Molecular and Cellular Biochemistry **182:** 169–175, 1998.
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TNF-α and insulin resistance: Summary and future prospects

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Abstract

While the causes of obesity remain elusive, the relationship between obesity and insulin resistance is a well-established fact [1]. Insulin resistance is defined as a smaller than normal response to a certain dose of insulin, and contributes to several pathological problems of obese patients such as hyperlipidemia, arteriosclerosis and hypertension. Several pieces of evidence indicate that the cytokine tumor necrosis factor a (TNF-α) is an important player in the state of insulin resistance observed during obesity. In this review we will try to summarize what is known about the function of TNF-α in insulin resistance during obesity and how TNF-α interferes with insulin signaling. (Mol Cell Biochem **182**: 169–175, 1998)

Key words: insulin resistance, TNF-α, obesity, insulin signaling, adipocyte, IRS-1

TNF-α: the cytokine

TNF-α was originally identified as an endogenous factor, induced after inflammation or mitogenic stimulus, that kills tumor cells [2–4]. TNF-α is produced mainly by macrophages, but also by other cell types such as T cells and fibroblasts [5]. Roughly one third of tumor cells are sensitive to the cytotoxic effect of TNF-α while non-tumorogenic cells are generally resistant to TNF-α. This cytokine also induces several pathological states such as the endotoxic shock observed after infection by gram-negative bacteria, fever, anorexia etc. TNF-α is also known to induce the synthesis of other proinflammatory molecules such as interleukin 1 and 6, interferon γ, prostaglandines, PDGF [6, 7].

TNF-α is produced as a pre-protein of 26 kDa bound to the cell membrane, probably as a trimer [8]. This membrane-bound protein is active, but only as an autocrine and paracrine factor. A cleavage releases the circulating form of TNF-α, a homotrimer of 51 kDa. Metalloprotease inhibitors have been produced to inhibit the maturation of TNF-α and have been used successfully to protect mice against a lethal dose of endotoxin [9, 10].

TNF-α: the receptors

Two TNF receptors have been identified and named according to their molecular mass, p55TNFR (also called TNFR1) and p75TNFR (or TNFR2). Both receptors are expressed in virtually all tissues but with different ratios [7, 11]. TNF receptors are expressed as a monomer at the cell surface; the ligand-induced homotrimerisation of the receptor triggers TNF-α signaling. Both receptors can be found as soluble proteins after proteolytic cleavage. These soluble receptors can bind TNF-α and their concentration is increased during bacterial infection. The function of these soluble receptors is thought to modulate the available circulating concentration of TNF-α.

p55TNFR and p75TNFR are totally unrelated proteins outside their ligand binding domains, suggesting that they control different intracellular signaling events [12]. So far, p55TNFR seems to be responsible for the majority of the biological effects of TNF-α. However, p75TNFR does possess signaling mechanisms. Indeed, p75TNFR activates the transcription factor NF-κB, and under certain conditions can mediate cell death. Moreover, according to the ligand passing idea, p75TNFR can increase locally TNF-α

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concentrations. Indeed, p75TNFR binds TNF-α with a higher affinity and a faster dissociation rate than p55TNFR (Kd of 100 pM vs. 500 pM and $t_{1/2}$ of 10 min. versus 3 h). Therefore, at low TNF-α concentration, p75TNFR binds TNF-α but releases it quickly and makes it potentially available for p55TNFR [13].

TNF-α: the signaling

TNF receptors are devoid of any intrinsic catalytic activity, and are not coupled to GTP binding proteins. However, TNF receptors activate several signaling pathways, such as the MAP kinase cascade, protein kinase C activation, sphingomyelinase activities, etc. [7].

This last two years have provided us with several insights in the early biological events triggered by TNF-α. Using fusion proteins and two-hybrid screen several laboratories have isolated proteins through their ability to bind to the cytoplasmic domain of p55TNFR and p75TNFR (Fig. 1). Indeed, the early steps of TNF-α signaling are totally dependent upon protein-protein interactions, in the absence of any phosphorylation mechanisms. TRAF1 and TRAF2 (TNF receptor associated protein) form homo- or heterodimers, through their 'TRAF' domains and bind to p75TNFR [14]. Only TRAF2 seems to bind directly to p75TNFR upon TNF-α treatment. TRAFs are sequestered in the cytosol by the ITRAF (inhibitor of TRAF) that maintained the TRAFs in an inactive form (i.e. unable to bind p75TNFR). TRAF proteins are also associated to the cIAP (inhibitor of apoptosis) through a domain of TRAF different from the one binding the I-TRAF [15, 16]. TRAF2 appears to be important for p75TNFR mediated NF-κB activation, but the function of TRAF1, and cIAP is still unknown.

