Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases

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ABSTRACT

Obesity and obesity-related disorders play an important role in clinical medicine. Adipose tissue, with its soluble mediators called adipocytokines, has emerged as a major endocrine organ. These adipocytokines comprise many mediators such as adiponectin, PBEF (pre-B-cell-enhancing factor)/visfatin, leptin, resistin, retinol-binding protein-4 and others. They play major roles in key aspects of metabolism, such as insulin resistance, fatty acid oxidation, inflammation and immunity. Adiponectin, a prototypic adipocytokine, is of importance in the regulation of insulin resistance, as circulating levels are decreased in obesity and diseases associated with insulin resistance. Besides its major role in regulation of insulin sensitivity, recent evidence suggests potent anti-inflammatory functions for adiponectin. These effects are paralleled by other immune-regulatory properties, such as regulation of endothelial cell function. The in vitro effects of adiponectin have been corroborated by several studies demonstrating potent in vivo anti-inflammatory effects. Many other adipocytokines, such as PBEF/visfatin, leptin, resistin or retinol binding protein-4, are involved in the physiology and pathophysiology of adipocytes, adipose tissue and related diseases. PBEF/visfatin, another recently characterized adipocytokine, has been linked to several inflammatory disease states beyond insulin resistance, such as acute lung injury or inflammatory bowel diseases. It has been recognized for many decades that obesity is accompanied by an increase in cancer and potentially some immune-mediated diseases. Understanding this new exciting world of adipocytokines will be of importance in the development of novel therapies for obesity-associated diseases.

INTRODUCTION

As a consequence of sustained overnutrition, obesity has become epidemic in many countries worldwide. The prevalence rates are continuing to increase, most rapidly in developing countries, and obesity is occurring in all age groups. Obesity predisposes individuals to an increased risk of developing several diseases, including atherosclerosis, diabetes, non-alcoholic fatty liver disease and certain cancers. Obesity, in particular

Key words: adiponectin, inflammation, insulin resistance, obesity, pre-B-cell-enhancing factor (PBEF), visfatin.

Abbreviations: AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; BMI, body mass index; CCL-2, CC chemokine ligand-2; CRP, C-reactive protein; DC, dendritic cell; DSS, dextran sulphate sodium; HMW, high-molecular-weight (‘mass’); IBD, inflammatory bowel disease; IFN-γ, interferon-γ; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; IR, insulin resistance; JNK, c-Jun N-terminal kinase; LMW, low-molecular-weight (‘mass’); LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MMW, middle-molecular-weight (‘mass’); NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor κB; NK, natural killer; PPAR, peroxisome-proliferator-activated receptor; PBEF, pre-B-cell-enhancing factor; RANTES, regulated upon activation, normal T-cell expressed and secreted; RPB-4, retinol-binding protein-4; T2DM, Type 2 diabetes mellitus; TLR, Toll-like receptor; TNF, tumour necrosis factor; Treg-cell, regulatory T-cell; WAT, white adipose tissue.

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visceral obesity, which is the accumulation of adipose tissue inside the abdominal cavity, is associated with resistance to the effects of insulin (IR, insulin resistance), often leading to the development of T2DM (Type 2 diabetes mellitus). Furthermore, although evidence so far is limited, obesity might be associated with some immune-mediated disorders, such as asthma. Research over the past number of years has identified important pathways that link metabolism with the immune system and vice versa. Many of these interactions between the metabolic and immune systems appear to be mediated by a complex network of soluble mediators derived from immune cells and adipocytes called adipocytokines.

Adipose tissue is found in mammals in two different forms: WAT (white adipose tissue) and BAT (brown adipose tissue). WAT is most familiar as the type of fat in which triacylglycerol (triglyceride) is stored and from which lipids are mobilized for systemic utilization when other tissues require energy. WAT is often divided into subcutaneous and abdominal depots, whose physiologies may be distinguished and whose roles in disease are distinct. In contrast, the main function of BAT is non-shivering thermogenesis, a process of heat production through the uncoupling of oxidative phosphorylation. In addition to adipocytes, which are the most abundant cell type in WAT, adipose tissue also contains pre-adipocytes, endothelial cells, fibroblasts and various leucocytes, including most importantly macrophages. Obese adipose tissue contains a considerable number of macrophages, making them an additional source of soluble mediators in the adipose tissue. These macrophages are bone-marrow derived and the number of these cells present in WAT directly correlates with obesity.

Adipose tissue is no longer considered to be an inert tissue functioning solely for energy storage. Various secreted products of adipocytes/adipose tissue, so called adipocytokines, have been recently characterized. These adipocytokines include adiponectin, PBEF (pre-B-cell-enhancing factor)/visfatin, leptin, resistin, TNF (tumour necrosis factor)-\(\alpha\), IL (interleukin)-6, CCL-2 (C-C chemokine ligand-2; also known as MCP-1), PAI-1 (plasminogen activator inhibitor-1), angiotensinogen, RBP-4 (retinol-binding protein-4), SAA (serum amyloid A) and others. Adiponectin and leptin are considered to be the primary adipocytokines because they appear to be produced mainly by adipocytes. Many of those...
adipocytokines in general are expressed in adipocytes as well as activated macrophages and/or other immune cells. The relative amounts of these adipocytokines produced either by adipocytes or macrophages in the adipose tissue is unclear. However, although macrophages in adipose tissue appear to be the main source of TNF-α, adipocytes contribute almost one-third of circulating IL-6 in patients who are obese [10]. CCL-2, also produced by adipocytes, has recently been identified as one potential factor contributing to macrophage infiltration into adipose tissue [11]. Therefore adipose tissue is a complex endocrine and immune organ that modulates, and is involved in, biological processes, such as insulin sensitivity, appetite, endocrine functions, inflammation, immunity and bone metabolism.

