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TOPIC HIGHLIGHT

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Fat: A matter of disturbance for the immune system

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Abstract

Obesity is increasingly being recognized as a risk factor for a number of benign and malignant gastrointestinal conditions. However, literature on the underlying pathophysiological mechanisms is sparse and ambiguous. There is compelling evidence that both overnutrition and undernutrition negatively interfere with the immune system. Overnutrition has been found to increase susceptibility to the development of inflammatory diseases, autoimmune diseases and cancer. In the regulation of immune and inflammatory processes, white adipose tissue plays a critical role, not only as an energy store but also as an important endocrine organ. The obese state is characterised by a low-grade systemic inflammation, mainly as a result of increased adipocytes as well as fat resident- and recruited-macrophage activity. In the past few years, various products of adipose tissue including adipokines and cytokines have been characterised and a number of pathways linking adipose tissue metabolism with the immune system have been identified. Activation of the innate immune system plays a major role in hepatic steatosis. Non-alcoholic fatty liver disease includes a wide spectrum of diseases, from pure steatosis to non-alcoholic steatohepatitis in the absence of significant alcohol consumption. Although steatosis is considered a non-progressive disease, non-alcoholic steatohepatitis may deteriorate in advanced chronic liver diseases, cirrhosis, and hepatocellular carcinoma. An important parallel between obesity-related pathology of adipose tissue and liver pertains to the emerging role of macrophages, and growing evidence suggests that Kupffer cells critically contribute to progression of non-alcoholic fatty liver disease. Moreover, a close link between specific immune activation and atherosclerosis has been well established, suggesting that fat can directly trigger immune responses. This review discusses the role of fat as "a matter of disturbance for the immune system" with a focus on hepatic steatosis.

Key words: Adipocytokine; Adipose tissue; Fat; Immune system; Kupffer cell; Natural killer; Steatosis

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INTRODUCTION

Obesity predisposes individuals to an increased risk of

developing many diseases, including atherosclerosis, diabetes, non-alcoholic fatty liver disease (NAFLD), cancer and immune-mediated disorders, such as asthma[1-3]. Obesity is typically assessed clinically with the surrogate measure of body mass index (BMI). Individuals with a BMI $\geqslant$ 30 kg/m^2 are considered obese. The incidence of obesity and its associated disorders is increasing markedly worldwide. Data from the most recent NHANES (National Health and Nutrition Examination Survey; 2005-2006) indicate that the prevalence of obesity was 33%-35% among US adults[4]. In another recent NHANES survey based on the combined years of 2003-2006, 16% of children or adolescents aged 2-19 years were obese[5]. In Europe, several surveys conducted since 2000 and using direct anthropometric measurements, showed that the prevalence of obesity ranges from 15% to 30% in men and from 11% to 34% in women, with considerable geographic variation (rates being higher in Central, Eastern, and Southern Europe)[6]. Urbanization and unbalanced diet, associated with genetic susceptibility have allowed the emergence of the obese phenotype.

In mammals, adipose tissue (AT) occurs in two forms: white adipose tissue (WAT) and brown adipose tissue (BAT). Most AT in mammals is WAT and this is thought to be the site of energy storage. In contrast, BAT is found mainly in human neonates and is important for the regulation of body temperature through non-shivering thermogenesis. In addition to adipocytes, which are the most abundant cell type in WAT, adipose tissue also contains pre-adipocytes or stromal vascular cells (which are non-fat cells): endothelial cells, fibroblasts, leukocytes and, most importantly, macrophages. Body fat distribution, rather than adiposity *per se*, is an important risk factor for obesity-related disorders. An excess of intra-abdominal fat rather than subcutaneous fat (central *vs* peripheral obesity) is associated with metabolic syndrome (MS) and cardiovascular disease (CVD). The mechanisms responsible for this association are still unknown, but several hypotheses, which are not mutually exclusive, have been formulated[7]. The first hypothesis proposed a direct effect of visceral AT depots on insulin resistance, lipoprotein metabolism, and blood pressure. Metabolic products of omental and mesenteric AT depots are released into the portal vein, which provides direct delivery to the liver. Lipolysis of omental and mesenteric AT depots releases free fatty acids (FFAs) that can induce hepatic insulin resistance and provide substrate for lipoprotein synthesis and neutral lipid storage in hepatocytes. In addition, specific proteins and hormones produced by omental and mesenteric AT, such as inflammatory molecules, angiotensinogen, and cortisol can also contribute to MS and CVD. Another hypothesis suggests that the limited capacity of subcutaneous fat to store excess energy results in overflow of fatty acids to intra-abdominal fat and "ectopic" sites such as liver, muscle, and islets. In this paradigm, excess intra-abdominal fat is merely a marker of fatty acid overflow from subcutaneous depots.

FAT AND THE IMMUNE SYSTEM

Adipose tissue was once thought to be an inert mass whose sole function was the storage of fat. However, it is now recognized that AT is an active endocrine organ that secretes numerous adipokines, cytokines and chemokines including leptin, adiponectin, resistin, retinol binding protein 4 (RBP4), tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-6, and monocyte chemotactic protein 1 (MCP-1)[8,9]. All of these play a central role in the regulation of energy and vascular as well as immune system homeostasis by acting both locally and at distant sites influencing various metabolic and immune processes. Moreover, organs other than AT may contribute to systemic levels of some adipokines.

Obesity is associated with a low-grade inflammation of WAT resulting from chronic activation of the innate immune system, which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes[10,11]. In addition to these associations between obesity and disease, research in the past few years has identified important pathways that link metabolism with the immune system and *vice versa*. Many of these interactions between metabolic and immune systems seem to be orchestrated by the complex network of soluble mediators derived from immune cells and adipocytes[1].

The effects of obesity on the immune system are not restricted to local effects within AT. Elevated levels of pro-inflammatory cytokines have been noted in the serum of asymptomatic obese individuals, the cytokine levels being related to the degree of obesity[12]. TNF-α is only present at very low levels in human blood suggesting that TNF-α released by adipose tissue has only autocrine/paracrine actions. IL-6, however, is present at much higher levels. Adipocyte-derived IL-6 has been estimated to comprise 30% of the circulating IL-6 suggesting an endocrine action[13]. Furthermore, these elevated levels of IL-6 are associated with increased circulating levels of C-reactive protein suggesting that although the elevation in levels is modest compared with those seen in sepsis, they could be having real effects on innate immune function.

Obesity is also associated with altered functioning of circulating immune cells[12,14]. Decreased T- and B-cell function, increased monocyte and granulocyte phagocytosis and oxidative burst, and an increase in leukocyte count have been described. More recently, circulating mononuclear cells from obese subjects have been shown to exhibit increased nuclear factor κB (NFκB) nuclear binding with decreased levels of NFκB inhibitor, together with increased mRNA expression of IL-6, TNF-α and migration inhibition factor. Furthermore, there is a good correlation between the markers of macrophage activation and plasma levels of FFAs[15]. It has previously been demonstrated that macronutrient challenges in normal subjects increase NFκB nuclear binding in circulating mononuclear cells, raising the possibility that the activated state of mononuclear cells is due to increased circulating levels of FFAs found in the obese. Indeed, hyperlipidaemia in mice mediates an inflammatory response by the same signalling cascade through which lipopolysaccharide activates the innate immune system (this engages a receptor complex comprising Toll 4 CD14, CD14 and MD-2)[16]. Table 1

Table 1 Adipocytokines, pro-inflammatory cytokines and chemokines, and other factors synthesised by adipocytes and macrophages in white adipose tissue

Adipocytes	Macrophages
Adiponectin	TNF-α
Leptin	IL-1β
Resistin	IL-6
RBP4	MCP-1
TNF-α	Resistin
IL-1β	
IL-6	
MCP-1	
Visfatin	
MIP	

RBP4: Retinol binding protein 4; TNF-α: Tumor necrosis factor α; IL: Interleukin; MCP: Monocyte chemotactic protein; MIP: Macrophage inflammatory protein.

summarises the secretion of adipokines, cytokines and other factors by adipocytes and macrophages in WAT. Finally, recent research has implicated the innate immune system in the pathophysiology of obesity-related liver damage[17,18].