After binding of TNF-α to p55TNFR, p55 binds to TRADD (p55TNF receptor associated death domain) and FAN (Factor associated with N-SMase activation). FAN binds to the NSD (N-sphingomyelinase domain) domain of p55TNFR and is responsible for the activation of the neutral sphingomyelinase [17]. TRADD binds to the 'death domain' of p55TNFR and is itself associated with FADD (Fas associated death domain protein), TRAF-2 and RIP [18–21]. The N-terminal domain of FADD binds to the protease MACH (also called FLICK) which is proposed to be a direct activator of the proteolysis cascade responsible of apoptosis [22, 23]. RIP is a serine/threonine kinase which is also involved in cell death [20, 21]. Therefore TRADD controls two different pathways; apoptosis (through its association with FADD and RIP) and NF-κB activation (through its association with TRAF-2). However, several reports indicate that these two pathways are to some extent antagonistic. Indeed, the transcription factor NF-κB protects cells from apoptosis induced by TNF-α but also from stress such as ionizing radiation and IL-1 [24–27]. The cell-specific effect of TNF-α on cell growth or apoptosis is therefore possibly regulated by the amount of NF-κB activation versus RIP/FADD activation.

TNF-α and glucose homeostasis

Although the function of TNF-α in the development of insulin resistance during obesity has been indicated recently by experiments performed in our laboratory, several earlier observations suggested that TNF-α affects glucose and lipid metabolism. In rat and human, TNF-α injection induces an increase in the concentration of plasma triglyceride and very low density lipoproteins [28, 29]. This hyperlipidemia is thought to be due to an increase in hepatic lipogenesis and lypolysis. TNF-α and other cytokines (such as interleukin 1 and interferon γ), affect glucose homeostasis in several tissues mainly by increasing non insulin dependent glucose transport through synthesis of the glucose transporter Glut-1, and by decreasing insulin stimulated glucose transport [29, 30]. In adipocytes, high concentration of TNF-α decreases the expression of lipogenic enzymes and can also induce adipocytes dedifferentiation [31]. Finally, an insulin resistant state is observed during certain cancers, infections, and trauma, such as burn injuries, conditions in which high level of circulating TNF-α has been detected [32–34].

TNF-α production during obesity

The first evidence for a function of TNF-α in the state of insulin resistance observed during obesity was provided by the observation that adipocytes of obese animals overexpress TNF-α [35, 36]. This overexpression of TNF-α messenger was observed in multiple models of rodent obesity such as fa/fa rats and ob/ob, tub/tub, KKAY mice and in a strain of transgenic mice lacking brown adipose tissue that develops spontaneously obesity [37]. In these rodents an increase in TNF-α messenger in the adipose tissue, as well as an increase in circulating TNF-α was observed. However, circulating levels were low in absolute terms.

An increase in TNF-α messenger is also observed in adipocytes from obese humans. TNF-α expression in humans is in positive correlation with the degree of obesity (body mass index) and of hyperinsulinemia, and in negative correlation with lipoprotein lipase activity in the adipose tissue [38, 39]. Moreover, after a weight reduction program (which is known to improve insulin sensitivity), a decrease in the level of TNF-α expression was observed. TNF-α overexpression is not completely restricted to fat, since by using RT-PCR TNF-α was also found to be expressed in skeletal muscle and the heart, although at lower levels than

