and then washed extensively on the following day. Immunoprecipitates were resuspended in 25 μL of kinase buffer supplemented with 200 μmol/L ATP and 2 μg ATP-2 fusion protein (Cell Signalling Technology) and incubated for 1 h at 30°C. After incubation, the reaction was terminated using Laemmli buffer (62.5 mmol/L Tris–HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol). The samples were boiled for 5 min and loaded onto a 10% SDS-polyacrylamide gel and probed with anti-phospho-ATF-2 rabbit polyclonal antibody (Cell Signaling Technology, 1:1000).

**Electrophoretic mobility shift assay**

DNA binding activity of NF-κB was determined by electrophoretic mobility shift assay (EMSA) with Gel shift assay systems (Promega, Beijing, China), according to manufacturer’s instructions. Double-stranded oligonucleotides containing the consensus sequence for NF-κB/DNA binding site (5’-TAGTGGAGGG ACTTTCGCCAGG-3’) were radiolabeled with [γ\textsuperscript{32}P]-ATP (Amersham Biosciences, Little Chalfont, Bucks, UK). After purification over a Sephadex G-25 column (Amersham Biosciences), [\textsuperscript{32}P]-labeled oligonucleotides (20000 r/min) were incubated with nuclear extracts at room temperature for 20 min in binding buffer containing 12 mmol/L HEPES (pH 7.9), 4 mmol/L Tris HCl (pH 7.9), 60 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L PMSF, 12% glycerol, 5 μg of BSA, and 2 μg of poly (dI/dC) poly (dI/dC) (Amersham Biosciences). DNA-
protein complexes were separated in 6% nondenaturing polyacrylamide gel. Signals were visualized by exposing the dried gel to X-ray film.

**Northern blot analysis**

Total RNA was isolated from whole murine liver tissue by the guanidinium isothiocyanate/cesium chloride procedure. Complementary DNA probes were obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) with specific primers, labeled with [α-32P]dCTP by random priming (Megaprime DNA labeling system, Amersham Biosciences). The sequences of the oligonucleotide primers used for PCR were designed using Primer 5.0 software as follows: mouse IFN-γ sense: 5'-AGCGGCTGACTGAACTCAGATTG AG-3', antisense: 5'-GCACAGTTTTCACTGTATAGGG-3', mouse IL-12 p40 sense: 5'-CAGAAGCTAACCATCTCTGG TTTG-3', antisense: 5'-TCCGGGATATTTG GTGCTTCACAC-3', mouse IL-1β sense prime: 5'-GCAAAGCTTCTGAAGACTCA-3', antisense prime: 5'-CTCCGGAAGCTGTAGTGCAG-3', mouse TNFα sense prime: 5'-GGCAAGTTCACTTTG GAG TCA TTTG C-3', antisense prime: 5'-ACATTCGAGGCTC CAGTGAATTCGG-3', mouse β-actin sense prime: 5'-TGGAAATCTCTGGTGTCCATGAA, antisense prime: 5'-TAAAAACGACGCTAGTAACGTCCG-3'. Thirty cycles of amplification were performed: denaturation at 94°C for 60 s, annealing at 57°C for 60 s, and extension at 72°C for 60 s. The identity of PCR products was confirmed by direct sequencing. For Northern blots, 10 µg of total RNA was fractionated on 1.2% formaldehyde-agarose gels and blotted onto Hybond-N+ nylon membranes (Amersham Biosciences), which were hybridized separately with individual probes overnight at 45°C in a solution containing 50% formamide, 5 × SSC, 2.5 × Denhardt’s solution, 25 mmol/L sodium phosphate buffer (pH 6.5), 0.1% SDS, and 250 µg/mL salmon sperm DNA. All Northern blots were subjected to stringent washing conditions prior to autoradiography with intensifying screen at -80°C for 2-7 d. Cytokine mRNA expressions were normalized to internal standards for 2 different constitutively expressed mRNAs (β-actin).

