Gastroenterol Hepatol. 2009;32(Espec Congr 1):83-9

Gastroenterología y Hepatología

www.elsevier.es/gastroenterologia

XXXIV CONGRESO ANUAL DE LA ASOCIACIÓN ESPAÑOLA PARA EL ESTUDIO DEL HÍGADO

The inflammatory response in liver injury

H. Tilg*

Christian Doppler Research Laboratory for Gut Inflammation, Medical University, Innsbruck, Austria

Abstract

Most liver diseases are characterized by inflammatory processes with enhanced local expression of various pro- and anti-inflammatory cytokines. These cytokines are the driving force of many inflammatory liver disorders often resulting in fibrosis and cirrhosis. Severe alcoholic hepatitis is the prototype of such a disease where tumor necrosis factor-alpha (TNFα) plays a key role. Anti-TNF treatment strategies might also improve other chronic inflammatory liver diseases such as primary sclerosing cholangitis or chronic hepatitis C infection. Adiponectin, the key adipocytokine, is another important mediator with mainly anti-inflammatory properties and beneficial effects in many experimental models of liver injury. The inflammatory injury plays a key role in most known liver diseases and specific neutralizing strategies are eagerly awaited.

Introduction

Cytokines are pleiotropic, regulatory peptides that can be produced by all types of liver cells. The cytokine family consists of several sub-families: the interleukins, the tumor necrosis factor (TNF) family, interleukin (IL)-6 and IL-6-related cytokines, interferons, chemokines such as IL-8, transforming growth factor-beta, colony-stimulating factors and others. In most tissues, including the liver, constitutive production of cytokines is absent or low. However, as physiologic and pathologic stimuli activate cells, the production of these autocrine, paracrine and endocrine effector molecules increases, and they, in turn, orchestrate the tissue's response to the stimulus. There is increasing evidence supporting a major role for several cytokines in various aspects of inflammatory liver diseases and liver tissue repair (table I). Cytokines are proximal mediators of hepatic inflammation, liver-cell death, cholestasis and fibrosis, but paradoxically also mediate regeneration of the liver after injury. Among the various cytokines, two cytokines from different cytokine families, namely the proinflammatory molecule TNF alpha (TNFα) and the anti-inflammatory adipocytokine adiponectin, have emerged as key cytokines in various aspects of liver diseases.

Cytokines and the normal liver

TNFα production is one of the earliest events in many types of liver injury, triggering the production of other cytokines that together recruit inflammatory cells, kill hepatocytes, and initiate an hepatic healing response that includes fibrogenesis. Although the liver's anatomic location and central role in drug and xenobiotic detoxification expose it to factors, such as reactive oxygen species and bacterial endotoxins, that induce TNFα production in other tissues, transcription of the genes for TNFα and other cytokines such as IL-10 is barely detectable in the normal liver. Moreover, administration of TNFα to animals or incubation of hepatocytes with TNFα in vitro promotes hepatocyte proliferation, rather than death. The same response (i.e., hepatocyte proliferation) also occurs after 70 percent partial hepatectomy, an insult that

*Autor para correspondencia.
E-mail: Herbert.Tilg@i-med.ac.at (H. Tilg).