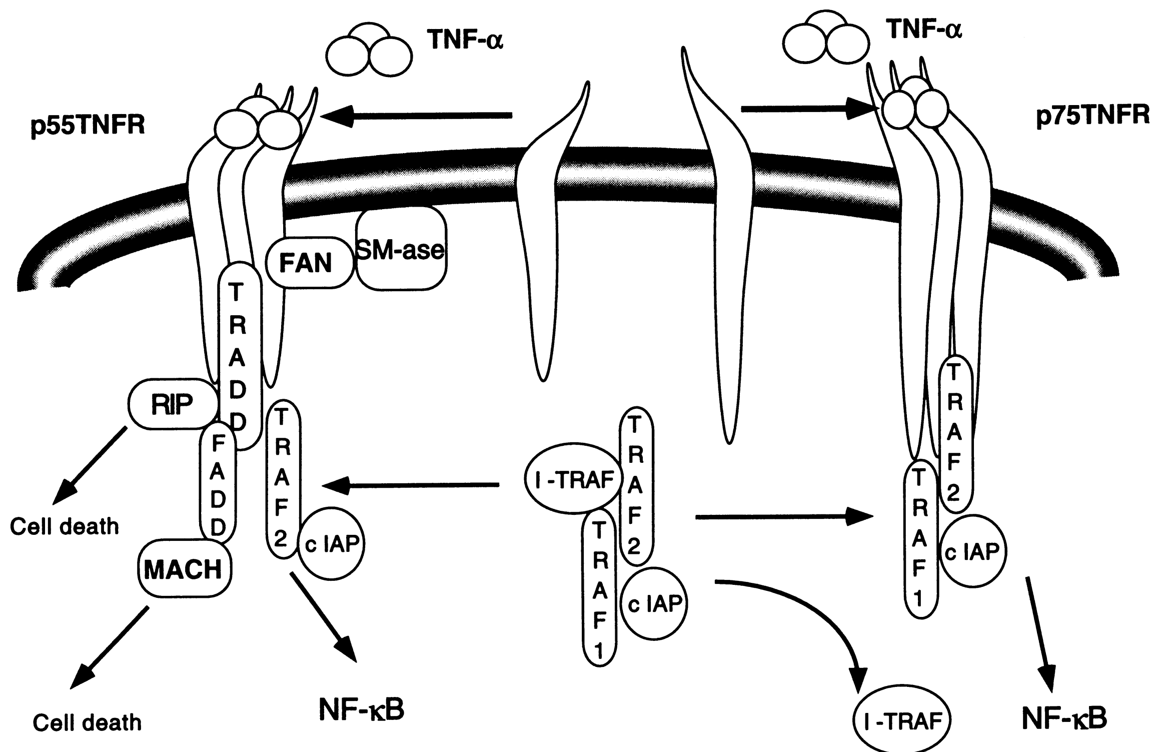

Fig. 1. TNF-α induces the trimerization of its receptors. This oligomerization induces the association of several intracellular proteins with the receptors and triggers TNF-α signaling.

those seen in adipocytes [40]. As muscle is the main post-prandial glucose utilization site, TNF-α expression in muscle could theoretically play an important role in the development of insulin resistance.

TNF-α and insulin resistance during obesity

Experiments neutralizing TNF-α (using immunoadhesins) in hyperinsulinemic, euglycemic clamps of obese animals have shown that this cytokine was causally involved in this syndrome of insulin resistance [35]. Indeed, fa/fa obese rats in which TNF-α is neutralized are more sensitive to insulin than untreated animals. This is due to a 2–3 fold increase in peripheric glucose uptake. On the other hand, hepatic glucose production was not affected. In non-clamped obese animals, three days of TNF-α neutralization results in decrease glucose, insulin and the circulating free fatty acid levels close to those observed in lean animals [41].

Additional evidence for a role of TNF-α in the development of insulin resistance has recently been provided by the study of the knock-out mice for the adipocyte-specific fatty acid binding protein (aP2). Indeed, these animals develop obesity as usual on a high fat diet, but they are the first known model in which obesity is not associated with development of insulin resistance or diabetes [42]. Interestingly, TNF-α overexpression in adipocytes was not observed, consistent with the notion that TNF-α has a function for the development of insulin resistance during obesity. Furthermore, these data prove that aP2 is linked to the state of insulin resistance observed during obesity, probably through the influence of fatty acid flux on the expression of TNF-α.

TNF-α and insulin signaling

Several laboratories have reported that the tyrosine kinase activity of the insulin receptor is decreased during obesity in muscle and fat. As the enzymatic activity of the insulin receptor is necessary for all of the known biological function of this hormone, this decrease is likely to be an important cause of the state of insulin resistance in obesity.