**Statistical analysis**

All data were expressed as mean ± SE. Differences between groups were assessed by one way ANOVA analysis. P < 0.05 was considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, version 101.4 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Activation of p38 MAPK and NF-κB in EAH**

In the current study, we observed the activation of p38 MAPK on d 1, 3, 7, 14, 21, 28 after the first immunization. The expression of phospho-p38 MAPK was progressively increased after injection of syngeneic S-100 antigen and reached its peak on d 14 to 21 after the first immunization (Figure 1A). The expression of total p38 MAPK, however, remained constant (Figure 1B), indicating that the activity of p38 MAPK was increased. To confirm our finding, we also determined the activity of p38 MAPK by immunoprecipitate kinase activity assay. As shown in Figure 1C, the p38 MAPK activity was increased and reached its peak on d 14 to 21 after administration of syngeneic S-100 antigen. These results showed that p38 MAPK activity was increased in mouse liver after IAH was induced by auto-antigens.

NF-κB DNA binding activity increased significantly after treatment with syngeneic S-100 antigen and reached its peak on d 21 after the first immunization (Figure 1D), consistent with that of p38 MAPK.

**Suppression of p38 MAPK and NF-κB activities in EAH by SB203580**

Because the activity of p38 MAPK and NF-κB was increased in mice with EAH, we tried to find whether SB203580, a specific p38 MAPK inhibitor, would reduce their activity and whether SB203580 would improve hepatic inflammation in this model. SB203580 significantly reduced the activity of p38 MAPK as reflected by reduced phospho-ATF-2 in immunoprecipitate kinase activity assay (Figure 2A). Similarly, the DNA binding activity of NF-κB also decreased significantly in SB203580-treated group.

**Reduction in expression of pro-inflammatory cytokines after SB 203580 treatment**

Since Th1 cytokines play a main role in adult[20] and children patients[21] with type I AIH, we investigated the expression of Th1 cytokine mRNA in the setting of SB203580 treatment which significantly reduced IFN-γ, IL-12, TNF-α and IL-1β expression in livers of EAH mice. Our results showed that Th1 cytokines (IFN-γ, IL-12, IL-1β) and TNF-α mRNA were up-regulated in EAH (Figure 3).

**Improvement in hepatic inflammation and injury in EAH after SB203580 treatment**

We observed the effect of SB203580 on hepatic inflammation in this animal model. After given syngeneic
S-100 antigen, the mice developed perivascular infiltrates as well as intralobular inflammatory and necrotic lesions (Figure 4A). SB203580 significantly attenuated perivascular infiltrates as well as intralobular inflammation or necrosis (Figure 4B). The severity of inflammation as reflected by histological inflammation score was markedly decreased from 2.0 ± 0.18 in the control group to 1.4 ± 0.18 in the SB203580-treated group (Figure 4C). SB203580 decreased the serum ALT level from 116 ± 14 U/L in PBS control group and 103 ± 11 U/L in vehicle control group to 75 ± 10 U/L in SB203580-treated group (Figure 4D). DMSO-treated animals did show the same histological picture of typical EAH as PBS- treated animals.

**DISCUSSION**

Our previous study and other studies showed that intraperitoneal injection of hepatic S100 antigen causes inflammatory cell infiltration and hepatocellular injury, which was accompanied with a mild increase in alanine aminotransferase (ALT) level, and peaked at 4 wk after the first immunization.\(^{[3,18,19]}\)

In this study, we investigated the role of p38 MAPK in an animal model of autoimmune liver injury. The activation of p38 MAPK signaling pathway was up-regulated in experimental autoimmune hepatitis, and the inhibition of p38 MAPK reduced hepatic inflammation and injury. The protection against hepatic injury induced by p38 MAPK was associated with a diminished expression of NF-κB and Th1 cytokines (IFN-γ, IL-12, IL-1β and TNF-α) known to promote hepatic injury in AIH, showing that activation of p38 MAPK plays an important role in promoting cytokine/chemokine production, which in turn results in autoimmune hepatic injury in EAH.

