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The role of TNFα and TNF receptors in obesity and insulin resistance

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Insulin resistance, a smaller than expected response to a given dose of insulin, is associated with many common diseases including, ageing, polycystic ovarian disease, syndrome X, cancer, infections, trauma and, most significantly, obesity and type 2 diabetes mellitus. The biochemical basis of insulin resistance in type 2 diabetes has been the subject of many studies. Earlier studies have indicated that quantitative regulation of the insulin sensitive glucose transporters (Glut-4) and insulin receptors themselves may contribute to this disorder, however, these two factors are probably inadequate to explain the extent of insulin resistance. This point also became apparent by the development of only mild hyperinsulinaemia in mice with a targeted mutation in the Glut-4 gene. Studies on postreceptor defects in type 2 diabetes has recently focused on the intrinsic catalytic activity of the insulin receptor and downstream signalling events. A reduction in tyrosine phosphorylation of both the insulin receptor (IR) and the insulin receptor substrate-1 (IRS-1) has been noted in both animal and human type 2 diabetes. Importantly, this appears to occur in all of the major insulin-sensitive tissues, namely the muscle, fat and liver. It is now clear that decreased signalling capacity of the insulin receptor is an important component of this disease. I will review some of the potential mechanisms underlying this deficiency.

Keywords: genetics, insulin action, obesity, transgenic mice, type 2 diabetes.

Insulin resistance in obesity and type 2 diabetes and the role of TNFα

Since 70–80% of all type 2 diabetes patients are obese, a central question in understanding type 2 diabetes is how obesity can bring about resistance to insulin in the key tissues. It is clear that the molecular aetiology of insulin resistance will turn out to be multifactorial where numerous independent mechanisms contribute to the final phenotype. Several such molecular targets involved in the inhibition of insulin action have been characterized in recent years [1, 2, 3, 4, 5]. These include Rad, a potential signalling molecule which is overexpressed in the muscle tissues of patients with type 2 diabetes [6], PC-1, an inhibitor of the insulin receptor tyrosine kinase [7], leptin [8], fatty acids [9] and tumour necrosis factor-α (TNFα) [10]. This

review presents recent advances in understanding the mechanism(s) by which TNFα interferes with insulin action.

Virtually all animal models of obesity and insulin resistance examined to date seem to produce significantly higher levels of TNFα mRNA and protein compared to their lean counterparts [11–14]. Neutralization studies in obese rats with a soluble TNF receptor-IgG fusion protein administered parenterally or via gene transfer resulted in increased insulin sensitivity, demonstrating the involvement of abnormal TNFα production in the insulin resistance of obesity [10, 15]. Recent reports also demonstrated elevated TNFα mRNA and protein expression in human obesity [16–21]. The expression of TNFα was in strong positive correlation with the degree of obesity (body mass index, BMI) and the level of hyperinsulinaemia and in negative correlation with the adipose tissue lipoprotein lipase activity. Recently, inhibition of insulin action and insulin receptor signalling by TNFα was demonstrated in cultured human adipocytes [22]. These results indicated that the regulation and function of TNFα expression in human obesity is quite similar to that in rodent models. An initial study using a single dose of a neutralizing antibody has not yielded changes in insulin sensitivity in patients with established type 2 diabetes [23]. Hence, the resolution of the role of TNFα in human disease, and the modalities with which to test this are not fully understood.

Mechanisms of TNFα-induced insulin resistance

Treatment of insulin-responsive cells with TNFα can clearly alter the catalytic activity of the insulin receptor (IR). In a variety of cell types, including mouse and human adipocytes, fibroblasts, hepatoma cells and myeloid 32D cells, TNFα treatment leads to a reduction of insulin-stimulated IR autophosphorylation and subsequent inhibition of IRS-1 phosphorylation without effecting the number of receptors or their insulin binding capacity [22, 24–27]. A similar TNFα-mediated inhibition of the insulin-induced tyrosine phosphorylations are also observed in the muscle and fat tissues of the obese and insulin resistant *fa*/*fa* rats [11]. The actual defect induced by TNFα is likely to be at or near the insulin receptor itself. Partially purified receptors isolated from TNFα-treated adipocytes show reduced autophosphorylation and phosphorylation of exogenously added substrate [24]. This suggests that the insulin receptor itself is modified or TNFα promotes the production of an inhibitor of the receptor that is associated with these preparations.