Neutralization of TNF-α in obese fa/fa rats restores the tyrosine kinase activity in fat and muscle, as well as the insulin-induced phosphorylation of IRS-1 to levels comparable to the ones observed in lean animals [41]. In lean animals, neutralization of TNF-α had no effect. These data suggest that TNF-α induces insulin resistance during obesity by interfering on the tyrosine kinase activity of the insulin receptor. Indeed, *in vitro* experiments have shown that

TNF-α treatment of adipocyte in culture induces a state of 'insulin resistance' [43]. This is due to an inhibition of the tyrosine kinase activity of the insulin receptor leading to a decrease to all the biological function of insulin such as the insulin-induced tyrosine phosphorylation of IRS-1 and glucose transport. While TNF-α induces a decrease of the expression of insulin receptor, IRS-1 and Glut-4 at high concentration [44], specific inhibition of the tyrosine kinase of the insulin receptor could be seen at concentration where insulin receptor, IRS-1, and the glucose transporter Glut-4 expression was unchanged. Since the concentration of circulating TNF-α during obesity are low (90 pg/ml in fa/fa rats, and undetectable in obese humans), it is likely that TNF-α induces insulin resistance by interfering with the tyrosine kinase of the insulin receptor and not by interfering with the expression of proteins, a process which need higher TNF-α concentration.

TNF-α-induced insulin receptor inhibition

The ability to inhibit insulin signaling by TNF-α in cell culture has facilitated the study of the mechanisms involved in this process. Several reports have shown that TNF-α interferes with insulin signaling in various cell lines such as hepatocytes, fibroblasts and myeloid cells [45–47].

Several steps of the signaling in the interaction between TNF receptors and insulin receptor have been elucidated (Fig. 2). IRS-1 appears to be a key molecule in this interaction. IRS-1 is one of the direct substrates of the insulin receptor and is necessary for several of the biological function of insulin [48]. The tyrosine phosphorylation of IRS-1 induce the binding of several SH2 domain containing proteins. These associations induce an activation of the protein (for example the PI3 kinase) or modify the compartmentalization of proteins, bringing them close to their substrates (for Grb2-Sos for example).

Treatment of adipocytes or hepatocytes with TNF-α induces an increase in the serine phosphorylation of IRS-1 [49, 50]. This phosphorylation is an important event since this modified form of IRS-1 act as an inhibitor of the insulin receptor *in vitro* [49]. This inhibition is dependent upon the phosphorylation of IRS-1 since dephosphorylation of IRS-1 causes it to lose its inhibitory activity. This mechanism probably happens in intact cells since TNF-α does not interfere with insulin receptor phosphorylation in myeloid cells that lack IRS-1 (32D cells). However, if IRS-1 is ectopically expressed in the same cells, insulin receptor and IRS-1 tyrosine phosphorylation become highly sensitive to TNF-α, indicating that IRS-1 is an important molecule in TNF-α-mediated inhibition of insulin-signaling. This mechanism may also be responsible for insulin resistance in obesity since IRS-1 from muscle and fat of obese animals is also an inhibitor of the insulin receptor tyrosine kinase activity. Hence, IRS-1 appears as a dual function protein involved in positive insulin signaling but also involved in a mechanism leading to the inhibition of insulin signaling.

The first steps of TNF-α signaling in insulin resistance have been resolved. It is mainly, if not entirely through stimulating p55TNFR that TNF-α inhibits insulin signaling in cell culture [46]. However, it is plausible that p75TNFR plays a role in obesity. Indeed, as previously described the concentration of circulating TNF-α during obesity are rather low, and one of the function of p75TNFR is to concentrate its ligand to make it available for p55TNFR by the ligand passing mechanism. Hence, although the signaling function of p75TNFR does not seem to be necessary for insulin resistance its 'concentrating' function could be important to allow p55TNFR to signal.

