Interleukin-6 and Diabetes

The Good, the Bad, or the Indifferent?

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Inflammatory mechanisms play a key role in the pathogenesis of type 1 diabetes. Individuals who progress to type 2 diabetes display features of low-grade inflammation years in advance of disease onset. This low-grade inflammation has been proposed to be involved in the pathogenetic processes causing type 2 diabetes. Mediators of inflammation such as tumor necrosis factor-α, interleukin (IL)-1β, the IL-6 family of cytokines, IL-18, and certain chemokines have been proposed to be involved in the events causing both forms of diabetes. IL-6 has in addition to its immunoregulatory actions been proposed to affect glucose homeostasis and metabolism directly and indirectly by action on skeletal muscle cells, adipocytes, hepatocytes, pancreatic β-cells, and neuroendocrine cells. Here we argue that IL-6 action—in part regulated by variance in the IL-6 and IL-6α receptor genes—contributes to, but is probably neither necessary nor sufficient for, the development of both type 1 and type 2 diabetes. Thus, the two types of diabetes are also in this respect less apart than apparent. However, the mechanisms are not clear, and we therefore propose future directions for studies in this field. *Diabetes* 54 (Suppl. 2):S114–S124, 2005

Since ancient Greece, differences in lethality and distinct clinical features have led physicians to discern between two main diabetic phenotypes. But it has only been since 1974 that what we now know as type 1 and type 2 diabetes could be objectively distinguished by their different associations to genes in the major histocompatibility complex (1). In the past 30 years, much effort has been devoted to prove the nosological differences between the two disease entities in terms of environmental, genetic, and pathogenetic features. In the last decade, the interest for the role of inflammation in a wide range of diseases not commonly regarded as immune-mediated disorders, such as atherosclerosis and adiposity, has fostered studies that indicate that inflammatory mediators may not only be markers of metabolic aberrancies in type 2 diabetes, but may directly contribute to β-cell dysfunction and insulin resistance (2,3).

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CNS, central nervous system; ERK, extracellular signal-regulated kinase; IL, interleukin; IRS, insulin receptor substrate; JAK, Janus kinase; PMA, phorbol myristate acetate; SHP-2, *Src* homology 2–containing tyrosine phosphatase; SNP, single nucleotide polymorphism; SOCS, suppressors of cytokine signaling; STAT, signal transducers and activator of transcription.

Type 1 diabetes is characterized by a complete or near-complete insulin deficiency caused by an immune-mediated selective destruction of the insulin-producing β-cells in the islets of Langerhans. Type 1 diabetes can be considered an inflammatory disease of the pancreatic islets in which a process of programmed cell death (apoptosis) is elicited in the β-cells by interaction of activated T-cells and proinflammatory cytokines in the immune infiltrate (4). The immune-mediated β-cell destruction is thought to be initiated by interaction between yet unknown environmental factors and type 1 diabetes susceptibility gene variants (5).

Type 2 diabetes is characterized by the failure of the β-cells to compensate for peripheral insulin resistance (6). Within the last decade, an increasing body of evidence has accumulated in favor of a putative role of immuno-related mechanisms and factors in the pathogenesis of type 2 diabetes, both with regard to the progressive β-cell failure and destruction and to the peripheral insulin resistance (2,3).

Interleukin (IL)-6 is a pleiotropic cytokine with a key impact on both immunoregulation and nonimmune events in most cell types and tissues outside the immune system (7). A vast number of epidemiological, genetic, rodent, and human in vivo and in vitro studies have investigated the putative role of action/lack of action of IL-6 in the pathogeneses underlying obesity, insulin resistance, β-cell destruction, type 1 diabetes, and type 2 diabetes. These studies suggest both protective and pathogenetic actions of IL-6 in diabetes.

In this review, we briefly review the biology of IL-6 and critically evaluate the role(s) of the IL-6 system in the pathogeneses of type 1 and type 2 diabetes (but not in obesity without type 2 diabetes) and suggest directions for future investigations of IL-6 in pathogenetic processes leading to the two diseases.