Obesity is associated with a chronic inflammatory response characterized by abnormal cytokine production, increased synthesis of acute-phase reactants, such as CRP (C-reactive protein), and activation of inflammatory signalling pathways [3]. Adipocytokines function as hormones to influence energy homeostasis and to regulate neuroendocrine function. As cytokines, they affect immune functions and inflammatory processes throughout the body. In this review, we provide an overview of recent advances in the understanding of adipose tissue with its secreted mediators and will focus on adiponectin, the main anti-inflammatory adipocytokine, and PBEF/visfatin, a mainly pro-inflammatory mediator.

**OBESITY-ASSOCIATED INFLAMMATION: INFECTION OF ADIPOSE TISSUE WITH VARIOUS TYPES OF LEUCOCYTES**

Obesity and related metabolic alterations are associated with a chronic inflammatory-response syndrome characterized by abnormal cytokine production, increased acute-phase reactant synthesis and activation of inflammation in adipose tissue (Figure 1). Macrophage recruitment to adipose tissue in obesity contributes to enhanced tissue inflammatory activity and, thus, may underlie obesity-associated metabolic dysfunction [12]. Two reports in 2003 [2,13] presented evidence for the first time that adipose tissue is infiltrated by macrophages. A large number of gene transcripts observed in ‘inflamed’ adipose tissue represent well-defined macrophage genes [2]. Thereby, macrophages may either differentiate from preadipocytes or may enter the adipose tissue attracted by certain chemokines. Macrophages can differentiate from bone-marrow-derived monocytes that reached the adipose tissue by diapedesis from the circulation. Secondly, macrophages might differentiate from preadipocytes and mesenchymal stem cells in the adipose tissue. Adipocytes secrete various chemoattractants that bring monocytes into this tissue. It has been demonstrated that obese adipose tissue exhibits increased CCL-2 expression, an important factor having the capability to recruit macrophages [11]. Inouye et al. [14] recently demonstrated that the absence of CCL-2 in mice does not limit obesity-associated infiltration of macrophages into adipose tissue. In this study, the authors used CCL-2-deficient mice, and adipose tissue was collected for analysis of macrophage infiltration after 34 weeks of a high-fat diet. Surprisingly, CCL-2-deficient mice on a high-fat diet had no decrease in adipose tissue macrophages, although they were glucose-intolerant and had mildly increased plasma glucose and decreased serum adiponectin levels compared with wild-type mice. These results suggest that CCL-2 is not critical for adipose tissue macrophage recruitment and, indeed, responsible factors for recruitment of macrophages into the adipose tissue have yet to be identified. There are, however, several other candidates that might play a role in the recruitment of monocytes/macrophages into the adipose tissue, such as MIF (migration inhibitory factor) or MIP-1α (macrophage inflammatory protein-1α) [15,16].

There is increasing evidence that besides macrophages other immune cells, such as T-cells, might infiltrate adipose tissue [17]. Wu and co-workers [17] recently presented evidence that, at least in mice, adipose tissue from diet-induced obese insulin-resistant mice is infiltrated by T-cells. This infiltration was accompanied by an increased expression of the T-cell chemoattractant RANTES (regulated upon activation, normal T-cell expressed and secreted) and, furthermore, adiponectin-deficient mice had higher RANTES expression compared with wild-type mice. Obese humans with the metabolic syndrome had higher RANTES expression levels of RANTES and CCR5 (CC chemokine receptor 5) in subcutaneous adipose tissue than lean humans. RANTES expression was again higher in human visceral fat and expression was correlated with CD3 and CD11b staining in human visceral adipose tissue. These findings suggest that T-cells accumulate in adipose tissue and activated T-cells might therefore contribute to overall immune dysfunction observed in obesity. It still has to be investigated whether other immune cells, such as NK (natural killer) cells or DCs (dendritic cells), are also infiltrating adipose tissue and could be part of the immune dysfunction observed in states of obesity.