Recent studies have shown that TNFα induces serine phosphorylation of IRS-1 in cultured adipocytes [26]and hepatoma cells [28] and this modified IRS-1 inhibits both IR autokinase and exokinase (measured using IRS-1 as a substrate) activity *in vitro* [26]. This effect is dependent upon IRS-1 serine phosphorylation, since enzymatic dephosphorylation of IRS-1 reduces its ability to inhibit the IR tyrosine kinase activity [26]. Myeloid 32D cells, which lack endogenous IRS-1, are resistant to the effect of TNFα on IR tyrosine phosphorylation [26]. When IRS-1 is expressed ectopically in these cells, insulin-stimulated IR autophosphorylation becomes very sensitive to TNFα, demonstrating that the presence of IRS-1 is necessary for the inhibition of the IR signalling by TNFα in intact cells [26]. In addition to the cultured cells, an inhibitory form of IRS-1 is also observed in muscle and fat tissues of obese *fa*/*fa* rats [26]. This inhibition is also reduced after enzymatic dephosphorylation of IRS-1. Although the exact mechanism by which TNFα induces IRS-1 phosphorylation is not clear, recent studies have suggested PKC isoforms as potential candidates [29, 30].

In hepatoma cells, alterations in the interaction of IRS-1 and insulin receptor have been demonstrated upon TNF treatment, providing further insight into both TNF-mediated blockade of insulin signalling and also the potential role of IRS-1 in this process [31]. The role of IRS-2 is more complicated. In contrast to IRS-1, IRS-2 appears not to play any role in the inhibition of IR signalling by TNFα in cultured white adipocytes [32] but plays an important role in other cell types such as cultured hepatoma cells and brown adipocytes [31, 33]. These results not only provided biochemical and genetic evidence for a novel mechanism by which TNFα induces insulin resistance but also demonstrated an unexpected role for IRS family in the attenuation of the IR signalling.

Genetic analysis of the role of TNFα in obesity and insulin resistance

To investigate the definitive role of TNFα in obesity-

induced insulin resistance, we have generated obese animals with no functional copy of the TNFα gene [34]. This was accomplished by placing mice homozygous for a targeted null mutation in the TNFα gene ($TNF\alpha^{-/-}$) and their control litter mates ($TNF\alpha^{+/+}$) on a high fat (50% of the total calories in the form of fat) and high caloric diet (5286 kcal kg^{-1}). On this diet, both $TNF\alpha^{-/-}$ and $TNF\alpha^{+/+}$ mice developed marked obesity as compared to mice kept on standard rodent diet. In both lean and obese animals (mice on standard diet and high fat diet, respectively), the total bodyweights of $TNF\alpha^{-/-}$ and $TNF\alpha^{+/+}$ mice were similar throughout the study, suggesting that the absence of TNFα did not have a significant affect on the development of dietary obesity [34].

Despite developing a similar degree of obesity, the $TNF\alpha^{-/-}$ mice remained highly insulin sensitive as compared to $TNF\alpha^{+/+}$ animals. For example, hyperinsulinaemia became apparent in obese $TNF\alpha^{+/+}$ mice 4 weeks after the start of the high fat diet and continued to increase in the following weeks. However, the insulin concentrations in obese $TNF\alpha^{-/-}$ mice were significantly lower than those of the obese $TNF\alpha^{+/+}$ animals and were indistinguishable from the lean mice, throughout the study [34]. Both insulin and glucose tolerance tests also revealed marked increase in insulin sensitivity in the obese $TNF\alpha^{-/-}$ mice compared to obese $TNF\alpha^{+/+}$ animals. These results clearly demonstrated that the genetic absence of TNFα can reduce development of insulin resistance associated with dietary obesity.