TABLE I **Key properties of cytokines involved in liver diseases**

Pro-inflammatory cytokines
Interleukin-1 (IL-1)-type cytokines (IL-1α, IL-1β, TNFα)
Prototype proinflammatory cytokines, stimulation of acute phase protein synthesis
Interferon-gamma (IFNγ)
Immunoregulatory T helper cell (Th)-1 cytokine, induces TNFα
Interleukin-12
Th-1-directing cytokine
Interleukin-17
Key cytokine directing neutrophil trafficking
Interleukin-18
IFNγ-inducing factor, proinflammatory at a very early step in the immune response
Gp130-signaling cytokines (IL-6, IL-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, cardiotrophin)
Pro- and anti-inflammatory activities, stimulation of most acute phase proteins
IL-6 regulates hepatic regeneration and immunoglobulin synthesis
IL-32
Anti-inflammatory cytokines
IL-1 Receptor antagonist
Member of the IL-1 family; blocks binding of IL-1 to cell-surface receptors
Prototype anti-inflammatory cytokine
Soluble IL-1 receptor type II
Binds circulating IL-1
Soluble tumor necrosis factor receptor (TNFR) p55 (I)/p75 (II)
Naturally occurring TNF inhibitors
Comprised of extracellular domains of the two known TNFRs, p55 and p75
Block TNF-regulated inflammatory processes
IL-18 binding protein
Neutralizes IL-18
Interleukin-10
Prototype anti-inflammatory cytokine, regulates B-cell function
IL-4, IL-13
Th-2 cytokines, regulate B-cell function, suppress synthesis of proinflammatory cytokines
Cytokines involved in immune responses
IL-2, IL-4, IL-7, IL-9, IL-12, IL-15, IL-17, IL-22, IL-23, IL-33
Th-1 cytokines (IL-2, IFNγ)
Direct anti-viral response, proinflammatory
Th2-cytokines (IL-4, IL-5, IL-10)
Mediate inflammation, allergic responses and immunoglobulin synthesis
Cytokines involved in acute liver failure
TNF and TNFR p55/p75
Death receptors (Fas, Fas ligand)
Critically involved in liver injury and apoptosis
IL-18
Mediates TNF-and Fas-related experimental liver failure
Fibrogenic cytokines
Transforming growth factor-beta
Prototype fibrogenic cytokine, upregulation by proinflammatory cytokines
Platelet derived growth factor
Fibroblast growth factor
Anti-fibrogenic cytokines
Hepatocyte growth factor
Anti-fibrogenic, anti-apoptotic, promotes liver regeneration
Interferon-alpha
Anti-viral, immunomodulatory, anti-inflammatory, anti-fibrogenic
Adipocytokines
Adiponectin
Key anti-inflammatory adipocytokine
Leptin
Mainly pro-inflammatory and immunoregulatory functions

acutely increases TNFα in the hepatic micro-environment. Indeed, antibody neutralization studies and experiments with mice that lack type 1 TNF receptors indicate that after partial hepatectomy liver regeneration is initiated by activation of type 1 TNF receptors by TNFα.

Alcoholic hepatitis—the prototypic cytokine-driven liver injury model

Pathophysiologic aspects in alcoholic hepatitis

Role of TNFα in AH

Recently, various proinflammatory cytokines have been proposed to play a role in this disease[1-3]. Among these cytokines, the proinflammatory cytokine TNFα has emerged as a key cytokine in the inflammatory process. The involvement of TNFα has been especially demonstrated in acute alcoholic hepatitis. Indeed, alcoholic hepatitis was one of the first diseases shown to exhibit increased circulating TNFα levels[4-6]. In addition, serum concentrations of various TNF-inducible cytokines such as interleukin-1 (IL-1) and IL-8 are also increased in patients with acute alcoholic hepatitis. Furthermore, plasma levels of both TNF and soluble TNF receptors are correlated with endotoxemia and stage of liver disease[7]. This finding, coupled with evidence that long-term ingestion of alcohol increases intestinal permeability and those patients with the highest serum concentrations have the highest in-hospital mortality, indicates that intestinally derived endotoxin and endotoxin-regulated cytokines such as TNFα, have a role in the pathogenesis of alcoholic steatohepatitis. Furthermore, Mookerjee *et al* demonstrated that TNFα is the key mediator of portal and systemic haemodynamic derangements in severe alcoholic hepatitis[8].

Animal models highlighting the role of TNFα

Human data supporting a key role for TNFα in alcohol-related liver diseases are further substantiated by data from animal experiments[9,10]. Several studies in rats, mice, and tissue culture focused on the role of cytokines, especially TNFα, in experimental models of alcoholic liver disease. Perhaps the most compelling evidence supporting a key role for this cytokine comes from studies using mice in which the type-1 TNF receptor gene has been disrupted as these mice are resistent to alcohol-induced liver disease[11]. Anti-TNF antibody treatment has also been successfully used to prevent liver injury in alcohol-fed rats. Furthermore, alcohol-associated liver injury is inhibited when the animals are treated with poorly absorbed oral antibiotics or lactobacillus to decrease endotoxemia, supporting the hypothesis that gut-derived bacterial products such as endotoxin might be important in activation of Kupffer cells and/or other cell types in the liver. This is in accordance with the recent observation that chronic ethanol feeding causes more severe liver injury in wild-type mice than in CD14 knockouts. These results further support the notion that gut-derived endotoxin acting via its cellular receptor CD14 plays a major role in the development of early alcohol-induced liver injury.