The p38 MAPK signaling transduction pathway plays an essential role in regulating many cellular processes, including inflammation, cell differentiation, cell growth and death.\(^{[22]}\) Activation of the p38 MAPK pathway plays an essential role in the production of proinflammatory cytokines. By early discovery of the p38 MAPK signaling pathway, specific inhibitors of p38 MAPK could be identified. Pyridinyl imidazole compounds were found to inhibit the production of TNF-α and IL-1 in lipopolysaccharide (LPS)-stimulated cells. These compounds can bind to a protein that has been proved to be p38 MAPK.\(^{[24]}\) The most widely used agents are SB203580 and SB202190. Because inhibiting p38 MAPK suppresses production of key mediators, it is an obvious target for the treatment of chronic inflammatory disease.\(^{[25]}\) In addition, MAP kinases play an important role in immune responses from the innate to the adaptive immune system, from the initiation of immune responses to activation-induced cell death.\(^{[26]}\) Recently, Hollenbach and colleagues\(^{[21]}\) found that SB203580 improves the clinical score, ameliorates histological alterations, and reduces mRNA levels of proinflammatory cytokines in dextran sodium sulphate (DSS)-induced experimental colitis model of mice. These results suggest that p38 MAPK is a therapeutic target for autoimmune diseases. In our study, the activation of p38 MAPK was found to increase in a time-dependent manner in mice with experimental autoimmune hepatitis, and reached its peak on d 14 after the first syngeneic S-100 administration, preceding that of histological lesions (about at 4 wk). Furthermore, inhibition of p38 MAPK activity by SB203580 administration could decrease the serum ALT level and improve hepatic lesion significantly. As expected, SB203580 could also inhibit the expression of Th1 proinflammatory cytokines (IFN-γ, IL-12, IL-1β and TNF-α), which are the key cytokines in inflammatory response. These results suggest that activation of p38 MAPK is one of the initial pathogenic...
factors for autoimmune liver injury and its inhibition provides a novel therapeutic strategy.

It was reported that Th1 cytokines play a main role in the pathogenesis of type 1 AIH\[20,21,22,23,24,25]. Hussain et al\[26] showed that TNF-α and IFN-γ producing cells are detectable in inflammatory cell infiltrates, revealing a significant correlation between the frequency of TNF-α and IFN-γ producing cells, the intensity of inflammatory cell infiltrates and transaminase level. Recently, Chernavsky et al\[27] showed that expression of IFN-γ and IL-12 is up regulated in liver, but is not detectable in control liver. In addition, Czaja et al\[28] and Cookson et al\[29] demonstrated that patients with type 1 AIH have a higher frequency than those with a normal TNF gene polymorphism associated with increased transcription of TNF-α. Patients with such a polymorphism have a lower frequency of remission during corticosteroid therapy and a higher occurrence of treatment failure and cirrhosis. Our results indicate that inhibition of the expression of Th1 cytokines could result in remission of EAH.

NF-κB is a pivotal transcription factor for the regulation of many genes, particularly those for inflammatory and immune responses, including IL-1β, IL-12 and TNF-α\[30,31,32,33,34,35]. Because a large variety of stimulations activate NF-κB and the transcription factor regulates the expression of inflammatory cytokines, chemokines, immunoreceptors, and cell adhesion molecules, NF-κB is often termed a central mediator of human immune response\[33\]. It has been reported that inhibition of NF-κB activation in vivo, using a selective proteasome inhibitor, attenuates chronic inflammation in experimental models of Crohn’s disease and rheumatoid arthritis\[34,35\]. Previous studies have clearly demonstrated that NF-κB might be an effector of p38 MAPK\[36\]. We found that NF-κB activation was also induced in mice with EAH, and reached its peak a little later than p38 MAPK. Furthermore, inhibition of p38 MAPK activation could reduce the activation of NF-κB, suggesting that p38 MAPK upregulation promotes release of inflammatory cytokines via a NF-κB-dependent mechanism in autoimmune liver injury.

In summary, p38 MAPK plays an important role in liver injury induced by autoimmunity, and inhibition of p38 MAPK attenuates EAH, thus providing a biochemical basis for the potential of using specific inhibitors of p38 MAPK for treating autoimmune hepatitis.