**ADIPONECTIN: THE PROTOTYPIC ANTI-INFLAMMATORY ADIPOCYTOKINE**

Although being primarily a product of adipocytes, adiponectin is also produced to a small degree by cardiac myocytes, muscle cells and endothelial cells [18–20]. It shares sequence homology with a family of proteins that are characterized by an N-terminal collagen-like region and a C-terminal complement factor C1q-like globular domain [21–23]. Adiponectin exists both as a full-length...
Adiponectin exists both as a full-length protein, as well as a proteolytic cleavage fragment (globular adiponectin). Full-length adiponectin is a trimer (LMW adiponectin) that forms hexamers (MMW adiponectin), which can further oligomerize to form polymers (HMW form). Adiponectin interacts with at least two known cellular receptors (AdipoR1 and AdipoR2). Activation of AdipoR1 and/or AdipoR2 by adiponectin stimulates PPAR-α, AMPK and p38 MAPK activation. Adiponectin regulates several pro- and anti-inflammatory cytokines. Its main anti-inflammatory function might be related to its capacity to suppress TNF and IFN-γ synthesis, and to induce anti-inflammatory cytokines, such as IL-10 and IL-1Ra. The anti-inflammatory properties of adiponectin have been demonstrated in various in vitro and in vivo models. ApoE, apolipoprotein E; CFU, colony forming units; FasL, Fas ligand; ICAM-1, intercellular adhesion molecule-1; IκB, inhibitor of NF-κB; IKK-β, IκB kinase-β; PGC-1α, PPAR-γ co-activator; ROS, reactive oxygen species; VCAM-1, vascular cell adhesion molecule-1.
using a hypocaloric diet improved insulin sensitivity, which was accompanied by a non-significant increase in the amount not only of HMW adiponectin, but also of MMW and LMW adiponectin [30].

TNF-α suppresses the transcription of adiponectin in adipocytes, which might explain the lower adiponectin levels in serum in individuals who are obese [31]. Weight loss induces adiponectin synthesis [32], as does activation of PPAR (peroxisome-proliferator-activated receptor)-γ by its ligands thiazolidinediones, which are used in the treatment of T2DM [33,34]. Circulating adiponectin levels, however, are affected by several factors, including gender, age and lifestyle. In severely obese women, surgical weight loss has been associated with improved liver insulin sensitivity, which was accompanied by increased adiponectin levels and improvement in peripheral insulin sensitivity, paralleled by decreases in CRP levels [35]. A causal association of these parameters, however, has not been proven.

Adiponectin in inflammation and immunity

Initial studies suggested that adiponectin exerted its anti-inflammatory effects on endothelial cells through the inhibition of TNF-α-induced adhesion molecule expression [36]. Adiponectin-deficient mice had higher levels of expression of mRNA encoding TNF-α in adipose tissue and higher TNF-α concentrations in plasma compared with wild-type mice [31]. Adiponectin inhibited NF-κB (nuclear factor κB) activation in endothelial cells and interfered with the function of macrophages [36,37]; treatment of cultured macrophages with adiponectin markedly inhibited their phagocytic activity and their production of TNF-α in response to LPS (lipopolysaccharide) stimulation [37].

Adiponectin induces the synthesis of the anti-inflammatory cytokines IL-10 and IL-1Ra (IL-1 receptor antagonist) by human monocytes, macrophages and DCs and suppresses the production of IFN-γ (interferon-γ) by LPS-stimulated human macrophages [38]. Through AdipoR1, globular adiponectin suppresses TLR (Toll-like receptor)-induced NF-κB activation [39], indicating that adiponectin negatively regulates macrophage responses to TLR ligands. LMW and HMW adiponectin share some biological effects on monocytes, such as the induction of apoptosis, the activation of AMPK (AMP-activated protein kinase) and the decrease in macrophage expression of scavenger receptors [40]. The exact roles of the different full-length and globular forms of adiponectin in inflammation and immunity need to be defined in the near future.

Adiponectin has been demonstrated to be a negative regulator of NK cell function [41], supporting further a role for this adipocytokine in immunity. It suppressed IL-2-enhanced cytotoxic activity of NK cells without affecting basal NK cell cytotoxicity through the AMPK-mediated inhibition of NF-κB activation. The production of IFN-γ was also suppressed by adiponectin, accompanied by the subsequent down-regulation of IFN-γ-inducible TRAIL (TNF-related apoptosis-inducing ligand) and Fas ligand expression. Adiponectin also acts as a haemopoietic stem cell factor [42]. Adiponectin stimulates the proliferation of haemopoietic stem cells, while retaining the cells in a functionally immature state as determined by in vitro and in vivo assays [42].

Adiponectin also modulated inflammatory reactions via calreticulin-receptor-dependent clearance of early apoptotic bodies [43]. In this study, adiponectin promoted the clearance of apoptotic cells by macrophages in both adiponectin-deficient and wild-type mice. This effect was mediated by the binding of adiponectin to calreticulin on the macrophage cell surface. This suggests another attractive pathway of how adiponectin might suppress inflammatory reactions.