Obesity is a high risk factor for NAFLD. Studies in an animal model of obesity-related liver disease revealed the involvement of dysfunctional hepatic immune cells[19]. In this review we analyse the relationship between hepatic steatosis and the immune system.

HEPATIC STEATOSIS AND THE IMMUNE SYSTEM

Hepatic steatosis is the histological hallmark of alcoholic liver disease (ALD) and NAFLD, which are among the commonest causes of cirrhosis and liver failure in the developed world[20-24]. Steatosis may also alter the natural history of other liver diseases such as chronic viral hepatitis[25]. Excessive consumption of alcohol in humans results in a spectrum of liver abnormalities, ranging from simple fatty liver to steatohepatitis and cirrhosis, which may be present independently or in combination. Infiltration of the liver by lymphocytes and neutrophils is an important feature of alcoholic hepatitis; it initiates a cascade of effector mechanisms that ultimately lead to hepatocyte death, fibrosis, and cirrhosis. Only a minority of consistently heavy drinkers with steatosis ever develop clinically important liver disease[24,26] implying that host or environmental factors determine the evolution of alcohol-related liver damage. Ingestion of alcohol leads to increased production of reactive oxygen species (ROS), which are generated during the metabolism of alcohol by cytochrome P450 2E1 enzyme, and excessive alcohol consumption is associated with increases in lipid, protein, and DNA peroxidation. Consistent with this disease model, risk factors for the development of progressive liver damage in alcohol drinkers include both polymorphisms in alcohol-metabolizing enzymes and polymorphisms in genes associated with a more vigorous inflammatory response in addition to exogenous factors including obesity, exposure to other hepatotoxins, and infection with hepatitis C and/or B virus[27-29].

NAFLD is increasingly recognized as a leading cause of liver dysfunction and cirrhosis in the developed world and is part of a spectrum of metabolic diseases associated with central (intra-abdominal) obesity, hypertension, dyslipidaemia, insulin resistance, and type 2 diabetes mellitus[22,30]. Similar to alcoholic liver disease, NAFLD is a spectrum of disorders, beginning as simple steatosis that is mostly considered an innocent condition. Being both the source and the result of insulin resistance, however, steatosis may be associated with an increased risk for cardiovascular morbidity[31]. Most importantly, in about 15% of all patients with NAFLD, steatosis may evolve into steatohepatitis (NASH), a medley of inflammation, hepatocellular injury, and fibrosis, often resulting in cirrhosis and even hepatocellular carcinoma[32]. Although this full sequence of progression is relatively rare, the overwhelming prevalence of NAFLD predicts a major healthcare burden. Epidemiology, pathogenesis, and approach to treatment of NAFLD follow the same trends as other metabolic disorders, and insulin resistance is the key event linking NAFLD to these diseases[33-35].

ROLE OF ADIPOCYTOKINES IN ALCOHOLIC AND NON-ALCOHOLIC STEATOHEPATITIS

Many of the initial proinflammatory changes seen in NAFLD may be the consequence of altered metabolism rather than the underlying immune pathogenic event, and adipokines provide a link between fat, inflammation, and immunity (for more details see review by Tilg *et al*[9]). More than 50 adipokines have been identified so far. Of these, leptin and adiponectin can influence the immune response, and their serum levels are increased and decreased, respectively, in NASH[9]. While many adipokines are associated with adverse biological functions, adiponectin, the most abundant adipose-derived hormone, seems to have a protective effect in NAFLD. Adiponectin inhibits TNF-α induced endothelial cell adhesion molecule expression, induces production of anti-inflammatory cytokines such as IL-10, and reduces T and B lymphocyte responses. In particular, full-length adiponectin (Acrp30) and its cleavage derivative, globular adiponectin (gAcrp), have been credited with anti-diabetic, anti-inflammatory and anti-atherogenic properties[36]. Adiponectin stimulates hepatic fatty acid oxidation and ketogenesis, while it inhibits cholesterol and triglyceride synthesis[36]. While these metabolic activities primarily occur in hepatocytes, adiponectin has potent anti-inflammatory effects in macrophages. Thus, adiponectin is able to suppress the effects of lipopolysaccharides (LPS) in macrophages, including activation of NFκB and ERK1/2[37-39]. Similarly, adiponectin prevents LPS-mediated inflammatory signalling in Kupffer cells[40]. These anti-inflammatory effects of adiponectin may involve IL-10 signalling pathways[41]. Interestingly, NADPH oxidase is a

major IL-10 target in various cell systems including macrophages[42].

Decreased levels of adiponectin are definitely related to a variety of unfavourable effects, but the precise origin of adiponectin reduction has not been clarified. TNF-α has been demonstrated to suppress the transcription of adiponectin in an adipocyte cell line, which might explain the lower levels of serum adiponectin in obese individuals[9]. Expression of adiponectin is also regulated by other pro-inflammatory mediators such as IL-6, which suppresses adiponectin transcription and translation in an adipocyte cell line[9].

In a recent study, Kolak *et al*[43] evaluated subcutaneous AT biopsies obtained from healthy women both with and without increased liver fat (LFAT) (2.3% ± 0.3% *vs* 14.4% ± 2.9%, respectively), with similar BMIs and percentage body fat. Expression of cytokines and chemokines including CD68 (which correlates with the number of macrophages), MCP-1, macrophage-inflammatory protein (MIP-1α), and PAI-1 were significantly increased, whereas peroxisome proliferator-activated receptors (PPAR)-γ and adiponectin were significantly decreased in women with high levels of LFAT compared with women with normal levels of LFAT, even though subcutaneous fat cell size, BMI, and percentage body fat were similar.

Leptin activates neutrophils, stimulates proliferation in human circulating monocytes, and appears to induce Th1-type cytokine production while inhibiting Th2-type cytokines. In addition, leptin has marked effects on the innate immune response by promoting activation and phagocytosis of macrophages, presumably through JAK/STAT signalling[44]. Expansion of adipocytes in obesity leads to the recruitment of macrophages and the release of TNF-α, IL-6, and MCP-1 from macrophages and lymphocytes. TNF-α and IL-6 suppress the transcription of adiponectin, and TNF-α and IL-1 stimulate the production of leptin[9,44]. Accordingly, hyperleptinaemia associated with obesity may contribute to progression of NAFLD, although this issue remains controversial[45].

Resistin is another pro-inflammatory adipokine secreted by monocytes/macrophages and adipocytes in response to pro-inflammatory signals. Resistin induces NFκB-dependent secretion of TNF-α and IL-6 by monocytes and increases ICAM-1 and VCAM-1 expression in endothelial cells, suggesting that it contributes to endothelial activation and leukocyte recruitment[46]. In particular, in pure steatosis there is no significant increase in adhesion molecule expression but distinctive patterns are associated with both alcoholic hepatitis and cirrhosis, and in murine models of NASH elevated ICAM-1 expression is seen[47]. Alcoholic hepatitis is characterized by increased expression of E-selectin and ICAM-1 on portal and hepatic venous endothelium and of ICAM-1, VCAM-1, and VAP-1 on sinusoidal endothelium as a consequence of local pro-inflammatory cytokines, particularly TNF-α[48-51]. In alcoholic cirrhosis, increased expression of endothelial adhesion molecules including ICAM-1, VCAM-1, and P-selectin is largely restricted to portal and septal vessels. Endothelial ICAM-1 expression is increased in periseptal areas where LFA-1 is also increased in leukocytes, however, in contrast to alcoholic hepatitis, there is little increased ICAM-1 expression on hepatocytes[48].