The role of the TNFα-activated pathway of insulin resistance was also tested in a more severe, genetic model of obesity by generating genetically obese mice (*ob*/*ob*) with targeted mutations in both p55 and p75 TNF receptors, effectively abolishing the TNFα signalling and function in these animals [34]. In this experiment the *ob*/*ob* animals developed early onset and severe obesity regardless of TNFR allele they carried. There was no significant difference in bodyweights between the obese animals lacking the TNF signalling (*ob*/*ob* -$p55^{-/-}$ $p75^{-/-}$) and obese control animals (*ob*/*ob*) [34]. Plasma glucose and insulin concentrations and glucose and insulin tolerance tests also revealed significantly increased insulin sensitivity in the *ob*/*ob* mice lacking TNFα signalling (*ob*/*ob* -$p55^{-/-}$ $p75^{-/-}$) compared to the *ob*/*ob* animals with functional TNF receptors [34]. Finally, using the individual receptor mutants, we have demonstrated that the p55 (TNFR1) TNF receptor was the dominant receptor involved in this action of TNFα [35]. This shows that interfering with TNFα signalling through null mutations in both TNF receptors results in a significant but incomplete protection from the insulin resistance associated with the *ob*/*ob* phenotype indicating the multifactorial aetiology of insulin resistance in obesity.

Recently, similar results were obtained in a third model of obesity induced by gold-thio-glucose (GTG) administration [36]. In an independently generated line of TNFα-deficient mice, the absence of TNFα has also resulted in significant but incomplete increase in insulin sensitivity in GTG-induced obesity [36]. Significant improvement was also noted in lipid metabolism of these animals [36].

Mechanism of TNFα-induced insulin resistance in obesity

The availability of obese mice which genetically lack TNFα function allowed definitive examination of the molecular parameters that might contribute to the understanding of TNFα-induced insulin resistance *in vivo*. Three potential sites of TNFα action that might mediate insulin resistance were examined in these animals; regulation of free fatty acid levels, leptin production, glucose transporter numbers and insulin receptor activity [34, 35, 37]. These studies demonstrated that the action of TNFα in obesity involves multiple mechanisms. First, the obese $TNF\alpha^{-/-}$ mice have lower free fatty acid levels compared to obese wild type animals [34]. This reduction in free fatty acids despite significant obesity might be the direct result of the loss of the lipolytic effects of TNFα in adipose tissue or alternatively, might reflect the increased efficiency of insulin to suppress lipolysis in the absence of TNFα. Secondly, the obese $TNF\alpha^{-/-}$ animals had higher levels of Glut-4 protein in their muscle tissues. This finding is interesting, as we did not observe a decrease in the levels of muscle Glut-4 in obese wild type animals. Therefore, the increased Glut-4 protein levels in $TNF\alpha^{-/-}$ animals was not reflective of a correction of the Glut-4 deficiency associated with obesity but rather represented an increase over the quantities observed in muscle

tissues of lean animals. These data raise the possibility that TNFα might be involved in the adaptive changes of muscle to obesity and/or insulin resistance by regulating Glut-4 protein levels in muscle tissue. Finally, the obese TNFα$^{-/-}$ animals were significantly spared from obesity-induced deficiencies in insulin receptor signalling in fat and muscle tissues. This observation probably represents the most significant effect of TNFα in the generation of obesity-induced insulin resistance due to the critical importance of insulin receptor signalling in the generation of the biological actions of insulin.

These recent studies with genetic ablation of function clearly demonstrated an important role for TNFα in the insulin resistance of obesity in at least three models of rodent obesity. Studies are currently ongoing in several laboratories examining additional models such as the KKA^{y} and *db* mutations and different genetic backgrounds to understand whether TNFα plays a role in other forms of obesity as well. These might have important implications in understanding and studying the potential role of TNF in human disease. If, for example, different models with different severity of the insulin-resistant or diabetic phenotype respond differently to the absence of TNFα, this might provide insights into the human populations that might benefit from potential anti-TNF treatment regimens.

In this regard, another important issue is the choice of treatment modalities. Neutralization attempts using antibodies against TNFα in obese rodent models including the *ob*/*ob* mice have generated inconsistent results. For example, administering a neutralizing antibody to TNFα did not generate any changes in insulin sensitivity of the *ob*/*ob* mice, whereas genetic absence of functional TNFα significantly improved insulin sensitivity of these animals [34, 35]. Studies employing short-term administration of neutralizing antibodies or soluble TNF receptors in a monotherapy format have not shown any effects of these treatments on the insulin sensitivity of obese individuals with insulin resistance and established diabetes [23]. Considered together, these observations might reflect the lack of effectiveness of these reagents or the duration of the treatments to block TNFα action, which primarily occurs in an autocrine/paracrine fashion. Therefore, long-term studies with alternative modalities might be necessary to definitively address the potential role of TNFα in human disease.

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