[Frontiers in Bioscience 5187-5194, May 1, 2008]

The implication of proinflammatory cytokines in type 2 diabetes

Christopher B. Guest[1,2,3], Min J. Park[1,2], Daniel R. Johnson[2,4] Gregory G. Freund[1-4]

[1]Division of Nutritional Sciences, Department of Pathology, University of Illinois at Urbana-Champaign, Urbana, IL, [2]Integrative Immunology and Behavior Program, [3]College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, [4]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL

TABLE OF CONTENTS

1. ABSTRACT

The incidence of type 2 diabetes (T2D) is rapidly expanding. Some of the more obvious pathologies associated with it include: defective glucose metabolism, obesity, cardiovascular disease and an inability to mount an effective immune response to infection by certain pathogenic organisms, leading to sepsis and death. A common tie linking these seemingly disparate complications is chronic inflammation. Today we know that inflammation is regulated locally and systemically by numerous biochemical signals. One of the most important of these signals is a class of molecules called cytokines. Cytokines can be generally classified as proinflammatory or anti-inflammatory and allow an organism to respond rapidly to an immune challenge by coordinating an appropriate immune response. In T2D, the balance between proinflammatory and anti-inflammatory cytokines is shifted toward proinflammation, potentially causing or exacerbating the health complications found in T2D. Over-nutrition has been shown to trigger the innate immune system but activation of the innate immune system, itself, induces hyperglycemia and insulin resistance. In all likelihood, diabetes and chronic inflammation are inseparable and act as a reciprocal feed-forward loop.

2. INTRODUCTION

T2D affects more than 150 million people worldwide (1) and is projected to increase to 300 million by the year 2020 (2). Unlike type 1 diabetes, which is characterized by an absolute lack of insulin, T2D is characterized by defective insulin function which progresses from subclinical impaired glucose intolerance and insulin resistance to overt diabetes over the course of years (3). Importantly, during this subclinical phase of the disease, health complications such as atherosclerosis and low grade chronic inflammation are already present (4). Inflammation is classically defined by four symptoms: swelling, redness, pain and heat. In 1941, Valy Menkin conducted a series of simple but elegant experiments establishing a firm link between diabetes and inflammation. Menkin found that pancreactomized dogs injected with an irritant into the pleural cavity showed a nearly 85% increase in blood glucose accompanied by proteolysis, enhanced gluconeogenesis and infiltration with vacuolized polymorphonuclear cells. Non-diabetic dogs showed no change in blood glucose and normal leukocytes after injection of the irritant. Importantly, Menkin was able to block this inflammatory reaction by administration of insulin (5). Menkin's findings illustrate that inflammation

enhances the degree of diabetes and diabetes enhances inflammation. Since this initial finding, thousands of studies have improved our understanding of the interaction between diabetes and inflammation. A decade ago, Pickup *et al.* suggested that T2D was a proinflammatory disease involving activation of the innate immune system (6). In support of this concept, T2D often presents serum elevations of acute-phase reactants including sialic acid, a-1 acid glycoprotein, amyloid A, IL-1beta, TNF-alpha, IL-6, C-reactive protein, and cortisol (7, 8). Additional studies have shown that altered innate immunity and chronic inflammation appeared strongly associated with insulin resistance in obesity. Uysal *et al.* reported that the proinflammatory cytokine TNF-alpha was synthesized by adipocytes and was a mediator of insulin resistance in obesity (9). Kim *et al.* demonstrated that inactivation of IKK-beta prevented fat-induced insulin resistance in skeletal muscle, suggesting it as a potential therapeutic target for T2D (10). Cai *et al.* demonstrated that NF-kappaB activation and proinflammatory cytokine production (including IL-6, IL-1 and TNF-alpha) were increased in the liver by obesity and high-fat diet, leading to insulin resistance and hyperglycemia (11). A study by Solinas and coworkers showed that inflammation but not obesity per se, triggered insulin resistance. In mice, high-fat diet-induced insulin resistance could be prevented through blocking an inflammatory pathway in macrophages by JNK1 deletion (12). These results connote that obesity-induced inflammation decreases high-fat diet-induced insulin resistance as well as resultant T2D. Recent data have shown that increased levels of inflammatory cytokines, such as IL-6 and high-sensitivity C-reactive protein (hsCRP), were linked to an elevated risk of clinical diabetes (13-15). Work by our group indicated that the enhanced proinflammatory phenotype in T2D not only affected complications like cardiovascular disease (16) but also exacerbated other pathologies such as depression and social withdrawal induced by activation of the innate immune system with lipopolysaccharide (17) or hypoxia (18).