BIOLOGY OF THE IL-6 SYSTEM

A complete review of the pleiotropic biological effects of IL-6 is beyond the scope of this review and has been the topic of a recent review (7). IL-6 belongs to the IL-6 family of cytokines, including IL-11, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor, cardiotrophin-1, and cardiotrophin-like cytokine. These cytokines are characterized by their common use of the gp130 (also known as IL-6Rβ or CD130) receptor as a signaling subunit. The two IL-6 receptors, gp130 and IL-6Rα (also known as gp80 or CD126), belong to the type I cytokine receptor family, which, in addition to the above cytokines, comprises leptin, growth hormone, prolactin, erythropoietin, thrombopoietin, and granulocyte- and granulocyte/macrophage-colony stimulating factors (7).

The genes encoding IL-6 and the IL-6α receptor. The human interleukin-6 gene (*IL6*, Online Mendelian Inheri-

tance in Man #147620) maps to chromosome 7p21. *IL6* has a high degree of sequence homology with the murine *Il6*, in particular, in regulatory proximal promoter sequences (8).

There are several polymorphisms in and close to *IL6* (8–10). Studies investigating the genetic association between *IL6* polymorphisms and disease—including type 1 diabetes, type 2 diabetes, insulin resistance, and other features of the metabolic syndrome—have mainly focused on the three common single nucleotide polymorphisms (SNPs) in the *IL6* promoter: the *IL6-174G>C*, *IL6-572A>G*, and *IL6-597A>G*. The *IL6-174G>C* promoter SNP, which has been suggested to functionally affect *IL6* promoter activity (10) (an issue discussed in further detail later), is a suitable haplotype marker for the common *IL6* promoter polymorphisms (9).

The human IL-6α receptor gene (*IL6R*, Online Mendelian Inheritance in Man #147880) maps to chromosome 1q21 in a region of replicated linkage to type 2 diabetes (11,12). The common genetic variants in *IL6R* have been identified recently, and a more general pattern of linkage disequilibrium of these variant needs to be established (11).

IL-6 and the IL-6Rα proteins. The human IL-6 protein comprises 212 amino acids with a signal peptide of 27 amino acids and two potential NH_2-linked glycosylation sites (7). The molecular weight ranges from 21 to 28 kDa.

The human IL-6Rα comprises 449 amino acids in its mature form but has only 82 amino acids in its cytoplasmic domain. IL-6Rα is found in a membrane-bound form and at least two soluble forms generated by proteolytic cleavage of the membrane-bound form or by alternative splicing.

IL-6 signaling and action. IL-6 induces signaling in all cells expressing the ubiquitous gp130 receptor. The IL-6Rα receptor subunit acts as an agonist in both its soluble and membrane-bound forms (7,13,14). Upon formation of the IL-6/IL-6Rα/gp130 hexameric signaling complex, two distinct signaling pathways are activated: *1*) Janus kinase (JAK)/signal transducers and activator of transcription (STAT) and *2*) the *Src* homology 2–containing tyrosine phosphatase (SHP-2)/extracellular signal–regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathways (Fig. 1).

The cellular response to IL-6 signaling depends on the signaling pathway that prevails in the individual cell type (7). The balance between the two pathways of signaling may further depend on the metabolic state of the cell and on the combination of other external stimuli (7). Thus, the biological outcome resulting from IL-6 exposure is complex and may result in a variety of physiological events such as cell proliferation, differentiation, survival, and apoptosis. For further details see the studies by Kamimura et al. (7) and Jones et al. (13).

Several cell types are reported to produce IL-6; these include most cells of the immune system, endothelial cells, skeletal and smooth muscle cells, adipocytes, islet β-cells, hepatocytes, microglial cells, astrocytes, and a number of other cell types (7). In contrast to IL-6, membrane-bound IL-6Rα is mainly found on hepatocytes, some endocrine cells such as pituitary and adrenal cortical cells, and leukocytes (7,13). As detailed above, membrane-bound IL-6Rα is not required for IL-6 signaling if sIL-6Rα (which is presumably mainly produced and released from hepatocytes and leukocytes and possibly by cells in the central nervous system [CNS]) complexed with IL-6 is available (7,13,14).