Anti-cytokine based therapies for alcoholic hepatitis

Pentoxifylline

The observation that TNFα levels are increased in patients with alcoholic hepatitis provided a rationale for the study of pentoxifylline (an inhibitor of TNF synthesis) in alcoholic hepatitis. So far, only one study has been performed. In this study, 101 patients with severe alcoholic hepatitis were included. Mortality at four weeks was significantly decreased in the group randomized to pentoxifylline (25 versus 46 percent)[12]. The benefit appeared to be related to a significant reduction in the risk of developing hepatorenal syndrome. These results are encouraging but need to be confirmed by others before this therapy can be routinely recommended. The study did not include a treatment arm with corticosteroids, although the survival benefit was higher than observed in several studies with corticosteroids.

Louvet and colleagues recently presented evidence that pentoxifylline therapy is not effective in patients not responding to a short course of corticosteroid therapy. In this study, 29 corticosteroid non-responders were treated with pentoxifylline over a month. At the end, there were no differences between pentoxifylline-treated patients versus matched controls. Therefore, this treatment approach might not be effective at least in the prognostically worst group which is not surprising as pentoxifylline is a weak anti-inflammatory drug without major effects on neutrophil dysfunction which has been demonstrated to be of importance in these patients.

Anti-TNF antibody treatment of acute alcoholic hepatitis

Based on studies of alcohol-related liver disease in animals alternative therapies for these diseases have been proposed. In animals, treatment with various anti-oxidants decreases alcohol-induced liver damage and improves the fatty liver that develops in rats fed choline- and methionine-deficient diets, but so far the benefits have been inconsistent in the small groups of patients with alcoholic liver disease.

Evidence that TNFα is important in animals with steatohepatitis suggests that TNFα is another potential therapeutic target in patients with these diseases. Recently various TNF-neutralizing agents have been successfully used in the treatment of chronic inflammatory disorders such as Crohn's disease, ulcerative colitis, rheumatoid arthritis and psoriasis. Based upon those experiences and the convincing preclinical and clinical data on the role of TNFα in alcoholic hepatitis, pilot trials with Infliximab, a TNFα-neutralizing antibody, have been performed and recently reported.

Spahr et al have randomized 20 patients with biopsy-proven severe alcoholic hepatitis and treated them with prednisone 40 mg/day for 28 days and either infliximab 5 mg/kg body weight or placebo[13]. Infliximab was administered once on day 0 and was well tolerated. At day 28, the Maddrey score improved significantly only in the prednisone/infliximab group. Furthermore, infliximab therapy was associated with decreased circulating levels of IL-6 and IL-10 at day 10 of treatment, whereas histology was not affected after a median time of 10 days (8-12 days). Even though the authors observed changes in prothrombin time and serum bilirubin, this pilot study gave no answer about survival due too limited sample size of included patients.

We recently reported results of a similar pilot study with infliximab[14]. In contrast to Spahr's trial we did not use steroids and treated 12 patients with biopsy-confirmed alcoholic hepatitis and a Maddrey score > 32. We also used a single dose of 5 mg/kg infliximab. All patients were hospitalized and received standard treatment including treatment of alcohol withdrawal with benzodiazepines, administration of fluid, calories, vitamins and minerals and management of ascites. In four patients we did a follow-up biopsy within 28 days of treatment. We observed a significant decrease in bilirubin levels, Maddrey score, neutrophil counts and C-reactive protein levels. Changes in circulating cytokine levels were only transient (IL-6, IL-8). More importantly, we observed remarkable changes in the expression of a mainly TNF-driven chemokine, namely IL-8 in the liver and also histological improvements with decreased fat content and reduced neutrophil infiltration in the liver. These changes suggest that this novel treatment could also alter histological features of the disease. Two patients, however, died of septicemia within 28 days, whereas 10 of 12 patients were alive at a median of 15 months.