Visfatin, the characteristic adipokine of mesenteric AT, was previously identified as a protein involved in immune B-cell maturation (pre-B colony enhancing factor)[52]. More recently, visfatin was described to be a highly expressed protein with insulin-like functions that was predominantly found in visceral AT, from which the name visfatin was derived[53]. Thus, visfatin was identified as nicotinamide phosphoribosyltransferase, the rate-limiting enzyme that converts nicotinamide (a form of vitamin B3) to nicotinamide mononucleotide, a NAD precursor[54]. Visfatin also has pro-inflammatory properties by inducing TNF-α and IL-6 in monocytes[55]. Further studies are needed to fully understand the effect of this adipokine in Kupffer cells.

Figure 1 summarises the effects of adipocytokines on the regulation of the immune response.

HEPATIC STEATOSIS AND NATURAL KILLER CELLS

One experimental model which has generated a significant body of evidence regarding potential mechanisms of NAFLD pathogenesis and its relationship with the immune system is the *ob/ob* mouse. *Ob/ob* mice, which are leptin deficient as a result of a spontaneous mutation in the leptin gene, exhibit a number of metabolic and inflammatory features which mimic human NAFLD[56] including insulin resistance, hyperlipidaemia, hepatic steatosis, and TNF-α elevation. One of the principal applications of the *ob/ob* mouse has been the identification of susceptibility of the steatotic liver to inflammatory insult (exemplified by the response to LPS) as a key factor in the development of NASH[57]. A number of immuno-regulatory abnormalities have been identified in *ob/ob* mice which may contribute to their increased susceptibility to inflammatory damage. These include selective depletion in the liver (but not other organs) of Natural Killer (NK) T cells, a key population of immuno-regulatory/effecter lymphocytes which express phenotypic features of both "classical" T cells (CD3) and NK cells [NK1.1 (CD161 in humans)][58,59]. In their most characteristic form, NKT cells show specificity, through a semi-invariant surface T-cell receptor, for highly conserved glycolipid antigens presented by the MHC class I homolog CD1d. NKT cells, which are specifically enriched within the liver, have characteristic cytokine release patterns {Th-1 dominant [interferon (IFN)-γ], mixed, and Th-2 dominant (IL-4) depending on the mechanism of stimulation} which endow, in addition to their effector function, significant immuno-regulatory properties[60]. The observation that liver NKT cells are depleted in steatosis in *ob/ob* mice has led to the suggestion that these cells play a key role in mediating and/or regulating inflammatory effects critical to the development of NAFLD. Although of potential value in the understanding of the pathogenesis of NAFLD, conceptual problems arise with regard to the *ob/ob* mouse

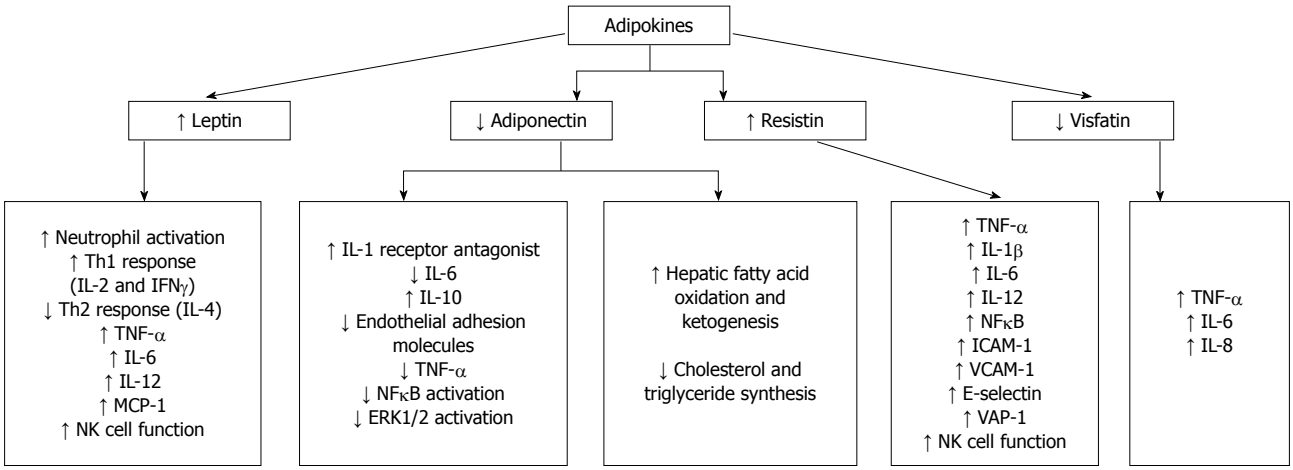

Figure 1 Effects of adipocytokines on regulation of the immune response. IL: Interleukin; IFN: Interferon; MCP: Monocyte chemotactic protein; NK: Natural Killer; TNF-α: Tumor necrosis factor α; NFκB: Nuclear factor κB.

as a model for human disease due to its markedly different leptin phenotype (absent *vs* elevated), and to the fact that leptin is itself a key immunomodulatory cytokine[61]. There are, therefore, potential mechanisms whereby leptin deficiency could modulate the immune response independent of its effects on hepatic fat accumulation. Li *et al*[62] used a natural obese/steatosis model to study the effects of hepatic steatosis on hepatic innate immune system function in leptin complete animals. C57Bl/6 mice fed a high-fat diet showed excess weight gain and the development of hepatic steatosis[63]. Although total hepatic mononuclear cell levels were similar in the high- and low-fat diet groups, the percentage of hepatic (but not splenic) NKT cells was significantly reduced. Within both the hepatic T-cell and NKT fractions, the numbers of cells showing cytoplasmic staining for TNF-α and IFN-γ were, conversely, increased in the high-fat diet group (and serum IFN-γ levels were elevated), suggesting Th1 skewing of the response phenotype resulting from induced NKT cell effects. Finally, the livers of obese mice appeared to be sensitized to LPS injury, presumably reflecting the augmented Th1-type inflammatory cytokine response. These observations suggest that the development of hepatic steatosis *per se* can be associated with significant changes in liver NKT cell function. This finding would be compatible with the NKT cell changes seen in the *ob/ob* mice occurring as a result of hepatic steatosis that occurs in these animals, rather than the specific absence of leptin. The findings do, however, raise a number of issues which will determine whether this model is suitable for the study of human NAFLD. The first issue is the mechanism responsible for liver NKT cell "loss", and Th-1 skewing of the residual cells, in obese C57Bl/6 mice. Theoretically, a reduction in liver NKT cells in obese C57Bl/6 mice could result from a decreased rate of NKT cell recruitment to, or development in, the liver, an increased rate of NKT cell death or migration from the liver, a loss of surface markers identifying the cells as NKT cells or any combination of these effects. The liver recruitment aspect of NKT cell homeostasis was not addressed in the Li's study[60,64,65]. Instead, the authors argue that increased cell loss is the dominant effect, with evidence presented to suggest increased NKT cell apoptosis and increased hepatic expression of IL-12 (postulated to be a promoter of NKT cell apoptosis). There is an emerging consensus, however, that NKT cells are in fact relatively resistant to activation-induced cell death[66]. An alternative (albeit non-mutually exclusive) explanation for the Li's data would be that endogenous IL-12 released by Kupffer cells (KC) at elevated levels in the context of obesity[62,67] acts as a cofactor for the stimulation of IFN-γ release (as opposed to IL-4 release which occurs in the absence of IL-12) by physiologically activated NKT cells, with the resulting "loss" of cells occurring as a consequence of post-activation surface phenotypic shift[68]. If elevation of KC-released IL-12 in response to steatosis were to prove to be a factor in human fatty liver development[69], its well-established ability to promote breakdown of self-tolerance may explain the increasingly recognised tendency towards autoantibody formation reported in NASH patients[70,71]. The possibility that NKT cell activation is responsible, through activation-induced cell death and/or post-activation phenotypic change, for "reduction" in hepatic NKT cells in obese C57Bl/6 mice, and through cytokine release, for liver damage, raises the important question of the mechanism of this activation. Most previous work on NKT cell activation has used non-physiological ligands (anti-CD3 and anti-TCR). Although the recent identification of α-galactosylceramide has highlighted the potential importance of glycolipids as natural ligands for NKT, it is unlikely, given its marine sponge origin, that this agent is a physiological ligand in mice or humans. At present, the identity of the *in vivo* physiological ligand for NKT cells, the extent to which TCR-mediated as opposed to cytokine-driven mechanisms (such as *via* IL-12) are required for activation, and the extent to which different activation pathways result in different cytokine response phenotypes, remain areas of speculation. One potentially highly intriguing link between hepatic steatosis and NKT cell activation has emerged