In addition to elevation of proinflammatory cytokines, T2D may be associated with a less effective anti-inflammatory response. The process of insulin resistance has been an area of prolific study. There are several factors that can lead to insulin resistance including increased degradation of the receptors by the proteasome, alteration of downstream signaling partners and phosphorylation at inhibitory serine and threonine residues (19). One of the critical regulators of this process is a class of molecules called suppressor of cytokine signaling (SOCS). Interestingly, several anti-inflammatory cytokines including IGF-1, IL-4 and IL-10 share key signaling components with the insulin receptor and are susceptible to similar resistance mechanisms.

3. PROINFLAMMATORY CYTOKINES

There are a variety of cytokines labeled as proinflammatory. Almost all immune cells as well as epithelial cells and adipocytes produce a subset of these cytokines. Generally, proinflammatory cytokines are important for initiating the innate immune response and for directing the subsequent adaptive immune response. The most studied contributors to the chronic inflammation seen in T2D are leptin, TNF-alpha, IL-1beta, and IL-6.

3.1. Leptin

Leptin was first discovered after a series of parabiosis experiments (20). Coleman infused the plasma of obese, hyper-leptinemic *db/db* mice into wild-type mice. Surprisingly, the mice became anorexic and died of starvation. Zhang *et al.* cloned the gene encoding the 16 kDa leptin protein (21) while the gene encoding the principal leptin receptor was identified by Leiter *et al.* (22). The crystal structure of leptin revealed a four-helix bundle similar to that of IL-6 (23). The action of leptin is primarily mediated through Janus kinase-2 (JAK-2) and signal transducer and activator of transcription-3 (STAT-3) (24). Targeted disruption of STAT-3 in the central nervous system induces a phenotype similar to mice lacking either leptin or the leptin receptor *ie*. obesity, diabetes and infertility (25). Importantly, leptin has also been shown to act on pathways that include those containing insulin receptor substrate (IRS), phosphotidylinositide 3'-kinase (PI3K), mitogen activated protein kinase (MAPK) (26) and, recently, AMP-activated protein kinase (27) (for a complete review see (28)).

Leptin is a multifunctional cytokine. It is secreted primarily by adipose tissue but many other tissues can produce it, including macrophages. Leptin is best known as a regulator of satiety and energy homeostasis (29). It acts as a permissive signal when energy levels are high, as represented by adequate fat stores. However, when energy stores are low, leptin secretion decreases and the orexigenic system is activated in the hypothalamus, causing feelings of hunger (29). Human studies that attempted to reduce food intake by exogenous administration of leptin have been disappointing (30). A number of theories were raised to explain this lack of appetite suppression. One theory is that leptin receptors are highly expressed in the satiety centers of the hypothalamus, but in order to bind to these receptors, circulating leptin must pass through the blood brain barrier via a saturable process (31). It is possible that the high circulating leptin levels observed in obese individuals do not result in similar increase in brain leptin. Interestingly, Faouzi et al. have shown that specific hypothalamic regions establish a direct contact with the general circulation, and thereby, display differential patterns of leptin uptake and responsiveness (32). Another mechanism potentially explaining the lack of therapeutic benefits of leptin is that individuals may acquire leptin resistance (33) in a manner similar to insulin resistance, including the disruption of downstream leptin receptor signaling by SOCS proteins (34).

Leptin has a number of important functions in immunity. It has been shown to induce the production of TNF-alpha, IL-1beta, IL-1RA, IL-R2 and IL-6 as well as that of reactive oxygen species, and to increase phagocytosis in some antigen presenting cells (35-37). Recently, a role of leptin in the regulation of emotions and depression has been suggested. *db/db* mice lacking a functional long form of the leptin receptor showed delayed recovery from LPS- or hypoxia-induced social withdrawal

(17, 18). This delayed recovery was accompanied by a failure to upregulate the anti-inflammatory cytokines IL-1RA and IL-1R2. Administration of exogenous leptin was also found to relieve anhedonia, demonstrating its potential to act as an antidepressant (38). Given these numerous functions of leptin, it is likely that its implication in T2D will be the subject of many new discoveries.

3.2. TNF-alpha

TNF-alpha is now recognized as an important modulator of immunity and metabolism, inducing loss of social exploration (39), production of acute phase proteins (40) and activation of dendritic cell migration (41). TNF-alpha is a 27 kDa protein that is processed into a 17 kDa active form. TNF-alpha has been shown to induce insulin resistance (6, 42) and to be implicated in the progression of obesity (43). Chronic exposure of adipocytes to TNF-alpha strongly inhibited insulin-stimulated glucose uptake and decreased the phosphorylation of the insulin receptor by insulin (44, 45). Some controversy exists as to whether TNF-alpha is a causative agent in T2D. In subjects suffering from impaired glucose tolerance (IGT), TNF-alpha levels were not elevated by contrast with IL-6 levels (46). However, TNF-alpha receptor knockout mice showed an improved glucose tolerance and increased insulin sensitivity (42). Leptin deficient ob/ob mice with an added p75 TNF-alpha receptor knockout exhibited improved glucose tolerance (47). Additionally, TNF-alpha is strongly linked with cardiovascular complications which are the leading cause of death in diabetes (48). TNF-alpha may accelerate the atherosclerotic process (49) through an increase in the expression of endothelin-1 (50) and by an alteration of lipid metabolism (9).