To further understand the effects of infliximab in severe alcoholic hepatitis Mookerjee and colleagues tested in infliximab-treated patients the hypothesis that TNFα is an important mediator of the circulatory disturbances in alcoholic hepatitis[15]. Cardiovascular haemodynamics, hepatic venous pressure gradient (HVPG) and hepatic and renal blood flow were measured before, 24 hours after infliximab, and prior to hospital discharge. Of the 10 reported patients, nine were alive at 28 days. Mean HVPG decreased significantly at 24 hours with a sustained reduction prior to discharge. Mean arterial pressure and systemic vascular resistance increased significantly paralleled by a reduction in cardia index. Furthermore, hepatic and renal blood flow also increased significantly suggesting that TNFα indeed is one of the key mediators in the observed circulatory disturbances in alcoholic hepatitis.

The French Infliximab Study

A French multicentre infliximab trial in alcoholic hepatitis was stopped in October 2002 by the French drug agency. In this study, the authors compared prednisolone (40 mg per day) either with placebo or infliximab given intravenously (10 mg/kg three times: week 0, 2 and 4). Mean end-point was 2-months mortality. After including 36 patients, an interim analysis revealed higher mortality in the infliximab group and the study was stopped[16]. It needs to be mentioned, however, that in this study unusual high doses of infliximab combined with short treatment intervals were used.

Etanercept in the treatment of ASH

Etanercept, a TNF receptor p75 fusion protein, showed promising results in a small pilot study in patients with moderate to severe ASH. Therefore, Boetticher and colleagues performed a placebo-controlled, randomized study which as been presented recently. In this study, 48 patients with moderate to severe AH (MELD score $\geq$ 15) received six doses of etanercept over a 3 week treatment period. The one month mortaility rates were similar whereas mortality rates at six months were significantly higher in the etanercept treated patients (58 vs 23%) raising serious concerns about such a treatment approach. Therefore, one may conclude that at least etanercept seems not to be a promising option for this disease. Whether this holds true for other anti-TNF agents is unclear.

So, what can learn from these conflicting results with anti-TNF blockers? The main issue treating patients with advanced alcoholic hepatitis is their high risk of severe infections. This aspect may be true using either steroids and/or anti-TNF agents. We now know from many clinical trials using infliximab in patients with various diseases that despite its potential to reactivate tuberculosis this treatment is associated with a remarkable benfit/risk ratio also with respect to infections. Certainly patients with alcoholic hepatitis may have an increased risk of developing tuberculosis or fungal infections compared to patients with rheumatoid arthritis or Crohn's disease. This may be, however, also true for treatment with steroids. There-

fore, it is probably more important to challenge our current concepts in treating alcoholic hepatitis as waiting in a smouldering disease until the patient fulfills a Maddrey score > 32. In our daily practice we often observe the patient over days using a watch-and-wait strategy until we finally decide to treat him e.g. with steroids. This concept should be challenged as we might think about earlier treatment dealing with a patient who is less immunocompromised using more selective treatment such as a neutralizing antibody. Certainly we need new concepts in better defining the patient population with alcoholic hepatitis which needs early treatment, a concept which is practiced in most diseases.

Adiponectin: the key adipocyte-derived cytokine controlling inflammation in the liver

Adipose tissue and its metabolic products have recently gained dramatic interest both from the scientific and the public community. Paralleled by the increasing prevalence of obesity various soluble mediators from the fat tissue have been identified clearly demonstrating that the fat tissue is not an «inert, lazy» tissue but acts as an endocrine organ communicating with many other tissues and especially with cells from the immune system. Various products of the fat tissue have been characterized including not only cytokines such as TNFα or IL-6, but also mediators involved in clotting processes such plasminogen-activator inhibitor type I and even more importantly so called adipokines such as leptin, resistin and adiponectin[17]. Adipocytokines such as adiponectin circulate in rather high concentrations and from a cytokine perspective have many features beyond a classical cytokine even reflecting more the nature of a hormone.