Anti-inflammatory activities of adiponectin in various in vivo models

Several studies have assessed the role of fat, inflammation and adiponectin in experimental liver disease. Adiponectin exerts anti-inflammatory effects in various animal models of liver inflammation. Administration of adiponectin has beneficial effects in both alcoholic and non-alcoholic fatty liver disease in mice by suppressing the expression of TNF in the liver. It also decreases hepatomegaly, steatosis and the levels of liver enzymes [44]. In addition, adiponectin attenuates liver fibrosis in the CCl₄ (carbon tetrachloride) liver fibrosis mouse model [45] and protects from endotoxin-induced liver injury in another model of fatty liver, the KK-A° obese mouse model [46]. Adiponectin-deficient mice developed a much more severe colitis compared with wild-type mice [47]. Adenovirus-mediated supplementation of adiponectin has been shown to be protective against this DSS (dextran sulphate sodium)-induced murine colitis due to the inhibition of chemokine production in intestinal epithelial cells and the following inflammatory responses, including infiltration of macrophages and release of pro-inflammatory cytokines [47]. Interpretation of this study has been challenged by another recently published study [48]. Fayad et al. [48] demonstrated that adiponectin-deficient mice were protected from DSS colitis and administration of adiponectin restored inflammation. In their experiments, adiponectin induced pro-inflammatory cytokines in colonic tissue and inhibited bioactivity of protective growth factors, such as HP-EGF (heparin-binding epidermal growth factor) and bFGF (basic fibroblast growth factor). Fayad et al. [48] therefore suggested that, in experimental colitis, adiponectin might exert an opposite, namely pro-inflammatory, role.
compared with atherosclerosis. Altogether, the role of adiponectin in this type of inflammation is unclear.

There is, however, further evidence that adiponectin might act in a pro-inflammatory manner outside of endocrine and vascular diseases. Adiponectin could be involved in critical pathways of inflammation and matrix degradation in the human joint by inducing IL-6 and MMP-1 (matrix metalloproteinase-1) via the p38 MAPK (mitogen-activated protein kinase) pathway [49]. Adiponectin therefore might also be a target for therapeutic strategies in inflammatory joint diseases.

**Adiponectin and IR**

In obese animals, treatment with adiponectin decreases hyperglycaemia and levels of non-esterified free fatty acids in the plasma, and improves insulin sensitivity [10,50]. Furthermore, adiponectin-deficient mice develop diet-induced IR on a high-fat/high-sucrose diet [31]. In other studies with adiponectin-deficient mice, however, these animals developed IR only if fed a high-fat diet [51] or failed to develop IR even when fed a high-fat diet [52]. Specific PPAR-γ agonists, such as thiazolidinediones, improve insulin sensitivity by unknown mechanisms. Circulating adiponectin levels are significantly up-regulated in vivo upon treatment with PPAR-γ ligands [33,34]. Adiponectin-deficient mice not only have decreased hepatic insulin sensitivity, but also reduced responsiveness to PPAR-γ agonists, suggesting that adiponectin is an important contributor to PPAR-γ-mediated improvements in glucose tolerance [53]. These studies together strongly support a major role for adiponectin in regulating insulin sensitivity.

Adiponectin appears to be of importance in the pathophysiology of atherosclerosis. Adiponectin-deficient mice had a 2-fold higher neointimal (i.e. inner vessel surface) formation in response to an external vascular injury than wild-type mice [51]. Furthermore, adiponectin protected ApoE (apolipoprotein E)-deficient mice (mice lacking a key component in cholesterol metabolism) from atherosclerosis [54]. This experimental evidence is paralleled by several clinical reports which support the observation that this abundant circulating protein affects the development of atherosclerosis, and hypoadiponectinaemia might directly play a role in its development [55–57]. Weight loss and treatment of T2DM with PPAR-γ ligands potently induce adiponectin synthesis [32,33].

**Adiponectin receptors and their regulation**

Two receptors for adiponectin have been identified (AdipoR1 and AdipoR2). AdipoR1 is widely expressed in mice, whereas AdipoR2 is mainly expressed in the liver [58]. Although globular adiponectin appears to activate mainly AdipoR1, AdipoR2 engages with the full-length variant of adiponectin [58]. In addition, T-cadherin, which is expressed by many cells, including endothelial cells and smooth muscle cells, appears to function as a receptor for MMW and HMW adiponectin, but not for the trimeric and globular adiponectin [59]. The overall role of this type of receptor needs to be clarified. Skeletal muscle AdipoR1 expression is down-regulated, whereas adipose tissue expression of this receptor is up-regulated during rosiglitazone therapy [60]. In contrast with AdipoR1 expression, AdipoR2 mRNA levels did not change during rosiglitazone therapy in either of the tissues. These findings suggest that AdipoR1 plays a role in mediating the effects of adiponectin in specific tissues in relation to insulin sensitization.

Expression of adiponectin and its receptors was studied in db/db mice after treatment with a β3-adrenoreceptor agonist [61]. Oral administration of this agent for 2 weeks improved parameters of IR and led to an increase in circulating adiponectin levels. This was paralleled by higher mRNA expression in epididymal WAT, soleus muscle and liver. AdipoR2 mRNA expression, in contrast, was significantly lower in the liver, suggesting that such a down-regulation occurs mainly at this location. We have shown that, in patients with NASH (non-alcoholic steatohepatitis), AdipoR2 expression is decreased compared with simple steatosis of the liver [62]. The abundantly expressed adiponectin receptor in the liver, namely the type 1 receptor, had similar expression in steatosis and NASH.

Adiponectin has been shown to be involved in the regulation of several placental functions [63]. Placental adiponectin mRNA expression is increased during pregnancy in the rat, whereas AdipoR2 had the contrary pattern. Furthermore, treatment with adiponectin during gestation decreased AdipoR2, GLUT 3 (glucose transporter 3), lipoprotein lipase and TGF-β (transforming growth factor-β) mRNA [63].