3.3. IL-1beta

Interleukin-1beta is a 17.4.-kDa protein derived from the cleavage of a 33 kDa inactive precursor by interleukin-1-beta-converting enzyme (51). IL-1beta signaling occurs through the evolutionarily conserved MyD88 pathway and the activation of NF-kappaB. IL-1beta is produced by a variety of tissues and cell types including macrophages, neurons, beta cells of the pancreas and adipose tissue. IL-1beta is known to induce sickness behavior, fever and the secretion of other cytokines (52). Like TNF-alpha and leptin, IL-1beta has important effects on metabolism. For instance, the activation of IL-1beta receptors in hypothalamic neurons caused a marked reduction in food intake (52, 53). The functions of IL-1beta are counter-regulated in part by competitive inhibition by IL-1RA and IL-1R2 (54). Importantly, these counter-regulatory mechanisms were deficient in type 2 diabetic *db/db* mice injected with LPS, IL-1beta or in hypoxic conditions (17, 18), by contrast with type 2 diabetic humans who tend to have higher basal serum levels of IL-1RA. Additionally, IL-1beta was shown to induce apoptosis in pancreatic beta cells. This finding was first described in type 1 diabetes but it was also demonstrated that beta cell loss in T2D was partially mediated by IL-1beta (55).

3.4. IL-6

IL-6 is a 27 kDa four helix-bundle cytokine with structural similarity to leptin (56). The IL-6 receptor is a heterodimer consisting of a gp130 subunit and IL-6R. IL-6 directly affects many tissues including B cells, T cells, megakaryocytes, macrophages, hepatocytes, osteoclasts, blood vessels, heart muscle, neuronal cells and the placenta (57). IL-6 is produced mainly by cells of the immune system, skeletal muscle and the liver, but other cells types such as glia and endothelial cells have been reported to produce IL-6 (58). The effects of IL-6 differ according to the target tissues. IL-6 is a key regulator of the acute phase response by the liver following infection. It induces the production of C-reactive protein, haptoglobin, serum amyloid A and fibrinogen (59). Like leptin, IL-6 signaling occurs through the MAP kinase, and the JAK/STAT pathways (60). IL-6 is a potent endogenous pyrogen and augments LPS induced sickness behavior (61).

The role of IL-6 in T2D is complex and appears to be tissue dependent. Circulating levels of IL-6 levels are increased in T2D (7). A chronic overexpression of IL-6 appears to reduce the action of insulin like growth factor in mice displaying growth defects. This effect was partially neutralized by the administration of anti-IL-6 receptor antibodies (62). In addition, IL-6 has been shown to promote insulin resistance in hepatocytes through the activation of STAT-3 (63). This mechanism was further elucidated by the finding that insulin resistance in hepatocytes was mediated by SOCS-3 and that mTOR played a critical role in SOCS-3 upregulation (64). Additionally, Cai *et al.* showed that T2D could be induced in mice by chronic activation of NF-kappaB in the liver or a high fat diet. These chronic inflammatory conditions induced steatosis of the liver and an increase production of proinflammatory cytokines by hepatocytes, including IL-6. Importantly, insulin resistance could be significantly improved by treatment with IL-6 neutralizing antibodies or salicylate (11). These findings suggested a causative role for IL-6 in the development of T2D. However, mice with a targeted deletion of IL-6 developed mature-onset insulin resistance, obesity and leptin resistance (65). It was speculated that the reason for this contrary finding was that the action IL-6 is tissue dependent. Indeed, the local administration of IL-6 into the brains of IL-6 deficient mice partially improved the aforementioned symptoms but it had no effect when administered into the brain of wild type control animals (66). The importance of tissue specificity was further emphasized by the finding that IL-6 enhanced insulin-stimulated glucose disposal and improved glucose metabolism in humans through the activation of AMPK, likely in skeletal muscle (67). IL-6 is clearly an important cytokine in the regulation of immunity and metabolism and it may be an important player in the development and complications of T2D. Further research will be necessary to clarify the absolute impact of IL-6 in T2D.