Adiponectin is a protein exclusively secreted from adipose tissue and shares sequence homology with a family of proteins that show a characteristic NH2-terminal collagen-like region and a COOH-terminal, complement factor C1q-like globular domain. This adipokine/cytokine is a relatively abundant plasma protein found in multimeric complexes in the circulation at relatively high concentrations in healthy human subjects. Plasma levels are markedly reduced in visceral obesity and states of insulin resistance such as non-alcoholic steatohepatitis[18] and diabetes mellitus type II.

Anti-inflammatory properties of adiponectin in vitro

Whereas adiponectin -/- mice show evidence of increased systemic TNFα production (ref), adiponectin itself also has an inhibitory effect on TNFα synthesis. Adiponectin suppresses endotoxin-induced TNFα mRNA expression in macrophages. Such a regulatory effect could also be found in animal experiments. Supplementation of plasma adiponectin using an adenoviral transfection system decreased TNFα in KO mice supporting the notion that adiponectin directly suppresses TNFα[19].

Three independent reports recently demonstrated the induction of Ithe anti-inflammatory cytokine IL-10 by adiponectin[20-22]. Kumada and co-workers demonstrated an upregulation of IL-10 in human monocyte-derived macrophages both at the transcriptional and protein level. Induction of IL-10 by adiponectin in their experiments furthermore upregulated tissue inhibitor of metalloproteinase-1 (TIMP-1), an important matrix metalloproteinase (MMP). Interestingly, other MMPs such as MMP-9 were not affected by adiponectin. Wulster-Radcliffe et al presented a similar finding in porcine macrophages where they also observed induction of IL-10 by adiponectin. They also found a suppressive effect of adiponecton on IL-6, a cytokine which shows proinflammatory actions in many situations but also has anti-inflammatory actions. Additionally they found that adiponectin led to an attenuation of translocation of NFkappaB (NFκB) to the nucleus. We recently reported a similar finding demonstrating that adiponectin induces IL-10 not only in macrophages but also in primary human monocytes and in dendritic cells (DC). We also could demonstrate for the first time that adiponectin suppresses another major proinflammatory macrophage product, namely IFNγ.

We recently presented clear evidence that adiponectin besides IL-10 also upregulates another anti-inflammatory cytokine, namely IL-1 receptor antagonist in human leukocytes. Therefore, it is now very clear that adiponectin indeed upregulates at least two critical anti-inflammatory cytokines (IL-10 and IL-1Ra).

Anti-inflammatory properties of adiponectin in experimental liver injury

Xu et al recently demonstrated a beneficial effect of adiponectin on alcoholic and non-alcoholic fatty liver disease in mice[23]. Administration of adiponectin into mice with alcoholic liver disease dramatically alleviated hepatomegaly and fatty liver steatosis and also significantly attenuated inflammation and the elevated levels of serum alanine aminotransferase. In these experiments, adiponectin suppressed both hepatic TNFα expression and plasma concentrations of this potent proinflammatory cytokine. Adiponectin was also effective in improving hepatomegaly, steatosis, and alanine aminotransferase abnormality in *ob/ob*

mice[24]. These data suggest that adiponectin might have a positive therapeutic effect not only in diseases linked with insulin resistance as NASH but also in alcoholic liver disease, a disease where fatty infiltration is obvious but insulin resistance not a common feature.

Kamada et al used the carbon-tetrachloride liver fibrosis model and demonstrated that adiponectin attenuates liver fibrosis in mice[25]. In this model carbon tetrachloride is injected twice a week for 12-weeks resulting in an extensive liver fibrosis. A single injection of adenovirus producing adiponectin either prior or at 6 weeks after staring the carbon-tetrachloride treatment prevented liver fibrosis in wild-type mice. Hepatic stellate cells transformed into myofibroblast cells under activation are considered the key cell type in fibrosis. Adiponectin suppresses platelet-derived growth factor-induced proliferation and migration of cultured hepatic stellate cells and attenuates the effect of fibrogenic transforming growth factor-beta 1 on the gene expression of transforming growth factor-beta.