ACKNOWLEDGMENTS

The authors thank Dr. Zhi-Ping Li, Department of Gastroenterology and Hepatology, Johns Hopkins University, for his critical review of this manuscript.

REFERENCES

1 Strassburg CP, Manns MP. Autoantibodies and autoantigens in autoimmune hepatitis. Semin Liver Dis 2002; 22: 339-352
2 Schramm C, Protschka M, Kohler HH, Podlech J, Reddehase MJ, Schirmacher P, Galle PR, Loheh AW, Blessing M. Impairment of TGF-beta signaling in T cells increases susceptibility to experimental autoimmune hepatitis in mice. Am J Physiol Gastrointest Liver Physiol 2003; 284: G525-G535
3 Loheh AW, Manns M, Dienes HP, Meyer zum Buschenfelde KH, Cohen IR. Experimental autoimmune hepatitis: disease induction, time course and T-cell reactivity. Hepatology 1990; 11: 24-30
4 Kohda H, Sekiya C, Kanai M, Yoshida Y, Uede T, Kikuchi K, Namiki M. Flow cytometric and functional analysis of mononuclear cells infiltrating the liver in experimental autoimmune hepatitis. Clin Exp Immunol 1990; 82: 473-478
5 Loheh AW, Brunner S, Kyratsoulis A, Manns M, Meyer zum Buschenfelde KH. Autoantibodies in experimental autoimmune hepatitis. J Hepatol 1992; 14: 48-53
6 Lohse AW, Dienes HP, Meyer zum Buschenfelde KH. Suppression of murine experimental autoimmune hepatitis by T-cell vaccination or immunosuppression. Hepatology 1998; 27: 1536-1543

7 Jaekel E. Animal models of autoimmune hepatitis. Semin Liver Dis 2002; 22: 325-38

8 Wang XS, Diener K, Manthey CL, Wang S, Rosenzweig B, Bray J, Delaney J, Cole CN, Chan-Hui PY, Mantlo N, Lichenstein HS, Zukiowski M, Yao Z. Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. J Biol Chem 1997; 272: 23668-23674

9 Nakamura H, Kawakami A, Yamasaki S, Kawabe Y, Nakamura T, Eguchi K. Expression of mitogen activated protein kinases in labial salivary glands of patients with Sjogren's syndrome. Ann Rheum Dis 1999; 58: 382-385

10 Schett G, Tohidast-Akrad M, Smolen JS, Schmid BJ, Steiner CW, Bitzan P, Zenz P, Redlich K, Xu Q, Steiner G. Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-JUN N-terminal kinase, and p38 mitogen-activated protein kinase, in synovial tissue and cells in rheumatoid arthritis. Arthritis Rheum 2000; 43: 2501-2512

11 Hollenbach E, Neumann M, Viet M, Roessner A, Malferttheiner P, Naumann M. Inhibition of p38 MAP kinase and RCK/NF-kappaB-signaling suppresses inflammatory bowel disease. FASEB J 2004; 18: 1550-1552

12 Iwata Y, Wada T, Furuichi K, Sakai N, Matsushima K, Yokoyama H, Kobayashi K. p38 Mitogen-activated protein kinase contributes to autoimmune renal injury in MRL-Fas lpr mice. J Am Soc Nephrol 2003; 14: 57-67

13 Aroor AR, Shukla SD. MAP kinase signaling in diverse effects of ethanol. Life Sci 2004; 74: 2339-2364

14 Panteva M, Korkaya H, Jamiel S. Hepatitis viruses and the MAPK pathway: is this a survival strategy? Viruses Res 2003; 92: 131-140

15 Tsutsusi T, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, Matsura Y, Koike K, Miymura T. Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. Hepatology 2003; 38: 820-828

16 Gilbert KM, Whitlow AB, Pumford NR. Environmental contaminant and disinfection by-product trichloroacetaldehyde stimulates T cells in vitro. Int Immunopharmacol 2004; 4: 25-36