4. CYTOKINES RESISTANCE

Counter-regulations are critical to maintain homeostasis. One of the most important mechanisms of hormone/cytokine counter-regulation is mediated by the SOCS family of proteins. While investigating the

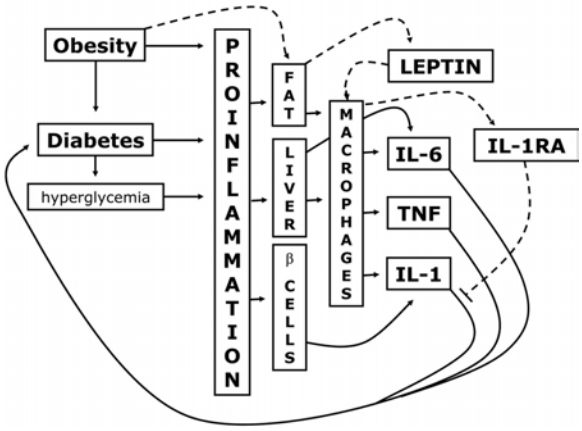

Figure 1. How obesity and diabetes may impact inflammation

downstream signaling cascade of IL-6, Kishimoto *et al.* discovered a protein that they called STAT-induced STAT inhibitor or SSI-1. As the name indicates, this protein inhibited IL-6 mediated STAT activation and was itself induced by activation of STAT (68). SSI-1 was later found to be part of a larger family of proteins which are now entitled SOCS proteins. This family of proteins contains an SH-2 domain that can interact with several receptors at phosphotyrosine residues to block signal transduction. Some of the important signaling molecules regulated by the SOCS are insulin, IGF-1, leptin, IL-6, IL-4 and IL-10 (64, 69, 70). Chronic activation of the aforementioned receptors can induce a state of functional resistance to the ligand responsible for that specific receptors activation. In a case of ligand-dependent chronic activation, ligand-specific receptor insensitivity occurs as does spillover insensitivity to other receptor pathways due to SOCS upregulation. This finding has led to the speculation that T2D was caused by chronic over expression of SOCS proteins (69). Work by our group indicated that hyperglycemia and hyperinsulinemia contributed to insulin resistance by activation of the nutrient sensing mTOR pathway (71). Chen *et al.* extended this finding by showing that inhibition of mTOR by rapamycin blocked IL-6 induced SOCS protein-mediated insulin resistance (64). Recently, it was demonstrated that T2D-dependent upregulation of SOCS proteins negatively impacted the efficacy of the anti-inflammatory cytokine IL-4 to induce IL-1RA by (70), adding to the growing body of evidence that implicates the SOCS proteins as key immune and metabolic regulators. Additionally, there now appears a direct mechanistic path to explain how dysregulation in certain immune pathways can adversely impact metabolic systems and vice versa.

5. ANTI-INFLAMMATORY INTERVENTIONS

The treatment of diabetes was very limited until the discovery and purification of insulin by Banting and Best in 1921. However, before the discovery of insulin, Ebstein showed that daily consumption of high doses of salicylates greatly reduced glucose elimination in the urine (72). There were a handful of additional studies that explored this finding further, as reviewed by Shoelson (73) but these early promising results were overshadowed by the tremendous success of insulin in the treatment of hyperglycemia. Recently, the emergence of the idea that T2D is an inflammatory disease has led to reexamining the use of anti-inflammatory agents in the treatment of T2D complications. Interestingly, some of the medications currently used as anti-hyperglycemic agents, such as the PPARgamma agonist rosiglitazone, may actually mediate at least part of their action through anti-inflammatory effects (74). Likewise, HMG-CoA reductase inhibitors (statins) have long been known to reduce cardiovascular disease, a serious complication of T2D, by reducing endogenous cholesterol production. Recently, a growing body of evidence suggests that statins exert potent anti-inflammatory effects (75). Metformin, one of the most commonly prescribed drugs in the treatment of T2D has been shown to act as an anti-inflammatory agent by activating AMPK (76). Use of metformin is interesting because AMPK is a key local and systemic metabolic regulator. In addition, these findings underscore the degree of integration between metabolism and immunity. Finally and very recently, IL-1RA has shown promise in improving glycemia, beta-cell secretory function and reducing markers of systemic inflammation (77). By expanding our understanding of T2D, we have increased the therapeutic options available to the individual with diabetes, and have, in some ways, returned to Ebstein's original observations with the archetypal anti-inflammatory aspirin (73).

6. CONCLUSIONS

Inflammation can be viewed as a homeostatic model with pro- and anti- inflammatory aspects. Proinflammatory cytokines are necessary in order to mount an initial effective immune response. However, this proinflammatory reaction must be balanced by an appropriate anti-inflammatory rejoinder in order to effectively direct the adaptive immune response and to avoid excessive damage to healthy tissues. Furthermore, the immune response must be in balance with the metabolic supplies of the organism. Immune and metabolic pathways are deeply intertwined (Figure 1) and require synchronicity in order to promote organismal survival (78). T2D is a key example of what happens when balance goes awry. Neither cytokine nor hormonal networks function in isolation so it is likely that many important future contributions to our understanding of T2D will be found by examining the complicated interaction and temporal variations of immune/metabolic balance. While tremendous strides have been made in understanding the nature of T2D and its complications, much more work needs to be done to improve the lives of these individuals living with the all too familiar quartet of swelling, redness, pain and heat.