Genetic variations in both AdipoR1 and AdipoR2 have been associated with T2DM [64]. In this study, three SNPs (single nucleotide polymorphisms) in the AdipoR1 and 16 in AdipoR2 were significantly associated with T2DM. These results provided the first evidence for an association between genetic variations in the adiponectin receptors and T2DM. Notably, these findings have been challenged by a study by Hara et al. [65]. The conclusion of this study was that genetic variations in AdipoR1 and AdipoR2 are unlikely to lead to and contribute to a common genetic predisposition to IR and T2DM, at least in the Japanese population.

The importance of targeted disruption of AdipoR1 and AdipoR2 has recently been demonstrated [66]. Adenovirus-mediated expression of both adiponectin receptors in the liver of lepr-deficient mice increased AMPK activation and PPAR-α signalling pathways. Activation of AMPK reduced gluconeogenesis, whereas expression of the receptors in both cases increased fatty
acid oxidation and led to an amelioration of diabetes. However, targeted disruption of AdipoR1 resulted in the abrogation of adiponectin-induced AMPK activation, whereas that of AdipoR2 resulted in decreased activity of PPAR-α signalling pathways. Disruption of both receptors abolished adiponectin binding and actions, resulting in increased triacylglycerol content, inflammation and oxidative stress, and thus leading to IR and marked glucose intolerance. This important finding also highlights that these two receptors should indeed be the two major adiponectin receptors [66].

**Conclusions**

Adiponectin is a key mediator in the regulation of IR and suppresses inflammation in various animal models. It also has a crucial role in suppressing macrophage activity not only in adipose tissue, but also in other tissues such as the liver. Its diminished synthesis, as is observed in individuals who are obese, might lead to the dysregulation of the control mechanisms that inhibit the production of pro-inflammatory cytokines. One of the main challenges in understanding the physiology of this adipocytokine will be to recognize how circulating levels decrease with the onset of obesity.

**PBEF/VISFATIN: A PRO-INFLAMMATORY (ADIPO)CYTOKINE**

PBEF/visfatin was originally cloned by Samal et al. [67] during a search for novel cytokine-like molecules secreted from human peripheral blood lymphocytes. They described a 52 kDa secreted molecule termed PBEF that was strongly induced by pokeweed mitogen and cycloheximide, and enhanced the effect of IL-7 and stem cell factor on pre-B-cell colony formation [67]. PBEF/visfatin is highly conserved in evolution, as homologous proteins have been described in bacteria [68], invertebrate sponges [69] and fish [70]. Intracellular PBEF/visfatin acts as a dimeric type II phosphoribosyltransferase (NAD biosynthesis) [68,71,72], and growth-phase-dependent changes in its subcellular distribution have been reported [73].

We have reported recently primarily pro-inflammatory activities exerted by PBEF/visfatin by showing that it dose-dependently up-regulated the production of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in human monocytes [74]. At higher concentrations, PBEF/visfatin also induced the expression of the anti-inflammatory cytokines IL-10 and IL-1Ra. We also demonstrated that PBEF/visfatin induces the expression of the co-stimulatory molecules CD80 (B7-1) and CD40 in human monocytes. Moreover, we observed a significant induction of ICAM-1 (intercellular adhesion molecule-1; CD54), another co-stimulatory ligand that binds to LFA-1 (lymphocyte function-associated antigen-1), thereby promoting the activation of T-cells [75]. Evidence that PBEF/visfatin affects primary lymphocyte responses was demonstrated by an increased dose-dependent proliferative response after pre-incubating monocytes with PBEF/visfatin. Notably, PBEF/visfatin was able to significantly increase mannose-receptor-mediated phagocytosis by human monocytes. Finally, in accordance with previous findings [76], APCs (antigen-presenting cells) might be a major source of PBEF/visfatin themselves, as identified by immunofluorescence double-staining with macrophage and DC markers (CD163, DC-Sign and MHC-II<sup>αβ</sup>) in colonic tissue samples of patients with IBD (inflammatory bowel disease). Trafficking of cells to sites of inflammation is another critical function of the immune system and largely orchestrated by chemokines [77]. In our studies [74], we have also shown that PBEF/visfatin is a potent chemotactic factor particularly for CD14<sup>+</sup> monocytes and CD19<sup>+</sup> B-cells.