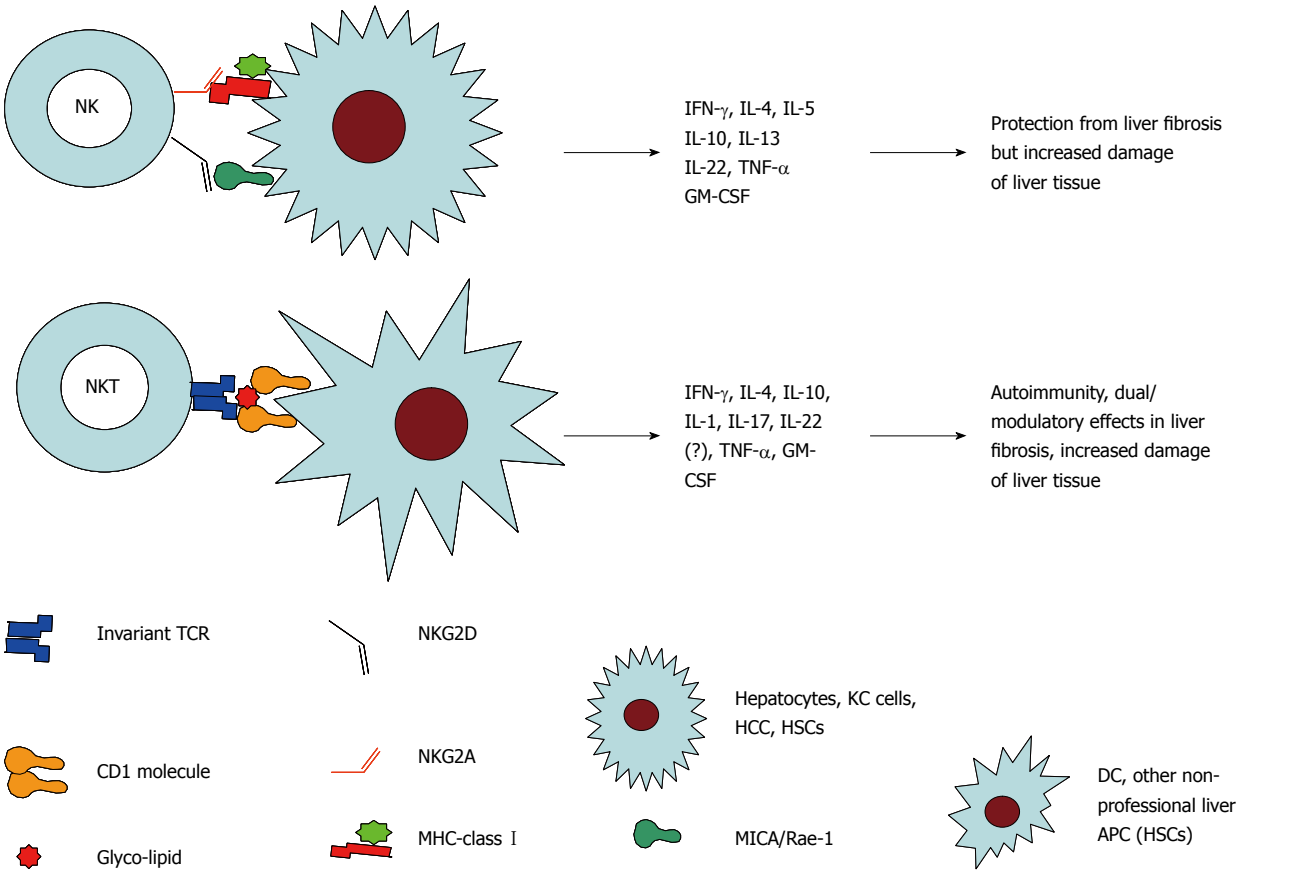

Figure 2 Simplified scheme of Natural Killer/Natural Killer T cell role in liver diseases. Natural Killer (NK) interacts with major and minor histocompatibility antigens expressed on several liver cells and kill and/or produce cytokines having several effects on the tissue. More complex is the role of NKT cells. These cells specifically recognize an antigen expressed in the context of a CD1 molecule and, upon recognition through an invariant TCR, secrete a large amount of cytokines having pleiotropic, sometimes controversial effects, whose overall results are due to the cytokine milieu and to the conditioning of the functions of other immune cells. This scenario is further complicated by the fact that many soluble factors (for instance cytokines) and hedgehog ligands may activate NK or NKT. IL: Interleukin; TNF-α: Tumor necrosis factor α; HCC: Hepatocellular carcinoma; HSCs: Hepatic stellate cells; GM-CSF: Granulocyte-macrophage colony stimulating factor; APC: Antigen presenting cell.

with the observation that microsomal triglyceride transfer protein, plays a key role in the acquisition of glycolipid antigens by CD1d[72]. In partial support of this concept, deficiency of microsomal triglyceride transfer protein in mice is associated with hepatic steatosis, and functional polymorphisms of its encoding gene have shown significant associations with NASH in humans[73]. One approach to dissecting out the mechanisms of NKT cell activation and loss in obese C57Bl/6 mice would be to utilize NKT cell adoptive transfer and tracking methodologies in recombinant NKT cell-deficient mice in combination with NKT cell activation and appropriate cytokine blocking.

In a recent paper, Hua *et al*[74] examined the mechanism of dietary fatty acid induced hepatic NKT cell deficiency and its causal relationship to insulin resistance and NAFLD, and found that dietary saturated fatty acids (SFA) or monounsaturated fatty acids (MUFA), but not polyunsaturated fatty acids (PUFA), caused hepatic NKT cell depletion with increased apoptosis. Dietary SFA or MUFA also impair hepatocyte presentation of endogenous, but not exogenous, antigen to NKT cells, indicating alterations of the endogenous antigen processing or presenting pathway. *In vitro* treatment of normal hepatocytes with fatty acids also demonstrates impaired ability of CD1d to present endogenous antigen by dietary fatty acids. Furthermore, dietary SFA and MUFA activate the NFκB signaling pathway and lead to insulin resistance and hepatic steatosis.