7. ACKNOWLEDGEMENTS

Christopher B. Guest, Min J. Park contributed equally to this work. This work was supported by grants from the National Institutes of Health (DK064862 to G.G.F) and University of Illinois Agricultural Experiment Station (to G.G.F.).

8. REFERENCES

1. S. Matthaei, M. Stumvoll, M. Kellerer and H. U. Haring: Pathophysiology and pharmacological treatment of insulin resistance. *Endocr Rev*, 21 (6), 585-618 (2000)

2. M. Crook: Type 2 diabetes mellitus: a disease of the innate immune system? An update. *Diabet Med*, 21 (3), 203-7 (2004)

3. P. Zimmet, K. G. Alberti and J. Shaw: Global and societal implications of the diabetes epidemic. *Nature*, 414 (6865), 782-7 (2001)

4. P. Z. Zimmet and K. G. Alberti: The changing face of macrovascular disease in non-insulin-dependent diabetes mellitus: an epidemic in progress. *Lancet*, 350 Suppl 1, SI1-4 (1997)

5. V. Menkin: Diabetes And Inflammation. *Science*, 93 (2419), 456-458 (1941)

6. J. C. Pickup and M. A. Crook: Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia*, 41 (10), 1241-8 (1998)

7. J. C. Pickup, M. B. Mattock, G. D. Chusney and D. Burt: NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia*, 40 (11), 1286-92. (1997)

8. J. C. Pickup: Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care*, 27 (3), 813-23 (2004)

9. K. T. Uysal, S. M. Wiesbrock, M. W. Marino and G. S. Hotamisligil: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature*, 389 (6651), 610-4 (1997)

10. J. K. Kim, Y. J. Kim, J. J. Fillmore, Y. Chen, I. Moore, J. Lee, M. Yuan, Z. W. Li, M. Karin, P. Perret, S. E. Shoelson and G. I. Shulman: Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest*, 108 (3), 437-46 (2001)

11. D. Cai, M. Yuan, D. F. Frantz, P. A. Melendez, L. Hansen, J. Lee and S. E. Shoelson: Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med*, 11 (2), 183-90 (2005)

12. G. Solinas, C. Vilcu, J. G. Neels, G. K. Bandyopadhyay, J. L. Luo, W. Naugler, S. Grivennikov, A. Wynshaw-Boris, M. Scadeng, J. M. Olefsky and M. Karin: JNK1 in Hematopoietically Derived Cells Contributes to Diet-Induced Inflammation and Insulin Resistance without Affecting Obesity. *Cell Metab*, 6 (5), 386-397 (2007)

13. S. Liu, L. Tinker, Y. Song, N. Rifai, D. E. Bonds, N. R. Cook, G. Heiss, B. V. Howard, G. S. Hotamisligil, F. B. Hu, L. H. Kuller and J. E. Manson: A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Arch Intern Med*, 167 (15), 1676-85 (2007)

14. A. D. Pradhan, J. E. Manson, N. Rifai, J. E. Buring and P. M. Ridker: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama*, 286 (3), 327-34. (2001)

15. A. D. Pradhan, N. R. Cook, J. E. Buring, J. E. Manson and P. M. Ridker: C-reactive protein is independently associated with fasting insulin in nondiabetic women. *Arterioscler Thromb Vasc Biol*, 23 (4), 650-5. (2003)

16. C. B. Guest, M. E. Hartman, J. C. O'Connor, K. S. Chakour, A. A. Sovari and G. G. Freund: Phagocytosis of cholesteryl ester is amplified in diabetic mouse macrophages and is largely mediated by CD36 and SR-A. *PLoS ONE*, 2, e511 (2007)

17. J. C. O'Connor, A. Satpathy, M. E. Hartman, E. M. Horvath, K. W. Kelley, R. Dantzer, R. W. Johnson and G. G. Freund: IL-1beta-mediated innate immunity is amplified in the db/db mouse model of type 2 diabetes. *J Immunol*, 174 (8), 4991-7 (2005)

18. D. R. Johnson, J. C. O'Connor, M. E. Hartman, R. I. Tapping and G. G. Freund: Acute hypoxia activates the neuroimmune system, which diabetes exacerbates. *J Neurosci*, 27 (5), 1161-6 (2007)