When administered to mice, murine PBEF/visfatin significantly increased the level of circulating IL-6 [74]. This increase was paralleled by an up-regulation of IL-6 mRNA levels in the intestine that appeared to be the major source, since no differences were observed in liver, spleen or lung. This result fits well with our in vitro results in human leucocytes [74], where IL-6 was the cytokine most prominently up-regulated. IL-6 is known to be a pleiotropic cytokine that is critically involved in a variety of immunological processes, such as activation of acute-phase responses [78], haemopoiesis [79], final B-cell maturation, T-cell activation and proliferation [80], induction of chemokines and leucocyte recruitment [81], and liver and neuronal regeneration [82,83]. Moreover, PBEF/visfatin-induced IL-6 expression might be involved in the pathogenesis of IR associated with visceral obesity [84]. IL-6 has been demonstrated to promote IR via induction of SOCS (suppressor of cytokine signalling) proteins [85]. Our results raise the possibility that obesity-related enhanced PBEF/visfatin expression [84,86] induces IL-6 production, which is likely to promote IR. Several studies now support the evidence that PBEF/visfatin is primarily a pro-inflammatory cytokine, as its serum/plasma levels are increased in various inflammatory disorders [76,87,88]. Plasma PBEF/visfatin levels have been demonstrated to be associated with endothelial function as a negative correlation between the log10-transformed plasma PBEF/visfatin concentration and flow-mediated vasodilation in patients with T2DM has been observed [89]. In addition, in this study, treatment with pioglitazone in 20 patients over 12 weeks did not affect plasma PBEF/visfatin levels. Overall, however, there is an ongoing debate as to whether this adipocytokine is mainly a ‘classical’ (adipo)cytokine with pro-inflammatory functions [90,91], as so far no further studies have clearly demonstrated its role in metabolic functions such as IR.
The potential roles of leptin and resistin as mediators linking adipose tissue, inflammation and immunity have been recently reviewed by the authors [1] and are not discussed further here.

**ADIPOCYTOKINES TARGETING THE NF-κB PATHWAY: INTERCONNECTION OF INFLAMMATION WITH OBESITY AND IR**

Systemic chronic inflammation has been proposed to have a key role in the pathogenesis of obesity-related IR [3,92]. Biomarkers of inflammation, such as TNF-α, IL-6 and CRP, are increased in individuals who are insulin-resistant and obese and predict the development of T2DM and cardiovascular diseases.

In searching for the mechanisms involved in inflammation-induced IR, Yuan and co-workers [93] identified the IKK-β (inhibitor of NF-κB kinase-β) pathway of NF-κB activation as a mediator of TNF-induced IR. The JNK (c-Jun N-terminal kinase) family of serine/threonine protein kinases, which are activated by many inflammatory stimuli (including TNF-α and ligation of the TLRs) are also important regulators of IR in mouse models of obesity [94]. In both genetic and dietary animal models of obesity, JNK activity is increased in the liver, muscle and adipose tissue, and loss of JNK1 prevents IR [94].

**TNF-α**

The first link between obesity, an increase in the expression of a pro-inflammatory cytokine, namely TNF-α, and insulin action came from a study more than a decade ago [92]. These findings led to the concept of inflammation in obesity and demonstrated that adipocytes express TNF-α. In these studies, expression of this cytokine in obese animals (fa/fa rat and ob/ob mouse) was increased and has been shown to regulate insulin action [92]. Further evidence supporting a key role of TNF-α in IR came from studies by Uysal et al. [95], where they showed that mice lacking TNF-α or TNF receptors had improved insulin sensitivity in both dietary and genetic (ob/ob) models of obesity. These observations were paralleled by similar findings in humans [96] with increased adipose tissue TNF-α expression in obesity, and improvement in this increased TNF-α expression following weight loss [97]. Furthermore, both TNF-α levels and its soluble receptors were positively correlated with BMI (body mass index) [98].

Importantly, studies using neutralizing anti-TNF antibodies in humans did not show improved insulin sensitivity [99,100]. In addition, single doses of TNF-α antagonists failed to improve IR in diabetic or obese subjects [99,101]. Subsequent placebo-controlled studies conducted over a treatment period of 4 weeks provided no improvements in insulin sensitivity in either obese diabetic subjects [102] or obese insulin-resistant subjects without diabetes [100]. Lo et al. [103] recently demonstrated, in a study involving 56 men and women with the metabolic syndrome, that etanercept therapy increased total adiponectin concentration, but concentrations of the HMW form, which is thought to mediate insulin sensitivity, was unchanged. Etanercept decreased muscle attenuation on computed tomography, suggesting an increase in muscle adiposity. Resistin levels tended to decrease, whereas leptin levels did not change. These findings may help to explain the lack of efficacy of this anti-TNF therapy on insulin sensitivity. Thus TNF-α blockade appears to have limited efficacy on IR in humans which, overall, might challenge the concept the role of TNF-α in these diseases.

**IL-1**

IL-1α and IL-1β were among the first cytokines identified and exert strong pro-inflammatory functions [104]. IL-1α-deficient mice have lower fasting glucose and insulin levels and improved insulin sensitivity, as determined by insulin tolerance testing, compared with wild type controls [105]. IL-1β, together with IL-6, concentrations predict the risk of T2DM in humans better than either cytokine alone [106]. The role of IL-1 as an adipocytokine is not clearly defined. More interestingly, and probably best highlighting the role of inflammation in T2DM and IR, treatment of patients with IL-1Ra improves glycaemic control [107]. More information is needed on the role of this important pro-inflammatory cytokine in obesity.