Adiponectin also protects from endotoxin-induced liver injury in another model of fatty liver, namely the KK-Ay obese mice[26]. The Galactosamine/endotoxin injury was more pronounced in KK-Ay obese mice compared to lean controls. Pretreatment with adiponectin ameliorated the Galactosamine/endotoxin-induced elevation of liver enzymes and apoptotic and necrotic changes in hepatocytes, resulting in reduced lethality. These adiponectin effects were paralleled by decreased levels of TNFα both systemically and in the liver.

These animal data together suggest that adiponectin seems to have considerable preventive potential in various models of liver toxicity and liver inflammation, all of which are characterized by increased TNFα synthesis, further supporting the importance of adiponectin-TNFα interaction.

References

1. McClain CJ, Barve S, Barve S, Deaciuc I, Hill DB. Tumor necrosis factor and alcoholic liver disease. Alcohol Clin Exp Res. 1998;22 Suppl:248S-52S.
2. McClain CJ, Barve S, Deaciuc I, Kugelmas M, Hill D. Cytokines in alcoholic liver disease. Semin Liver Dis. 1999;19:205-19.
3. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med. 2000;343:1467-76.
4. Bird GL, Sheron N, Goka AK, Alexander GJ, Williams RS. Increased plasma tumor necrosis factor in severe alcoholic hepatitis. Ann Intern Med. 1990;112:917-20.
5. McClain CJ, Cohen DA. Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. Hepatology. 1989;9:349-51.
6. Khoruts A, Stahnke L, McClain CJ, Logan G, Allen JI. Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. Hepatology. 1991;13: 267-76.
7. Hanck C, Rossol S, Bocker U, Tokus M, Singer MV. Presence of plasma endotoxin is correlated with tumour necrosis factor receptor levels and disease activity in alcoholic cirrhosis. Alcohol Alcohol. 1998;33:606-8.
8. Mookerjee RP, Sen S, Davies NA, Hodges SJ, Williams R, Jalan R. Tumour necrosis factor alpha is an important mediator of portal and systemic haemodynamic derangements in alcoholic hepatitis. Gut. 2003;52:1182-7.
9. Iimuro Y, Gallucci RM, Luster MI, Kono H, Thurman RG. Antibodies to tumor necrosis factor alfa attenuate hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat. Hepatology. 1997;26:1530-7.
10. Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, et al. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. Gastroenterology. 1999;117: 942-52.
11. Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, et al. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. Gastroenterology. 1999;117: 942-52.
12. Akriviadis E, Botla R, Briggs W, Han S, Reynolds T, Shakil O. Pentoxifylline improves short-term survival in severe acute alcoholic hepatitis: a double-blind, placebo-controlled trial. Gastroenterology. 2000;119:1637-48.
13. Spahr L, Rubbia-Brandt L, Frossard J, Giostra E, Rougemont A, Pugin J, et al. Combination of steroids with infliximab or placebo in severe alcoholic hepatitis: a randomized controlled pilot study. J Hepatol. 2002;37:448.
14. Tilg H, Jalan R, Kaser A, Davies NA, Offner FA, Hodges SJ, et al. Anti-tumor necrosis factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis. J Hepatol. 2003;38:419-25.
15. Mookerjee RP, Sen S, Davies NA, Hodges SJ, Williams R, Jalan R. Tumour necrosis factor alpha is an important mediator of portal and systemic haemodynamic derangements in alcoholic hepatitis. Gut. 2003;52:1182-7.
16. Poynard T, Thabut D, Chryssostalis A, Taieb J, Ratziu V. Anti-tumor necrosis factor-alpha therapy in severe alcoholic hepatitis: are large randomized trials still possible? J Hepatol. 2003;38:518-20.
17. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. Trends Endocrinol Metab. 2000;11:327-32.
18. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology. 2004;40:46-54.
19. Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, et al. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. Hepatology. 2004;40:177-84.
20. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, et al. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation. 2004;109:2046-9.
21. Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. Biochem Biophys Res Commun. 2004;316: 924-9.

22. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochem Biophys Res Commun. 2004;323: 630-5.
23. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest. 2003;112:91-100.
24. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest. 2003;112:91-100.
25. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology. 2003;125:1796-807.
26. Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, et al. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. Hepatology. 2004;40:177-84.