17 Griffin JM, Gilbert KM, Lamps LW, Pumford NR. CD4+ T-cell activation and induction of autoimmune hepatitis following trichloroethylene treatment in MRL+/+ mice. Toxicol Sci 2000; 57: 345-352

18 Ma X, Qiu D, Peng Y, Chen X. Expression of neural cell adhesion molecule in murine livers with experimental autoimmune hepatitis. Zhonghua Ganzangbing Zazhi 2001; 9: 226-228

19 Ma X, Qiu DK, Li EL, Peng YS, Chen XY. Changes in T-cell population in murine experimental autoimmune hepatitis. Zhonghua Ganzangbing Zazhi 2004; 12: 44-46

20 Vergani D, Choudhuri K, Bogdanos DP, Mieli-Vergani G. Pathogenesis of autoimmune hepatitis. Clin Liver Dis 2002; 6: 727-737

21 Chernavsky AC, Paladino N, Rubio AE, De Biasio MB, Periolo N, Cuarterolo M, Goni J, Galoppo C, Canero-Velasco MC, Munoz AE, Fainboim H, Fainboim L. Simultaneous expression of TH1 cytokines and IL-4 confers severe characteristics to type I autoimmune hepatitis in children. Hum Immunol 2004; 65: 683-691

22 Ono K, Han J. The p38 signal transduction pathway: activation and function. Cell Signal 2000; 12: 1-13

23 Perregraux DG, Dean D, Cronan M, Connelly P, Gabel CA. Inhibition of interleukin-1 beta production by SKF86002: evidence of two sites of in vitro activity and of a time and system dependence. Mol Pharmacol 1995; 48: 433-442

24 Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 1994; 372: 739-746

25 Saklatvala J. The p38 MAP kinase pathway as a therapeutic target in inflammatory disease. Curr Opin Pharmacol 2004; 4: 372-377

26 Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. Annu Rev Immunol 2002; 20: 55-72

27 Al-Khalidi JA, Czaia AJ. Current concepts in the diagnosis, pathogenesis, and treatment of autoimmune hepatitis. Mayo Clin Proc 2001; 76: 1237-1252

28 Hussain MJ, Mustafa A, Gallati H, Mowat AP, Mieli-Vergani G, Vergani D. Celluar expression of tumour necrosis factor-alpha and interferon-gamma in the liver biopsies of children with chronic liver disease. J Hepatol 1994; 21: 816-821

29 Czaia AJ, Cookson S, Constantini PK, Clare M, Underhill JA, Donaldson PT. Cytokine polymorphisms associated with clinical features and treatment outcome in type 1 autoimmune hepatitis. Gastroenterology 1999; 117: 645-652

30 Cookson S, Constantini PK, Clare M, Underhill JA, Bernal W, Czaia AJ, Donaldson PT. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. Hepatology 1999; 30: 851-856

31 Baueerle PA, Baltimore D. NF-kappa B: ten years after. Cell 1996; 87: 13-20

32 Sun Z, Andersson R. NF-kappaB activation and inhibition: a review. Shock 2002; 18: 99-106

33 Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999; 18: 6853-6866

34 Conner EM, Brand S, Davis JM, Laroux FS, Palombella VJ, Fuseler JW, Kang DY, Wolf RE, Grisham MB. Proteasome inhibition attenuates nitric oxide synthase expression, VCAM-1 transcription and the development of chronic colitis. J Pharmacol Exp Ther 1997; 282: 1615-1622

35 Palombella VJ, Conner EM, Fuseler JW, Destree A, Davis JM, Larou FX, Wolf RE, Huang J, Brand S, Elliott PJ, Lazarus D, McCormack T, Parent L, Stein R, Adams J, Grisham MB. Role of the proteasome and NF-kappaB in streptococcal cell wall-induced polyarthritis. Proc Natl Acad Sci USA 1998; 95: 15671-15676

36 Baeza-Raja B, Munoz-Canoves P. p38 MAPK-induced nuclear factor-kappaB activity is required for skeletal muscle differentiation: role of interleukin-6. Mol Biol Cell 2004; 15: 2013-2026