The p55TNFR stimulates a neutral sphingomyelinase which hydrolyzes sphingomyelin into ceramide and choline [51]. Ceramides activates directly several enzymes such as PKC-ζ [52, 53], a ceramide-activated kinase which phosphorylates and activates Raf-1 [54], and a ceramide-activated phosphatase which belongs to the phosphatase 2A family [55]. Synthetic cell permeant ceramides and exogenous sphingomyelinase mimic the effect of TNF-α on insulin signaling in cell culture [46]. Like TNF-α, these molecules convert IRS-1 into an inhibitor of insulin receptor, suggesting that stimulation of sphingomyelinase and production of ceramides are the first step of TNF-α signaling leading to insulin signaling inhibition.

The questions

A complete understanding of the function of TNF-α during obesity is an important task since TNF-α production and signaling could be a potential therapeutic target for the treatment of insulin resistance during obesity. Indeed, several questions remains to be addressed:

– What is the physiological component of obesity that triggers the production of TNF-α by adipocytes? TNF-α overexpression is observed in all the models of obesity, and in adipocytes (and muscles to a lower extent). Therefore is it unlikely that TNF-α overexpression is linked proximally to a particular genetic defect. It is more likely that a common modification of the hormonal or the metabolic balance in obesity is involved. As aP2 knock out mice develop obesity without insulin resistance and without TNF-α production, it is likely that the aP2 protein plays a role in TNF-α synthesis [42]. The only known function of the aP2 protein is to bind fatty acids, suggesting that fatty acids are good candidates to regulate the level of TNF-α during obesity.

– What is the tissue basis of TNF-α actions? Circulating concentration of TNF-α are very low. It is therefore unlikely that TNF-α could act as an endocrine factor in obesity. In

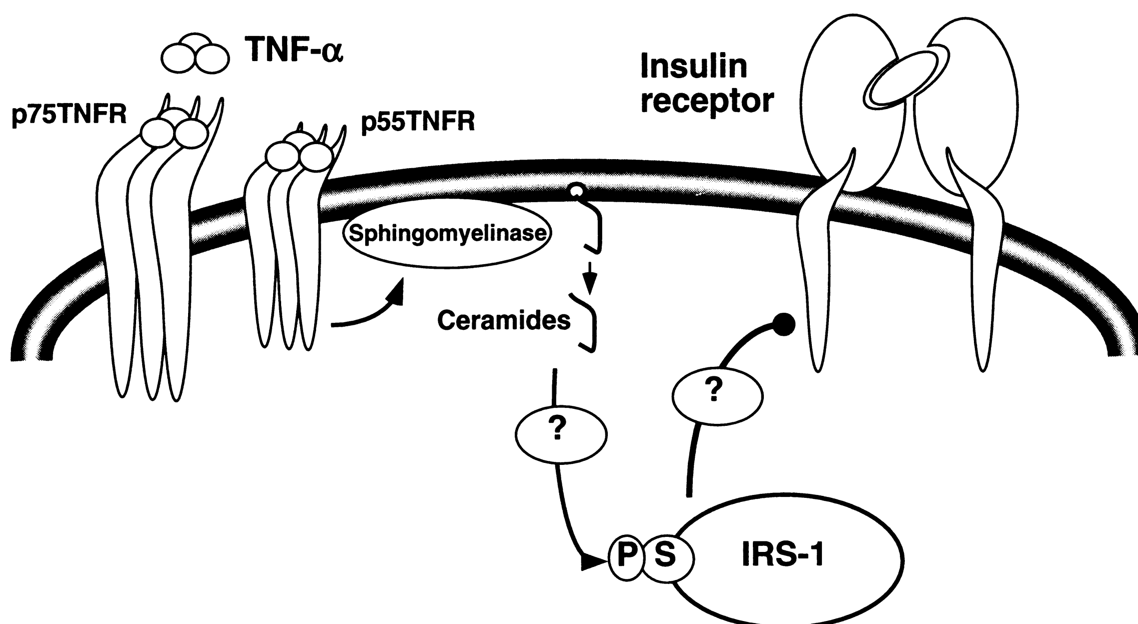

Fig. 2. By binding to p55 TNFR and activating sphingomyelinase (which produces ceramides) TNF-α increases IRS-1 serine phosphorylation. This phosphorylated IRS-1 act as an inhibitor of the insulin receptor by an unknown mechanism.

adipose tissue TNF-α could act as an autocrine and/or paracrine factor. The state of insulin resistance in muscle is probably due to a paracrine action of TNF-α provided by the fat, and/or an autocrine function of TNF-α produced by muscle itself. Of interest would be to treat animals with inhibitors of TNF-α processing. If the function of TNF-α is only autocrine these inhibitors could be without influence on insulin resistance, however if the function is paracrine the inhibitors should improve insulin sensitivity.