19. K. Morino, K. F. Petersen and G. I. Shulman: Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes*, 55 Suppl 2, S9-S15 (2006)

20. D. L. Coleman: Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia*, 9 (4), 294-8 (1973)

21. Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold and J. M. Friedman: Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372 (6505), 425-32 (1994)

22. E. H. Leiter, D. L. Coleman, A. B. Eisenstein and I. Strack: A new mutation (db3J) at the diabetes locus in strain 129/J mice. I. Physiological and histological characterization. *Diabetologia*, 19 (1), 58-65 (1980)

23. F. Zhang, M. B. Basinski, J. M. Beals, S. L. Briggs, L. M. Churgay, D. K. Clawson, R. D. DiMarchi, T. C. Furman, J. E. Hale, H. M. Hsiung, B. E. Schoner, D. P. Smith, X. Y. Zhang, J. P. Wery and R. W. Schevitz: Crystal structure of the obese protein leptin-E100. *Nature*, 387 (6629), 206-9 (1997)

24. H. Baumann, K. K. Morella, D. W. White, M. Dembski, P. S. Bailon, H. Kim, C. F. Lai and L. A. Tartaglia: The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci U S A*, 93 (16), 8374-8 (1996)

25. Q. Gao, M. J. Wolfgang, S. Neschen, K. Morino, T. L. Horvath, G. I. Shulman and X. Y. Fu: Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. *Proc Natl Acad Sci U S A*, 101 (13), 4661-6 (2004)

26. C. Martin-Romero and V. Sanchez-Margalet: Human leptin activates PI3K and MAPK pathways in human peripheral blood mononuclear cells: possible role of Sam68. *Cell Immunol*, 212 (2), 83-91 (2001)

27. Y. Minokoshi, Y. B. Kim, O. D. Peroni, L. G. Fryer, C. Muller, D. Carling and B. B. Kahn: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, 415 (6869), 339-43 (2002)

28. G. Fruhbeck: Intracellular signalling pathways activated by leptin. *Biochem J*, 393 (Pt 1), 7-20 (2006)

29. J. M. Friedman and J. L. Halaas: Leptin and the regulation of body weight in mammals. *Nature*, 395 (6704), 763-70 (1998)

30. S. B. Heymsfield, A. S. Greenberg, K. Fujioka, R. M. Dixon, R. Kushner, T. Hunt, J. A. Lubina, J. Patane, B. Self, P. Hunt and M. McCamish: Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *Jama*, 282 (16), 1568-75 (1999)

31. G. H. Lee, R. Proenca, J. M. Montez, K. M. Carroll, J. G. Darvishzadeh, J. I. Lee and J. M. Friedman: Abnormal splicing of the leptin receptor in diabetic mice. *Nature*, 379 (6566), 632-5 (1996)

32. M. Faouzi, R. Leshan, M. Bjornholm, T. Hennessey, J. Jones and H. Munzberg: Differential accessibility of circulating leptin to individual hypothalamic sites. *Endocrinology*, 148 (11), 5414-23 (2007)

33. M. Van Heek, D. S. Compton, C. F. France, R. P. Tedesco, A. B. Fawzi, M. P. Graziano, E. J. Sybertz, C. D. Strader and H. R. Davis, Jr.: Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest*, 99 (3), 385-90 (1997)

34. J. C. O'Connor, D. R. Johnson and G. G. Freund: Psychoneuroimmune implications of type 2 diabetes. *Neurol Clin*, 24 (3), 539-59 (2006)

35. Q. L. Lam and L. Lu: Role of leptin in immunity. *Cell Mol Immunol*, 4 (1), 1-13 (2007)

36. C. Gabay, M. Dreyer, N. Pellegrinelli, R. Chicheportiche and C. A. Meier: Leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes. *J Clin Endocrinol Metab*, 86 (2), 783-91 (2001)

37. M. G. Dreyer, C. E. Juge-Aubry, C. Gabay, U. Lang, F. Rohner-Jeanrenaud, J. M. Dayer and C. A. Meier: Leptin activates the promoter of the interleukin-1 receptor antagonist through p42/44 mitogen-activated protein kinase and a composite nuclear factor kappa B/PU.1 binding site. *Biochem J*, 370 (Pt 2), 591-9 (2003)

38. X. Y. Lu, C. S. Kim, A. Frazer and W. Zhang: Leptin: a potential novel antidepressant. *Proc Natl Acad Sci U S A*, 103 (5), 1593-8 (2006)

39. R. M. Bluthe, S. Laye, B. Michaud, C. Combe, R. Dantzer and P. Parnet: Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. *Eur J Neurosci*, 12 (12), 4447-56 (2000)