Recently, a new subset of T helper cells, named Th17 due to the ability to produce IL-17 and other cytokines, has been correlated to processes underlying hepatic steatosis. In particular, Th17 largely express a NKT marker, CD161, and they have been described to be closely involved in the immune responses in several anatomical sites including skin, liver and gut[75,76]. Th17 produce cytokines besides IL-17 such as IL-22 which is indicated to play a pivotal role in hepatic steatosis as recently shown[77].

Figure 2 shows a simplified Scheme of NK/NKT cell role in liver diseases.

KUPFFER CELLS AND STEATOSIS

Hepatocellular accumulation of lipids is a key morphologic feature of NAFLD. Lipidomic analysis of human liver tissue is a promising novel approach to associate abnormal fat composition with various stages of NAFLD. Thus, total and damaged phospholipids are more abundant in simple steatosis at the expense of triglycerides[78], while the increased ratio of stearic to arachidonic acid in NASH may

correlate with fibrosis[79]. Altered abundance and composition of liver tissue lipids may modulate the biological activity of KC in NAFLD through a number of mechanisms. First, the space-occupying effect of fat-laden hepatocytes may lead to impaired sinusoidal perfusion[80]. Leukocytes trapped in narrowed sinusoids may increasingly engage KC in the microvascular inflammatory response[80]. Second, excessive exposure of KC to fatty acids may modulate pathways of inflammation and insulin resistance through interaction with cell surface receptors and intracellular mediators[81]. Third, anomalous deposition of lipids in the plasma membrane may alter the structure of lipid raft domains and interfere with clustering and function of cell surface receptors[82]. Altered lipid composition may also affect proper functioning of intracellular membranes as seen with free cholesterol loading of mitochondria[83]. Finally, abundant or abnormal lipids may confound recognition of fatty hepatocytes as dangerous and promote adverse interactions with KC[17]. Nevertheless, the existence of a lipid-derived quintessential alarm expressed or released by steatotic hepatocytes remains speculative.

Recent findings indicate that TLR-mediated recognition of fatty acid moieties is an important mechanism by which lipids regulate pathways of inflammation and innate immunity[82]. Depending on fatty acid composition, the outcome of this effect may be highly variable. Saturated fatty acids, implicated in the development of chronic conditions such as atherosclerosis, have been shown to activate TLR4 signalling in adipocytes and macrophages through both Myd88-dependent and TRIF-dependent pathways[84,85]. In contrast, polyunsaturated fatty acids inhibit these events in several cell types including macrophages[85]. Consequently, TLR4 is a sensor of endogenous fatty acid levels and composition, and KC most likely benefit from this ability.

Emerging evidence indicates that altered cholesterol metabolism may directly affect the function of KC. Thus, high-fat diet fed to LDL receptor deficient mice rapidly results in significant hepatic inflammation, but only if the diet contains cholesterol[86]. The presence of "foamy" KC suggests that scavenging of modified lipoproteins may induce this early inflammatory response[86]. While these findings need to be extrapolated to human NAFLD with caution, they point to the importance of altered cholesterol metabolism. In addition, some of these observations challenge the "second-hit" concept since steatosis is not necessarily a forerunner of hepatic inflammation as these events may develop simultaneously[86,87].

There is evidence that steatosis promotes Th1 polarization of the cytokine balance favouring innate or classic activation of macrophages in NAFLD[88]. PPAR-α, PPAR-γ, and PPAR-σ and liver X receptors LXR-α and LXR-β are members of the nuclear hormone receptor superfamily of transcription factors that coordinate complex genetic programs of metabolism[89,90]. Therapeutic use of synthetic ligands to target these receptors and exploit their biological functions is increasing. The beneficial effects of PPAR-γ in hepatocellular lipid homeostasis have prompted large clinical trials to assess impact on NAFLD and these efforts have been recently reviewed elsewhere[91]. However, the recognition that nuclear hormone receptors link lipid metabolism to alternative activation of macrophages adds a new dimension to their potential use in the treatment of NAFLD[88,92]. While PPAR-γ promotes alternative activation of macrophages that contribute to valuable metabolic changes such as improved insulin sensitivity[93,94], recent research indicates that PPAR-σ is specifically required for a similar program in KC[95,96]. Thus, signature gene expression of PPARσ-deficient KC is greatly reduced in the livers of obese mice and in response to IL-4 stimulation[95,96]. Moreover, PPARσ ablation results in severe steatosis and insulin resistance[95,96]. Notably, the effect of PPARσ in KC is modulated by fatty acids[95] and may fail due to altered lipid homeostasis and hepatic microenvironment in NAFLD. Thus, hepatocytes as a previously unsuspected source of Th2 cytokines stimulate M2 gene expression in KC and this important regulatory circuit may be altered in steatosis[96]. These findings raise the intriguing possibility that specific targeting of PPAR-σ in KC to induce alternative activation may improve both inflammation and steatosis in NAFLD. One important caveat is that the M2 phenotype includes stimulation of the extracellular matrix that may contribute to hepatic fibrosis[97]. Figure 3 shows the effects of activation of Kupffer cells in NAFLD.

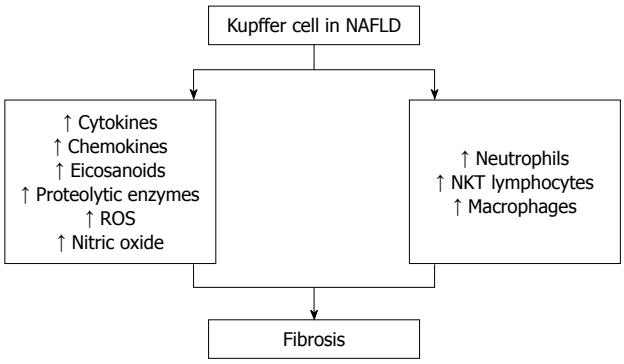

Figure 3 Effects of the activation of Kupffer cells in non-alcoholic fatty liver disease. NAFLD: Non-alcoholic fatty liver disease; NKT: Natural Killer T cells; ROS: Reactive oxygen species.

In the last few years, there is increasing evidence that ligands of Hedgehog (Hh) may have a critical role in processes leading to liver fibrosis. The Hh mediated activity is quite low in healthy liver but increases during the course of several liver diseases, as recently reviewed[98]. In particular, it has recently been shown that damaged/dying hepatocytes may produce Hh ligands that mediate proliferation of myofibroblasts in the liver, thus promoting fibrosis[99]. Moreover, Hh seems to be critical due to its properties in regulating NKT growth and functions in liver fibrosis[100,101].

IMMATURE MYELOID CELLS AND STEATOSIS

Immature myeloid cells ($CD11b^{+}Gr\text{-}1^{+}$) play a role in the

induction of inflammatory cytokines[102] through activation of innate immune pathways. The role that immature myeloid cell populations play in obesity-related liver disease is unknown. In a recent study, Deng *et al*[103] hypothesize that accumulation of immature myeloid cells in the liver may be an important component in the development of inflammatory responses in liver tissue that are triggered by obesity, which in turn contributes to metabolic consequences, such as steatohepatitis. In this study, the liver of obese mice was demonstrated as the major organ where $CD11b^{+}Ly6C^{+}$-$Ly6G^{-}$ immature myeloid cells accumulate. It is not clear why these cells are preferentially recruited into the liver. Chemotactic cytokines and chemokines could direct the migration of immune cells including myeloid cells and may be responsible for the cell accumulation. Several hepatic cell populations, including hepatocytes, KC, sinusoidal endothelial cells, and hepatic stellate cells, can secrete chemokines upon activation. High-fat diet-derived products could activate one of these cells in the liver, resulting in the recruitment of these circulating activated immature myeloid cells into the liver. IL-6 is overexpressed in the NAFLD patient[104], and IL-6 has been shown to block immature myeloid cell differentiation[105]. As a result, these activated cells are accumulated in the liver. The specific role of chemokines or other factors in the recruitment of these cells to the liver warrants further investigation.