**IL-6**

Besides TNF-α, other cytokines, such as IL-6 [108], and cytokine-regulated molecules, such as CRP [109], also correlate with obesity and BMI, enhancing further the concept that obesity and IR are inflammatory conditions. IL-6 was among the first cytokines to be implicated as a predictor or pathogenetic marker of IR and cardiovascular disease. Concentrations of IL-6 decrease in parallel with weight loss and improvement in IR in patients undergoing bariatric surgery [110]. Visceral fat has been demonstrated as an important site for IL-6 secretion in humans, and these results provide a potential mechanistic link between visceral fat and systemic inflammation in subjects with abdominal obesity [111]. IL-6 production in abdominal adipose tissue is at least 3-fold higher compared with subcutaneous adipose tissue, thereby potentially contributing to hepatic IR. This cytokine may indeed be involved in the pathogenesis of hepatic IR, as insulin sensitivity increases in diet-induced obese mice treated with anti-(IL-6) antibodies [112]. IL-6-deficient mice are insulin-resistant and develop mature-onset obesity and diabetes, questioning whether IL-6 might indeed be involved in the development of IR [113]. A clear answer to this question will only be
possible when patients with diabetes and/or IR receive treatment with an IL-6-neutralizing antibody.

RBP-4

Serum RBP-4 is another characterized adipocytokine [114]. Until recently, the function of RBP-4 was thought to be the delivery of retinol to tissues; however, in patients with T2DM, serum levels of RBP-4 are increased. Transgenic overexpression of human RBP-4 or injection of recombinant RBP-4 in normal mice causes IR [114]. Therefore lowering RBP-4 levels could be an interesting strategy for the treatment of individuals with T2DM. Several studies have been published assessing the role of circulating RBP-4, as outlined below.

Stefan et al. [115] reported a positive correlation between RBP-4 levels with HOMA-IR (homeostatic model assessment of IR), suggesting a close relationship between circulating RBP-4 with not only liver fat, but also hepatic IR. In another recently published study, however, serum RBP-4 levels were not statistically different between lean, overweight and obese subjects [116]. Gavi and co-workers [117] found a relationship between RBP-4, insulin sensitivity and the percentage trunk fat in individuals without features of IR. Serum RBP-4 has been shown to be decreased after weight loss in morbidly obese subjects [118]. Another study presented evidence that severe calorie restriction promotes a decrease in adipose tissue and plasma levels of RBP-4; however, this study could not provide evidence for a role of RBP-4 in the regulation of diet-induced changes in insulin sensitivity [119]. Although there is now more evidence that RBP-4 might be associated with obesity-related disorders such as IR, the exact role of RBP-4 levels in diabetes and related IR remains controversial.

OTHER NEW ADIPOCYTOKINES

A new adipocytokine termed vaspin (visceral adipose-tissue-derived serine protease inhibitor) has been recently identified [120]. This molecule has similarities with adiponectin in that it improves insulin sensitivity. Preliminary studies indicate that it might also have anti-inflammatory effects, as it suppresses the production of TNF-α, leptin and resistin [120].

Omentin, another new adipocytokine, is a protein expressed and secreted from visceral, but not subcutaneous, adipose tissue that increases insulin sensitivity in human adipocytes [121]. Decreased omentin levels are associated with increasing obesity and IR. Omentin-1 was shown to be the major circulating isofrom of two known isoforms. Lean subjects had significantly higher omentin levels than obese and overweight subjects. Therefore omentin levels may be predictive of the metabolic consequences or co-morbidities associated with obesity and behave similar to adiponectin levels. Much more knowledge is needed on this new adipocytokine.

ROLE OF ADIPOCYTOKINES IN CANCER

In humans, the prevalence of cancer is increased or disease activity is more severe in individuals who are obese [7,8]. Although currently there is only limited evidence, mainly derived from correlative studies, adipocytokines could be attractive candidates linking obesity with these diseases. Epidemiological studies indicate that obesity represents a significant risk factor for the development of cancer, although the exact mechanisms of this have still not been identified [8]. It has been shown that patients with various cancers, including gastric, endometrial, prostate and breast cancers, have low circulating adiponectin levels [122–125]. Another study indicated an association between obesity and decreased adiponectin serum concentrations with colorectal adenomas and a higher risk of colorectal cancer in men with low adiponectin levels [126].

Adiponectin has anti-angiogenic effects through the inhibition of endothelial cell proliferation and migration [127]. In a mouse tumour model, adiponectin substantially inhibited primary tumour growth in a caspase-dependent manner and it resulted in endothelial cell apoptosis [128]. Prostate cancer is also associated with obesity, and full-length adiponectin inhibits prostate cancer cell growth at physiological concentrations [129]. Adiponectin has been demonstrated to modulate the GSK3β (glycogen synthase kinase-3β)/β-catenin signalling pathway and to attenuate mammary tumorigenesis of MDA-MB-231 cells in nude mice [71]. Adiponectin also has antiproliferative effects on another breast cancer cell line, the MCF7 cell line [130]. Adiponectin inhibits the growth and peritoneal metastasis of gastric cancer through both AdipoR1 and AdipoR2 [131]. Cancer cell lines expressing both receptors and engagement with adiponectin induced apoptosis and inhibited proliferation of the cell lines. Moreover, local injection of adiponectin markedly inhibited the growth of AZ521, a gastric cancer cell line, after subcutaneous inoculation into nude mice [131]. In addition, the continuous intraperitoneal infusion of adiponectin effectively suppressed the development of metastasis. Interestingly, Kamada et al. [132] recently reported that hypo-adiponectinaemia might accelerate hepatic tumour formation in a mouse model of NASH [132]. In this study, NASH was induced in adiponectin-deficient mice by a choline-deficient l-amino-acid-defined diet and markers of oxidative stress were assessed. A total of six out of 24 mice developed liver cirrhosis and hepatic tumours within 24 weeks, whereas wild-type mice only had steatosis. This study clearly demonstrated that the lack of adiponectin enhances the degree of steatosis and might favour the development of cirrhosis.
and tumour formation, a phenomenon which is also observed in patients with NASH and diabetes [132]. Many studies have investigated the effect of leptin on different cancer types in experimental cellular and animal models [133]. Most of the studies indicate that adiponectin can decrease the growth of cancer cells (breast, oesophageal, gastric, pancreatic, colorectal, prostate, ovarian and lung carcinoma cell lines), whereas leptin appears to increase cell proliferation. Although these first studies are only descriptive and do not allow further conclusions, adipocytokines could be attractive candidates as the missing link between obesity and cancer.