– By what mechanism(s) does serine-phosphorylated IRS-1 inhibit insulin receptor activity? Several hypotheses are possible. It is possible that the affinity of serine phosphorylated IRS-1 for insulin receptor is modified, and inhibits insulin receptor activity by steric hindrance. On the other hand, IRS-1 is known to be a docking protein. Therefore, it is conceivable that IRS-1 associates with an inhibitor of insulin receptor upon TNF-α treatment. This inhibitor (a tyrosine phosphatase for example) could be activated after IRS-1 binding, or physically brought to the insulin receptor by IRS-1. Of interest would also be to identify the kinase stimulated by TNF-α that phosphorylates IRS-1, since an inhibitor of this kinase could uncouple TNF-α binding from insulin resistance.

The future

If the observations performed in obese animals can be extended to human obesity, inhibition of TNF-α synthesis or signaling in adipocytes and muscle could provide a novel way to increase insulin sensitivity in the obese patient. So far only one report has been published concerning the neutralization of TNF-α in a population of obese patients and the results have been disappointing [56]. However, this study has been performed in a population of adults with established diabetes. These patients had hyperglycemia and elevated free fatty acid level, both being known to induce a state of insulin resistance by themselves [57, 58]. In addition, this population was not examined for the production of TNF-α. It has been shown that in very obese people TNF-α production tends to go down, the insulin resistance state being maintained in all likelihood by hyperglycemia and high free fatty acid level. Therefore it is difficult to draw any conclusion on the function of TNF-α in the development of insulin resistance based only on these data. Of interest will be to study the effect of TNF-α neutralization in a population of insulin resistant obese subjects, having not yet developed diabetes. To be fully efficient in humans the neutralization should happen in the adipose tissue, in order to inhibit the autocrine and paracrine effect of TNF-α production by the fat. Ideally, the molecule used should be relatively stable and should diffuse through the blood vessels to reach the adipocytes and/or muscle tissue. Theoretically, three strategies are possible: inhibiting the binding of TNF-α to p55TNFR, inhibiting TNF-α production in fat and interfering with TNF-α signaling. As previously described, the first strategy has been used in obese rats by neutralizing circulating TNF-α. However, it requires frequent injection of antibody and this

approach does not affect the autocrine and paracrine effect of TNF-α, which could have a more important function in obese humans who have lower circulating level of TNF-α. Theoretically, inhibition of ceramide production during obesity could reverse insulin resistance, however to our knowledge no specific inhibitors of sphingomyelinase have been produced. We still need a better understanding of the mechanisms of TNF-α production during obesity and the signaling pathways of TNF-α leading to insulin resistance to develop specific drugs. Neutralization of the effect of TNF-α could increase glucose tolerance of obese people and delay or even thwart the development of diabetes.