CONCLUSION

It is now recognized that adipose tissue is an active endocrine organ that secretes numerous molecules that play a central role in the regulation of energy and vascular as well as immune system homeostasis by acting both locally and at distant sites influencing various metabolic and immune processes. Many of these interactions between metabolic and immune systems seem to be orchestrated by this complex network of soluble mediators derived from immune cells and adipocytes that are briefly summarized in Figure 4.

NAFLD is becoming an increasingly relevant clinical issue, especially in the developed world. One of the unmet challenges of NAFLD is to satisfactorily predict its progression from simple steatosis into steatohepatitis. This transition represents a milestone in the natural history with a considerable probability for developing end-stage liver disease. Elucidation of molecular and cellular events that may lead to this outcome is therefore critically important. Fortunately, the past few years have brought remarkable advances in our understanding of NAFLD pathogenesis, often by extension of research in adipose tissue biology, obesity, and insulin resistance. These efforts point to the intricate relationship of the innate immune system and lipid homeostasis in NAFLD with a prominent role for Kupffer, myeloid and NKT cells and a number of biochemical and cellular mechanisms involved.

However, a number of questions regarding the role of macrophage infiltration in human obesity remain to be answered. For example, what is the cause/s of macrophage infiltration? Does moderate fat gain alter macrophage number and/or macrophage phenotype in humans? Are some individuals predisposed to this? Is macrophage infiltration causal in the development of insulin resistance? The activation of NKT cells exacerbates macrophage infiltration in adipose tissue and glucose intolerance with obesity. Therefore, NKT cells enhance chronic inflammation in visceral adipose tissue and contribute to the development of metabolic disorders in obesity. The NKT cells may be the novel therapeutic targets in atherosclerosis, metabolic syndrome, and type 2 diabetes.

Further studies are needed to fully understand the interaction between fat, the immune system and steatosis.

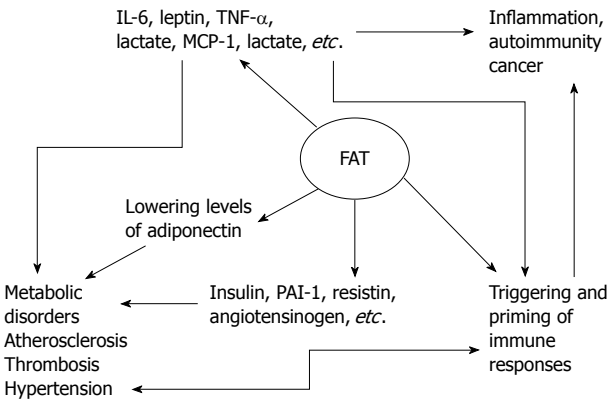

Figure 4 Complex network of soluble mediators derived from immune cells and adipocytes. MCP: Monocyte chemotactic protein; TNF-α: Tumor necrosis factor α; IL: Interleukin.

REFERENCES

1 **Wellen KE**, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; **115**: 1111-1119
2 **Calle EE**, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004; **4**: 579-591
3 **Mannino DM**, Mott J, Ferdinands JM, Camargo CA, Friedman M, Greves HM, Redd SC. Boys with high body masses have an increased risk of developing asthma: findings from the National Longitudinal Survey of Youth (NLSY). *Int J Obes* (Lond) 2006; **30**: 6-13
4 **Ogden CL**, Carroll MD, McDowell MA, Flegal KM. Obesity among adults in the United States--no statistically significant chance since 2003-2004. *NCHS Data Brief* 2007; 1-8
5 **Ogden CL**, Carroll MD, Flegal KM. High body mass index for age among US children and adolescents, 2003-2006. *JAMA* 2008; **299**: 2401-2405
6 **Berghöfer A**, Pischon T, Reinhold T, Apovian CM, Sharma AM, Willich SN. Obesity prevalence from a European perspective: a systematic review. *BMC Public Health* 2008; **8**: 200
7 **Klein S**, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C, Kahn R. Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Diabetes Care* 2007; **30**: 1647-1652
8 **Berg AH**, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 2005; **96**: 939-949
9 **Tilg H**, Moschen AR. Adipocytokines: mediators linking

adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006; **6**: 772-783
10 **Gil A**, María Aguilera C, Gil-Campos M, Cañete R. Altered signalling and gene expression associated with the immune system and the inflammatory response in obesity. *Br J Nutr* 2007; **98** Suppl 1: S121-S126
11 **Bastard JP**, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2006; **17**: 4-12
12 **Mendall MA**, Patel P, Asante M, Ballam L, Morris J, Strachan DP, Camm AJ, Northfield TC. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart* 1997; **78**: 273-277
13 **Tataranni PA**, Ortega E. A burning question: does an adipokine-induced activation of the immune system mediate the effect of overnutrition on type 2 diabetes? *Diabetes* 2005; **54**: 917-927
14 **Nieman DC**, Henson DA, Nehlsen-Cannarella SL, Ekkens M, Utter AC, Butterworth DE, Fagoaga OR. Influence of obesity on immune function. *J Am Diet Assoc* 1999; **99**: 294-299
15 **Ghanim H**, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P. Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation* 2004; **110**: 1564-1571
16 **Björkbacka H**, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, Means T, Halmen K, Luster AD, Golenbock DT, Freeman MW. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. *Nat Med* 2004; **10**: 416-421
17 **Maher JJ**, Leon P, Ryan JC. Beyond insulin resistance: Innate immunity in nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 670-678
18 **Szabo G**, Mandrekar P, Dolganiuc A. Innate immune response and hepatic inflammation. *Semin Liver Dis* 2007; **27**: 339-350
19 **Sahai A**, Malladi P, Pan X, Paul R, Melin-Aldana H, Green RM, Whitington PF. Obese and diabetic db/db mice develop marked liver fibrosis in a model of nonalcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G1035-G1043
20 **Kojima H**, Sakurai S, Uemura M, Takekawa T, Morimoto H, Tamagawa Y, Fukui H. Difference and similarity between non-alcoholic steatohepatitis and alcoholic liver disease. *Alcohol Clin Exp Res* 2005; **29**: 259S-263S
21 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112
22 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-S10
23 **Day CP**. From fat to inflammation. *Gastroenterology* 2006; **130**: 207-210
24 **Lieber CS**. Alcoholic liver disease: new insights in pathogenesis lead to new treatments. *J Hepatol* 2000; **32**: 113-128
25 **Cheung O**, Sanyal AJ. Hepatitis C infection and nonalcoholic fatty liver disease. *Clin Liver Dis* 2008; **12**: 573-585, viii-ix
26 **Molina PE**, McClain C, Valla D, Guidot D, Diehl AM, Lang CH, Neuman M. Molecular pathology and clinical aspects of alcohol-induced tissue injury. *Alcohol Clin Exp Res* 2002; **26**: 120-128
27 **Lumeng L**, Crabb DW. Genetic aspects and risk factors in alcoholism and alcoholic liver disease. *Gastroenterology* 1994; **107**: 572-578
28 **Harrison SA**, Diehl AM. Fat and the liver--a molecular overview. *Semin Gastrointest Dis* 2002; **13**: 3-16
29 **Yang S**, Lin H, Diehl AM. Fatty liver vulnerability to endotoxin-induced damage despite NF-kappaB induction and inhibited caspase 3 activation. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G382-G392
30 **Day CP**. Natural history of NAFLD: remarkably benign in the absence of cirrhosis. *Gastroenterology* 2005; **129**: 375-378
31 **Ioannou GN**. Implications of elevated serum alanine aminotransferase levels: think outside the liver. *Gastroenterology* 2008; **135**: 1851-1854
32 **Marrero JA**, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 2002; **36**: 1349-1354
33 **Alberti KG**, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet* 2005; **366**: 1059-1062
34 **Klein S**, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C, Kahn R. Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Diabetes Care* 2007; **30**: 1647-1652
35 **Zimmet P**, Magliano D, Matsuzawa Y, Alberti G, Shaw J. The metabolic syndrome: a global public health problem and a new definition. *J Atheroscler Thromb* 2005; **12**: 295-300
36 **Kadowaki T**, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006; **116**: 1784-1792
37 **Yokota T**, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000; **96**: 1723-1732
38 **Wulster-Radcliffe MC**, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun* 2004; **316**: 924-929
39 **Park PH**, Huang H, McMullen MR, Mandal P, Sun L, Nagy LE. Suppression of lipopolysaccharide-stimulated tumor necrosis factor-alpha production by adiponectin is mediated by transcriptional and post-transcriptional mechanisms. *J Biol Chem* 2008; **283**: 26850-26858
40 **Thakur V**, Pritchard MT, McMullen MR, Nagy LE. Adiponectin normalizes LPS-stimulated TNF-alpha production by rat Kupffer cells after chronic ethanol feeding. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G998-G1007
41 **Huang H**, Park PH, McMullen MR, Nagy LE. Mechanisms for the anti-inflammatory effects of adiponectin in macrophages. *J Gastroenterol Hepatol* 2008; **23** Suppl 1: S50-S53
42 **Kuga S**, Otsuka T, Niiro H, Nunoi H, Nemoto Y, Nakano T, Ogo T, Umei T, Niho Y. Suppression of superoxide anion production by interleukin-10 is accompanied by a downregulation of the genes for subunit proteins of NADPH oxidase. *Exp Hematol* 1996; **24**: 151-157
43 **Kolak M**, Westerbacka J, Velagapudi VR, Wågsäter D, Yetukuri L, Makkonen J, Rissanen A, Häkkinen AM, Lindell M, Bergholm R, Hamsten A, Eriksson P, Fisher RM, Oresic M, Yki-Järvinen H. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes* 2007; **56**: 1960-1968
44 **La Cava A**, Matarese G. The weight of leptin in immunity. *Nat Rev Immunol* 2004; **4**: 371-379
45 **Angulo P**, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease. *J Hepatol* 2004; **41**: 943-949
46 **Verma S**, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, Mickle DA. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation* 2003; **108**: 736-740
47 **Haydon G**, Lalor PF, Hubscher SG, Adams DH. Lymphocyte recruitment to the liver in alcoholic liver disease. *Alcohol* 2002; **27**: 29-36
48 **Burra P**, Hubscher SG, Shaw J, Elias E, Adams DH. Is the intercellular adhesion molecule-1/leukocyte function associ-

ated antigen 1 pathway of leukocyte adhesion involved in the tissue damage of alcoholic hepatitis? *Gut* 1992; **33**: 268-271
49 **Kurkijärvi R**, Yegutkin GG, Gunson BK, Jalkanen S, Salmi M, Adams DH. Circulating soluble vascular adhesion protein 1 accounts for the increased serum monoamine oxidase activity in chronic liver disease. *Gastroenterology* 2000; **119**: 1096-103
50 **Adams DH**, Burra P, Hubscher SG, Elias E, Newman W. Endothelial activation and circulating vascular adhesion molecules in alcoholic liver disease. *Hepatology* 1994; **19**: 588-594
51 **Kurkijärvi R**, Adams DH, Leino R, Möttönen T, Jalkanen S, Salmi M. Circulating form of human vascular adhesion protein-1 (VAP-1): increased serum levels in inflammatory liver diseases. *J Immunol* 1998; **161**: 1549-1557
52 **Samal B**, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol* 1994; **14**: 1431-1437
53 **Fukuhara A**, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 2005; **307**: 426-430
54 **Revollo JR**, Körner A, Mills KF, Satoh A, Wang T, Garten A, Dasgupta B, Sasaki Y, Wolberger C, Townsend RR, Milbrandt J, Kiess W, Imai S. Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. *Cell Metab* 2007; **6**: 363-375
55 **Moschen AR**, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, Tilg H. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. *J Immunol* 2007; **178**: 1748-1758
56 **Pelleymounter MA**, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; **269**: 540-543
57 **Yang SQ**, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; **94**: 2557-2562
58 **Guebre-Xabier M**, Yang S, Lin HZ, Schwenk R, Krzych U, Diehl AM. Altered hepatic lymphocyte subpopulations in obesity-related murine fatty livers: potential mechanism for sensitization to liver damage. *Hepatology* 2000; **31**: 633-640
59 **Godfrey DI**, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? *Nat Rev Immunol* 2004; **4**: 231-237
60 **Godfrey DI**, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. *J Clin Invest* 2004; **114**: 1379-1388
61 **Matarese G**, Moschos S, Mantzoros CS. Leptin in immunology. *J Immunol* 2005; **174**: 3137-3142
62 **Li Z**, Soloski MJ, Diehl AM. Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 880-885
63 **Black BL**, Croom J, Eisen EJ, Petro AE, Edwards CL, Surwit RS. Differential effects of fat and sucrose on body composition in A/J and C57BL/6 mice. *Metabolism* 1998; **47**: 1354-1359
64 **Klugewitz K**, Adams DH, Emoto M, Eulenburg K, Hamann A. The composition of intrahepatic lymphocytes: shaped by selective recruitment? *Trends Immunol* 2004; **25**: 590-594
65 **Johnston B**, Kim CH, Soler D, Emoto M, Butcher EC. Differential chemokine responses and homing patterns of murine TCR alpha beta NKT cell subsets. *J Immunol* 2003; **171**: 2960-2969
66 **Seino K**, Harada M, Taniguchi M. NKT cells are relatively resistant to apoptosis. *Trends Immunol* 2004; **25**: 219-221
67 **Li Z**, Lin H, Yang S, Diehl AM. Murine leptin deficiency alters Kupffer cell production of cytokines that regulate the innate immune system. *Gastroenterology* 2002; **123**: 1304-1310
68 **Schmieg J**, Yang G, Franck RW, Van Rooijen N, Tsuji M. Glycolipid presentation to natural killer T cells differs in an organ-dependent fashion. *Proc Natl Acad Sci USA* 2005; **102**: 1127-1132
69 **Kremer M**, Thomas E, Milton RJ, Perry AW, van Rooijen N, Wheeler MD, Zacks S, Fried M, Rippe RA, Hines IN. Kupffer cell and interleukin-12-dependent loss of natural killer T cells in hepatosteatosis. *Hepatology* 2010; **51**: 130-141
70 **Trembleau S**, Germann T, Gately MK, Adorini L. The role of IL-12 in the induction of organ-specific autoimmune diseases. *Immunol Today* 1995; **16**: 383-386
71 **Adams LA**, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2004; **99**: 1316-1320
72 **Brozovic S**, Nagaishi T, Yoshida M, Betz S, Salas A, Chen D, Kaser A, Glickman J, Kuo T, Little A, Morrison J, Corazza N, Kim JY, Colgan SP, Young SG, Exley M, Blumberg RS. CD1d function is regulated by microsomal triglyceride transfer protein. *Nat Med* 2004; **10**: 535-539
73 **Namikawa C**, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, Akisawa N, Saibara T, Hiroi M, Enzan H, Onishi S. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in nonalcoholic steatohepatitis. *J Hepatol* 2004; **40**: 781-786
74 **Hua J**, Ma X, Webb T, Potter JJ, Oelke M, Li Z. Dietary fatty acids modulate antigen presentation to hepatic NKT cells in nonalcoholic fatty liver disease. *J Lipid Res* 2010; **51**: 1696-1703
75 **Billerbeck E**, Kang YH, Walker L, Lockstone H, Grafmueller S, Fleming V, Flint J, Willberg CB, Bengsch B, Seigel B, Ramamurthy N, Zitzmann N, Barnes EJ, Thevanayagam J, Bhagwanani A, Leslie A, Oo YH, Kollnberger S, Bowness P, Drognitz O, Adams DH, Blum HE, Thimme R, Klenerman P. Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. *Proc Natl Acad Sci USA* 2010; **107**: 3006-3011
76 **Cosmi L**, De Palma R, Santarlasci V, Maggi L, Capone M, Frosali F, Rodolico G, Querci V, Abbate G, Angeli R, Berrino L, Fambrini M, Caproni M, Tonelli F, Lazzeri E, Parronchi P, Liotta F, Maggi E, Romagnani S, Annunziato F. Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. *J Exp Med* 2008; **205**: 1903-1916
77 **Yang L**, Zhang Y, Wang L, Fan F, Zhu L, Li Z, Ruan X, Huang H, Wang Z, Huang Z, Huang Y, Yan X, Chen Y. Amelioration of high fat diet induced liver lipogenesis and hepatic steatosis by interleukin-22. *J Hepatol* 2010; **53**: 339-347
78 **Elizondo A**, Araya J, Rodrigo R, Poniachik J, Csendes A, Maluenda F, Díaz JC, Signorini C, Sgherri C, Comporti M, Videla LA. Polyunsaturated fatty acid pattern in liver and erythrocyte phospholipids from obese patients. *Obesity* (Silver Spring) 2007; **15**: 24-31
79 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
80 **Farrell GC**, Teoh NC, McCuskey RS. Hepatic microcirculation in fatty liver disease. *Anat Rec* (Hoboken) 2008; **291**: 684-692
81 **Kim JK**. Fat uses a TOLL-road to connect inflammation and diabetes. *Cell Metab* 2006; **4**: 417-419
82 **Lee JY**, Hwang DH. The modulation of inflammatory gene expression by lipids: mediation through Toll-like receptors. *Mol Cells* 2006; **21**: 174-185
83 **Marí M**, Caballero F, Colell A, Morales A, Caballeria J, Fernandez A, Enrich C, Fernandez-Checa JC, García-Ruiz C. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metab* 2006; **4**: 185-198
84 **Shi H**, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 2006; **116**: 3015-3025
85 **Lee JY**, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, Sizemore N,