40. I. Gresser, F. Delers, N. Tran Quangs, S. Marion, R. Engler, C. Maury, C. Soria, J. Soria, W. Fiers and J. Tavernier: Tumor necrosis factor induces acute phase proteins in rats. *J Biol Regul Homeost Agents*, 1 (4), 173-6 (1987)

41. M. Cumberbatch and I. Kimber: Tumour necrosis factor-alpha is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization. *Immunology*, 84 (1), 31-5 (1995)

42. G. S. Hotamisligil, P. Peraldi, A. Budavari, R. Ellis, M. F. White and B. M. Spiegelman: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science*, 271 (5249), 665-8. (1996)

43. B. M. Spiegelman and G. S. Hotamisligil: Through thick and thin: wasting, obesity, and TNF alpha. *Cell*, 73 (4), 625-7. (1993)

44. G. S. Hotamisligil, A. Budavari, D. Murray and B. M. Spiegelman: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J Clin Invest*, 94 (4), 1543-9 (1994)

45. G. S. Hotamisligil, D. L. Murray, L. N. Choy and B. M. Spiegelman: Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A*, 91 (11), 4854-8 (1994)

46. S. Muller, S. Martin, W. Koenig, P. Hanifi-Moghaddam, W. Rathmann, B. Haastert, G. Giani, T. Illig, B. Thorand and H. Kolb: Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. *Diabetologia*, 45 (6), 805-12 (2002)

47. G. Voros, E. Maquoi, D. Collen and H. R. Lijnen: Influence of membrane-bound tumor necrosis factor (TNF)-alpha on obesity and glucose metabolism. *J Thromb Haemost*, 2 (3), 507-13 (2004)

48. J. C. Kleinman, R. P. Donahue, M. I. Harris, F. F. Finucane, J. H. Madans and D. B. Brock: Mortality among

diabetics in a national sample. *Am J Epidemiol*, 128 (2), 389-401. (1988)

49. M. F. Lopes-Virella and G. Virella: Cytokines, modified lipoproteins, and arteriosclerosis in diabetes. *Diabetes*, 45 (Suppl 3), S40-4. (1996)

50. P. Klemm, T. D. Warner, R. Corder and J. R. Vane: Endothelin-1 mediates coronary vasoconstriction caused by exogenous and endogenous cytokines. *J Cardiovasc Pharmacol*, 26 Suppl 3, S419-21 (1995)

51. D. P. Cerretti, C. J. Kozlosky, B. Mosley, N. Nelson, K. Van Ness, T. A. Greenstreet, C. J. March, S. R. Kronheim, T. Druck, L. A. Cannizzaro and *et al.*: Molecular cloning of the interleukin-1 beta converting enzyme. *Science*, 256 (5053), 97-100 (1992)

52. R. M. Bluthe, R. Dantzer and K. W. Kelley: Central mediation of the effects of interleukin-1 on social exploration and body weight in mice. *Psychoneuroendocrinology*, 22 (1), 1-11. (1997)

53. C. R. Plata-Salaman: Meal patterns in response to the intracerebroventricular administration of interleukin-1 beta in rats. *Physiol Behav*, 55 (4), 727-33 (1994)

54. R. M. Bluthe, R. Dantzer and K. W. Kelley: Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. *Brain Res*, 573 (2), 318-20. (1992)

55. K. Bendtzen, T. Mandrup-Poulsen, J. Nerup, J. H. Nielsen, C. A. Dinarello and M. Svenson: Cytotoxicity of human pI 7 interleukin-1 for pancreatic islets of Langerhans. *Science*, 232 (4757), 1545-7 (1986)

56. W. Somers, M. Stahl and J. S. Seehra: 1.9. A crystal structure of interleukin 6: implications for a novel mode of receptor dimerization and signaling. *Embo J*, 16 (5), 989-97 (1997)

57. T. Kishimoto, S. Akira, M. Narazaki and T. Taga: Interleukin-6 family of cytokines and gp130. *Blood*, 86 (4), 1243-54 (1995)

58. T. Kishimoto: Interleukin-6: from basic science to medicine--40 years in immunology. *Annu Rev Immunol*, 23, 1-21 (2005)

59. J. V. Castell, M. J. Gomez-Lechon, M. David, T. Hirano, T. Kishimoto and P. C. Heinrich: Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. *FEBS Lett*, 232 (2), 347-50 (1988)

60. Z. Zhong, Z. Wen and J. E. Darnell, Jr.: Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*, 264 (5155), 95-8 (1994)

61. R. M. Bluthe, B. Michaud, V. Poli and R. Dantzer: Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. *Physiol Behav*, 70 (3-4), 367-73 (2000)

62. F. De Benedetti, T. Alonzi, A. Moretta, D. Lazzaro, P. Costa, V. Poli, A. Martini, G. Ciliberto and E. Fattori: Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. *J Clin Invest*, 99 (4), 643-50 (1997)