References

1. Sigal RJ, Warram JH: The interaction between obesity and diabetes. Curr Opin Endocrinol Diab 3: 3–9, 1996
2. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Dernick R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV: Human tumor necrosis factor: Precursor structure, expression and homology to lymphotoxin. Nature 312: 724–727, 1984
3. Gray PW, Aggarwal BB, Benton CV, Bringman TS, Henzel WJ, Jarret JA, Leung DW, Moffat BNP, Svedersky LP, Palladino MA, Nedwin GE: Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumor necrosis activity. Nature 312: 721–724, 1984
4. Fiers W: Tumor necrosis factor. FEBS Lett 285: 199–212, 1991
5. Vassalli P: The pathophysiology of tumor necrosis factors. Annu Rev Immunol 10: 411–452, 1992
6. Tracey KJ, Cerami A: Tumor necrosis factor, and other cytokines and disease. Annu Rev Cell Biol 9: 317–343, 1993
7. Vandenabeele P, Declercq W, Beyaert R, Fiers W: Two tumor necrosis factor receptors: Structure and function. Trends Cell Biol 5: 392–399, 1995
8. Kriegler M, Perez C, DeFay K, Albert I, Lu S: A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: Ramifications for the complex physiology of TNF. Cell 53: 45–53, 1988
9. Mohler K, Sleath P, Fitzner J, Cerretti D, Alderson M, Kerwar S, Torrance D, Otten-Evans C, Greenstreet T, Weerawarna K, Al E: Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. Nature 370: 218–220, 1994
10. Gearing A, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson A, Drummond A, Galloway W, Gilbert R, Gordon J, Al E: Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature 370: 555–558, 1994
11. Bazzoni F, Beutler B: How do tumor necrosis factor receptors work? J Inflam 45: 221–238, 1995
12. Tartaglia LA, Weber RF, Figari IS, Reynolds C, Palladino JMA, Goeddel DV: The two different receptors for tumor necrosis factor mediates distinct cellular responses. Proc Natl Acad Sci USA 88: 9292–9296, 1991
13. Tartaglia L, Pennica P, Goeddel D: Ligand passing: The 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem 268: 18542–18548, 1993
14. Rothe M, Wong SC, Henzel WJ, Goeddel DV: A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. Cell 78: 681–692, 1994
15. Rothe M, Pan M, Henzel W, Ayres T, Goeddel D: The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell 83: 1243–1252, 1995
16. Rothe M, Xiong J, Shu H-B, Williamson K, Goddard A, Goeddel DV: I-TRAF is a novel TRAF-interacting protein that regulates TRAF-mediated signal transduction. Proc Natl Acad Sci USA 93: 8241–8246, 1996
17. Adam-Klages S, Adam D, Wiegman K, Struve S, Kolanus W, Schneider-Mergener J, Kronke M: FAN, a novel WD-repeat protein, couples the p55TNF-receptor to neutral sphingomyelinase. Cell 86: 937–947, 1996
18. Hsu H, Xiong J, Goeddel D: The TNF receptor 1-associated protein TRADD signals death and NF-κB activation. Cell 81: 495–504, 1995
19. Hsu H, Shu HB, Pan MG, Goeddel DV: TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signaling transduction pathways. Cell 84: 299–308, 1996
20. Stanger B, Leder P, Lee T, Kim E, Seed B: RIP: A novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 81: 513–523, 1995
21. Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV: TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 4: 387–396, 1996
22. Boldin MP, Goncharov TM, Goltsev YV, Wallach D: Involvement of MACH, a novel MORT1/FADD-interacting protease, in FAS/APO-1 and TNF receptor induced cell death. Cell 85: 803–815, 1996
23. Muzio M, Chinnaiyan A, Kischkel F, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz J, Zhang M, Gentz R, Mann M, Krammer P, Peter M, Dixit V: FLICK, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell 85: 817–827, 1996
24. Wang C-Y, Mayo MW, Baldwin AS: TNF- and cancer therapy-induced apoptosis: potential by inhibition of NF-κB. Science 274: 784–786, 1996
25. Beg AA, Baltimore D: An essential role for NF-κB in preventing TNF-α-induced cell death. Science 274: 782–784, 1996
26. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM: Supression of TNFα-induced apoptosis by NF-κb. Science 274: 787–789, 1996
27. Liu Z-g, Hsu H, Goeddel DV, Karin M: Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. Cell 87: 565–576, 1996
28. Van Dongen CJ, Zwiers H, Gispen WH: Purification and partial characterization of the phosphatidylinositol 4-phosphate kinase from rat brain. Biochem J 223: 197–203, 1984
29. Lang CH, Dobrescu C, Bagby GJ: Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology 130: 43–52, 1992
30. Grunfeld C, Feingold K: The metabolic effect of tumor necrosis factor and other cytokines. Biotherapy 3: 143–158, 1991
31. Torti FM, Dieckmann B, Beutler B, Cerami A, Ringold GM: A macrophage factor inhibits adipocyte gene expression: An *in vitro* model for cachexia. Science 229: 867–869, 1985
32. Grunfeld C, Feingold K: Metabolic disturbance and wasting in the acquired immunodeficiency syndrome. N Engl J Med 327: 329–337, 1992
33. Copeland GP, Leinster SJ, Davis JC, Hipkin LJ: Insulin resistance in patient with colorectal cancer. Br J Surg 74: 1031–1035, 1987
34. Marano M, Moldawer L, Fong Y, Al E: cachectin/TNF production in experimental burns and pseudonomas infection. Arch Surg 123: 1383–1388, 1988
35. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. Science 259: 87–91, 1993
36. Hofmann C, Lorenz K, Braithwaite SS, Colca JR, Palazuk BJ, Hotamisligil GS, Spiegelman BM: Altered gene expression for tumor necrosis factor-α and its receptors during drug and dietary modulation of insulin resistance. Endocrinology 134: 264–270, 1994
37. Lowell BB, Susulic S, Hamann A, Lawitts JA, Himms H-J, Boyer BB, Kozak LP, Flyers JS: Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature 366: 740–742, 1993

38. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 95: 2409–2415, 1995
39. Kern P, Saghizadeh M, Ong J, Bosch R, Deem R, Simsolo R: The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95: 2111–2119, 1995
40. Saghizadeh M, Ong JM, Garvey TW, Henry R, Kern PA: The expression of TNF-α by human muscle. J Clin Invest 97: 1111–1116, 1996
41. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. J Clin Invest 94: 1543–1549, 1994
42. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM: Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 274: 1377–1379, 1996
43. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM: Tumor necrosis factor α inhibits signaling from the insulin receptor. Proc Natl Acad Sci 91: 4854–4858, 1994
44. Stephens J, Pekala P: Transcriptional repression of the C/EBP-alpha and GLUT4 genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. Regulations is coordinate and independent of protein synthesis. J Biol Chem 267: 13580–13584, 1992
45. Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A: Tumor necrosis factor-α suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. J Biol Chem 268: 26055–26058, 1993
46. Peraldi P, Hotamisligil GS, Buurman WA, White MF, Spiegelman BM: Tumor necrosis factor (TNF)-α inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. J Biol Chem 271: 13018–13022, 1996
47. Kroder G, Bossenmayer B, Kellerer M, Capp E, Stoyanov B, Muhlhofer A, Berti L, Horikoshi H, Ullrich A, Haring H: Tumor necrosis factor α and hyperglycemia-induced insulin resistance. J Clin Invest 97: 1471–1477, 1996
48. White MF, Kahn CR: The insulin signaling system. J Biol Chem 269: 1–4, 1994
49. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α-and obesity-induced insulin resistance. Science 271: 665–668, 1996
50. Kanety H, Feinstein R, Papa M, Hemi R, Karasik A: Tumor necrosis factor-α-induced phosphorylation of insulin receptor substrate-1 (IRS-1). J Biol Chem 270: 23780–23784, 1995
51. Kolesnick R, Golde D: The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 77: 325–328, 1994
52. Lozano J, Berra E, Municio M, Diaz-Meco M, Dominguez I, Sanz L, Moscat J: Protein kinase C isoform is critical for kappa B-dependent promoter activation by sphingomyelinase. J Biol Chem 269: 19200–19202, 1994
53. Muller G, Ayoub M, Storz P, Rennecke J, Fabbro D, Pfizenmaier K: PKC ζ is a molecular switch in signal transduction of TNF-α bifunctionally regulated by ceramide and arachidonic acid. EMBO J 14: 1156–1165, 1995
54. Yao B, Zhang Y, Delikat S, Mathias S, Basu S, Kolesnick R: Phosphorylation of Raf by ceramide-activated protein kinase. Nature 378: 307–310, 1995
55. Dobrowsky R, Hannun Y: Ceramide stimulates a cytosolic protein phosphatase. J Biol Chem 267: 5048–5051, 1992
56. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R: Effects of an engineered human anti-TNF-α antibody (CPD571) on insulin sensitivity and glycemic control in patients with NIDDM. Diabetes 45: 881–885, 1996
57. Boden G, Chen X, Ruiz J, White JV, Rosseti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 93: 2438–2446, 1994
58. Muller HK, Kellerer B, Ermel A, Muhlhofer B, Obermaier-Kusser B, Vogt B, Haring HC: Prevention by protein kinase C inhibitors of glucose-induced insulin receptor tyrosine kinase resistance in rat fat cells. Diabetes 40: 1440–1448, 1991