Hwang DH. Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. *J Biol Chem* 2003; **278**: 37041-37051
86 **Wouters K**, van Gorp PJ, Bieghs V, Gijbels MJ, Duimel H, Lütjohann D, Kerksiek A, van Kruchten R, Maeda N, Staels B, van Bilsen M, Shiri-Sverdlov R, Hofker MH. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 474-486
87 **Shiri-Sverdlov R**, Wouters K, van Gorp PJ, Gijbels MJ, Noel B, Buffat L, Staels B, Maeda N, van Bilsen M, Hofker MH. Early diet-induced non-alcoholic steatohepatitis in APOE2 knock-in mice and its prevention by fibrates. *J Hepatol* 2006; **44**: 732-741
88 **Gordon S**. Alternative activation of macrophages. *Nat Rev Immunol* 2003; **3**: 23-35
89 **Mangelsdorf DJ**, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. *Cell* 1995; **83**: 835-839
90 **Sugden MC**, Holness MJ. Role of nuclear receptors in the modulation of insulin secretion in lipid-induced insulin resistance. *Biochem Soc Trans* 2008; **36**: 891-900
91 **Younossi ZM**. Review article: current management of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2008; **28**: 2-12
92 **Rigamonti E**, Chinetti-Gbaguidi G, Staels B. Regulation of macrophage functions by PPAR-alpha, PPAR-gamma, and LXRs in mice and men. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1050-1059
93 **Vats D**, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ, Chawla A. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* 2006; **4**: 13-24
94 **Odegaard JI**, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vats D, Brombacher F, Ferrante AW, Chawla A. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* 2007; **447**: 1116-1120
95 **Odegaard JI**, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, Goforth MH, Subramanian V, Mukundan L, Ferrante AW, Chawla A. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. *Cell Metab* 2008; **7**: 496-507
96 **Kang K**, Reilly SM, Karabacak V, Gangl MR, Fitzgerald K, Hatano B, Lee CH. Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. *Cell Metab* 2008; **7**: 485-495
97 **Omenetti A**, Diehl AM. The adventures of sonic hedgehog in development and repair. II. Sonic hedgehog and liver development, inflammation, and cancer. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G595-G598
98 **Jung Y**, Witek RP, Syn WK, Choi SS, Omenetti A, Premont R, Guy CD, Diehl AM. Signals from dying hepatocytes trigger growth of liver progenitors. *Gut* 2010; **59**: 655-665
99 **Syn WK**, Witek RP, Curbishley SM, Jung Y, Choi SS, Enrich B, Omenetti A, Agboola KM, Fearing CM, Tilg H, Adams DH, Diehl AM. Role for hedgehog pathway in regulating growth and function of invariant NKT cells. *Eur J Immunol* 2009; **39**: 1879-1892
100 **Syn WK**, Oo YH, Pereira TA, Karaca GF, Jung Y, Omenetti A, Witek RP, Choi SS, Guy CD, Fearing CM, Teaberry V, Pereira FE, Adams DH, Diehl AM. Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1998-2007
101 **Reiman RM**, Thompson RW, Feng CG, Hari D, Knight R, Cheever AW, Rosenberg HF, Wynn TA. Interleukin-5 (IL-5) augments the progression of liver fibrosis by regulating IL-13 activity. *Infect Immun* 2006; **74**: 1471-1479
102 **Delano MJ**, Scumpia PO, Weinstein JS, Coco D, Nagaraj S, Kelly-Scumpia KM, O'Malley KA, Wynn JL, Antonenko S, Al-Quran SZ, Swan R, Chung CS, Atkinson MA, Ramphal R, Gabrilovich DI, Reeves WH, Ayala A, Phillips J, Laface D, Heyworth PG, Clare-Salzler M, Moldawer LL. MyD88-dependent expansion of an immature GR-1(+)CD11b(+) population induces T cell suppression and Th2 polarization in sepsis. *J Exp Med* 2007; **204**: 1463-1474
103 **Deng ZB**, Liu Y, Liu C, Xiang X, Wang J, Cheng Z, Shah SV, Zhang S, Zhang L, Zhuang X, Michalek S, Grizzle WE, Zhang HG. Immature myeloid cells induced by a high-fat diet contribute to liver inflammation. *Hepatology* 2009; **50**: 1412-1420
104 **van der Poorten D**, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, George J. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; **48**: 449-457
105 **Yu S**, Liu C, Su K, Wang J, Liu Y, Zhang L, Li C, Cong Y, Kimberly R, Grizzle WE, Falkson C, Zhang HG. Tumor exosomes inhibit differentiation of bone marrow dendritic cells. *J Immunol* 2007; **178**: 6867-6875

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