ROLE OF OBESITY AND ADIPOCYTOKINES IN IMMUNE-MEDIATED DISEASES

An association between obesity and increased asthma incidence and severity has been reported in some studies [7]. Recent studies have reported a higher incidence of atopy and asthma in overweight children [134] and a higher degree of IR in morbidly obese asthma patients compared with morbidly obese non-asthma patients [135]. Asthma patients are also less likely to respond to anti-inflammatory medication than non-obese control patients [136]. Administration of leptin increases airway hyper-responsiveness and the production of Th2 (T-helper 2) cytokines in ovalbumin-sensitized mice [137]. High leptin levels have been observed in asthmatic children compared with a control group with similar BMI, indicating that, besides BMI, other factors might affect leptin levels in these patients [10]. Todd et al. [138] recently reported that, in 126 adult asthmatic and non-asthmatic participants, there was no association between BMI and airway inflammation measured by sputum cell counts. Therefore the association of obesity with asthma might also be influenced by other factors, such as mechanical factors, which could be reflected by the increased prevalence of gastro-oesophageal reflux disease observed in this population.

Overexpression of adipocytokines, including adiponectin, leptin and resistin, in mesenteric adipose tissue of patients with Crohn’s disease after ileocolic surgical resection has been reported [139]. In Crohn’s disease, leptin and adiponectin are highly expressed in the mesenteric fat tissue, indicating that both pro- and anti-inflammatory adipocytokines are overexpressed in this type of inflammation [139,140]. There is no clear association between obesity and IBD, although obesity has been associated with increased disease severity [141].

In patients with rheumatoid arthritis, serum leptin levels remain correlated with BMI, rather than disease stage. In addition, increased levels of adiponectin and resistin are observed in the synovial fluid of patients with rheumatoid arthritis compared with that seen in patients with osteoarthritis [142,143]. Intra-articular injection of resistin also induces arthritis in healthy mouse joints [142]. An association of obesity with presence and development of rheumatoid arthritis, however, is less clear [144].

T_{reg}-cells (regulatory T-cells), obesity and immunity

T_{reg}-cells have attracted a lot of interest over the last few years as they might play an overwhelming role in the control of immune homoeostasis. Naturally occurring T_{reg}-cells comprise a subset of T-cells derived from the thymus which constitute 5–10 % of peripheral CD4+ T-cells. These cells constitutively express the high-affinity IL-2 receptor and are able to inhibit T-cell effector functions in vitro and in vivo. Development and function of these cells is under the control of the key forkhead transcription factor Foxp3. As these cells, therefore, might control many immune-mediated conditions, many strategies have been applied to control their expansion in the periphery. T_{reg}-cells can be expanded successfully in vitro by IL-2 and are able to suppress proliferative responses to either anti-CD3 or allogeneic stimuli, in a fashion similar to naturally occurring T_{reg}-cells. Freshly isolated human T_{reg}-cells constitutively express high amounts of leptin and leptin receptor (ObR), and the leptin pathway can act as a negative signal for the proliferation of T_{reg}-cells [145]. Interestingly, leptin neutralization reversed the anergic state of T_{reg}-cells. Together with the finding of enhanced proliferation of T_{reg}-cells observed in leptin- and ObR-deficient mice, these studies suggest a potential for therapeutic interventions in various immune-mediated diseases where the leptin pathway might be involved. Effects of other adipocytokines, such as adiponectin or PBEF/visfatin, on T_{reg}-cells are currently not known.

CONCLUSIONS

The discovery of adipocytokines has defined a new way of thinking how obesity, inflammation and immunity might be connected to each other. It is now evident that there are key adipocytokines, such as adiponectin and leptin, which are synthesized mainly in the adipose tissue, circulate at high concentrations (especially adiponectin), function in a hormone-like manner and have many features of classical cytokines. These two mediators have recently dominated the field of adipocytokine research, and there is increasing evidence that they are involved in many diseases beyond obesity and its related disorders. One of the challenging questions remains whether this new knowledge on adipocytokines might lead to new therapies for obesity-related diseases.
ACKNOWLEDGMENTS

The authors’ work was supported by a grant from the Austrian Science Foundation (P17447), and the Christian-Doppler Research Society.

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Received 8 June 2007/20 August 2007; accepted 24 September 2007
Published on the Internet 15 January 2008, doi:10.1042/CS20070196

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