63. J. J. Senn, P. J. Klover, I. A. Nowak and R. A. Mooney: Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes*, 51 (12), 3391-9 (2002)

64. J. H. Kim, J. E. Kim, H. Y. Liu, W. Cao and J. Chen: Regulation of IL-6 induced hepatic insulin resistance by mtor through the STAT3-SOCS3 pathway. *J Biol Chem* (2007)

65. V. Wallenius, K. Wallenius, B. Ahren, M. Rudling, H. Carlsten, S. L. Dickson, C. Ohlsson and J. O. Jansson: Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med*, 8 (1), 75-9 (2002)

66. K. Wallenius, V. Wallenius, D. Sunter, S. L. Dickson and J. O. Jansson: Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem Biophys Res Commun*, 293 (1), 560-5 (2002)

67. A. L. Carey, G. R. Steinberg, S. L. Macaulay, W. G. Thomas, A. G. Holmes, G. Ramm, O. Prelovsek, C. Hohnen-Behrens, M. J. Watt, D. E. James, B. E. Kemp, B. K. Pedersen and M. A. Febbraio: Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation *in vitro* via AMP-activated protein kinase. *Diabetes*, 55 (10), 2688-97 (2006)

68. T. Naka, M. Narazaki, M. Hirata, T. Matsumoto, S. Minamoto, A. Aono, N. Nishimoto, T. Kajita, T. Taga, K. Yoshizaki, S. Akira and T. Kishimoto: Structure and function of a new STAT-induced STAT inhibitor. *Nature*, 387 (6636), 924-9 (1997)

69. R. A. Mooney, J. Senn, S. Cameron, N. Inamdar, L. M. Boivin, Y. Shang and R. W. Furlanetto: Suppressors of cytokine signaling-1 and -6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. *J Biol Chem*, 276 (28), 25889-93 (2001)

70. J. C. O'Connor, C. L. Sherry, C. B. Guest and G. G. Freund: Type 2 diabetes impairs insulin receptor substrate-2-mediated phosphatidylinositol 3-kinase activity in primary macrophages to induce a state of cytokine resistance to IL-4 in association with overexpression of suppressor of cytokine signaling-3. *J Immunol*, 178 (11), 6886-93 (2007)

71. M. E. Hartman, J. C. O'Connor, J. P. Godbout, K. D. Minor, V. R. Mazzocco and G. G. Freund: Insulin receptor substrate-2-dependent interleukin-4 signaling in macrophages is impaired in two models of type 2 diabetes mellitus. *J Biol Chem*, 279 (27), 28045-50 (2004)

72. W. Ebstein: Invited comment on W. Ebstein: On the therapy of diabetes mellitus, in particular on the application of sodium salicylate. *J Mol Med*, 80 (10), 618; discussion 619 (2002)

73. S. E. Shoelson, J. Lee and A. B. Goldfine: Inflammation and insulin resistance. *J Clin Invest*, 116 (7), 1793-801 (2006)

74. P. Mohanty, A. Aljada, H. Ghanim, D. Hofmeyer, D. Tripathy, T. Syed, W. Al-Haddad, S. Dhindsa and P. Dandona: Evidence for a potent antiinflammatory effect of rosiglitazone. *J Clin Endocrinol Metab*, 89 (6), 2728-35 (2004)

75. P. M. Ridker, N. Rifai, M. Clearfield, J. R. Downs, S. E. Weis, J. S. Miles and A. M. Gotto, Jr.: Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med*, 344 (26), 1959-65 (2001)

76. Y. Hattori, K. Suzuki, S. Hattori and K. Kasai: Metformin inhibits cytokine-induced nuclear factor kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. *Hypertension*, 47 (6), 1183-8 (2006)

77. C. M. Larsen, M. Faulenbach, A. Vaag, A. Vølund, J. A. Ehses, B. Seifert, T. Mandrup-Poulsen, M. Y. Donath. Interleukin-1–Receptor Antagonist in Type 2 Diabetes Mellitus. *N Engl J Med*, 356 (15), 1517-26 (2007)

78. C. B. Guest, Y. Gao, J. C. O'connor, G. G. Freund: Obesity and Immunity. In (Ed.), R. Ader. Psychoneuroimmunology 4th Edition. Elsivier, Rochester, NY (2006)

Key Words: Diabetes, Inflammation, Cytokine, Macrophage, Insulin Resistance, Review

Send correspondence to: Professor Gregory G Freund, University of Illinois at Urbana-Champaign (UIUC), College of Medicine, 190 Medical Sciences Building, 506 South Mathews Avenue, Urbana, Illinois, 61801, Tel : 217-244-8839, Fax :217-244-5617, E-mail: freun@uiuc.edu