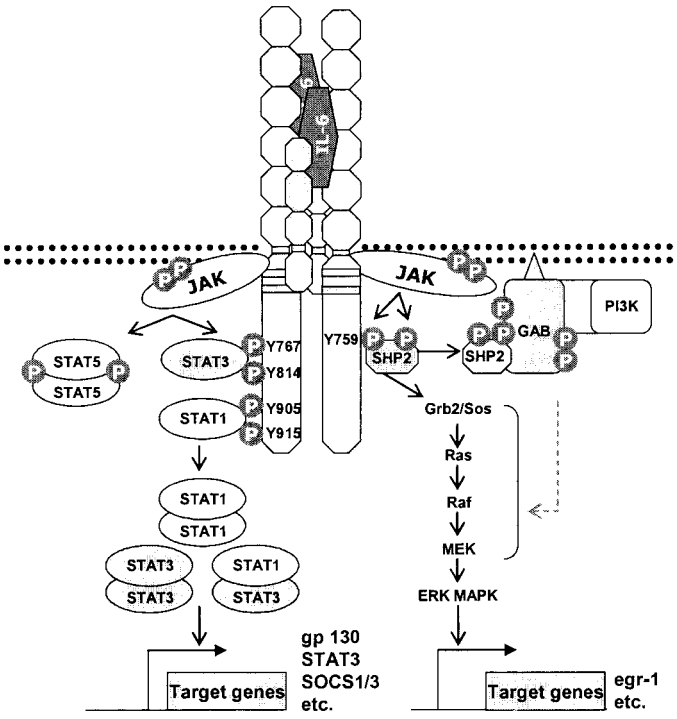

FIG. 1. The intracellular IL-6 signal transduction pathways. Modified from Kamimura et al. (7). The formation of the hexameric IL-6/IL-6Rα/gp130 complex initiates signal transduction by activating JAK kinases. Phosphorylated tyrosine residues (P) in gp130 are recognized by SHP-2 and STAT molecules, leading to generation of the two major gp130 signaling pathways: the Y759-derived SHP-2/ERK mitogen-activated protein kinase (MAPK) cascade (*right*) and the YXXQ-mediated STAT pathway (*left*). GAB, Grb-associated binder; PI3K, phosphatidylinositol 3-kinase.

IL-6 was initially thought to be a proinflammatory cytokine mainly with effects within the immune system, but this understanding of IL-6 was soon found to be too simplistic (7). In the adaptive and innate immune systems, IL-6 is involved in both amplification of and protection against inflammation (7,13). Thus, inappropriate regulation of IL-6 may play a direct protective or deleterious role in both antigen-specific immune-mediated diseases and in diseases where IL-6 or other inflammatory factors cause a low-grade inflammation (as seen in obesity and type 2 diabetes), which is likely to be involved in the pathogeneses of these diseases (2,3,7,13). IL-6–induced activation of the JAK/STAT pathway in both immune and non–immune-related cell types can, as illustrated in Fig. 1, lead to increased expression of suppressors of cytokine signaling (SOCS) proteins and in particular SOCS-3, which will exert negative feedback on JAK/STAT signaling.

GENETIC STUDIES OF *IL6* AND *IL1R* IN TYPE 1 AND TYPE 2 DIABETES

Genetic studies of a disease candidate protein-encoding gene may reveal whether genetic variance in the candidate gene confers susceptibility to the disease in question. Several genetic studies of *IL6* in relation to type 1 and type 2 diabetes and studies of *IL6R* in relation to type 2 diabetes have been reported.

Is the functional *IL6-174*G>C promoter SNP associated with type 1 diabetes, and how does it contribute to type 1 diabetes proneness? The *IL6* locus was not linked to type 1 diabetes in the human genome-wide scans (8). In contrast, the murine *Il6* maps to chromosome 5

TABLE 1
Studies of the *IL6−174G>C* SNP in type 1 diabetes

Population (ref. no.)	Design	Study cohort	Sex	Associated allele	*P*
UK (15)	C-C	257 type 1 diabetic/120 control	M/F	*IL6-174G*	0.0001
UK (15)	TDT	53 trios	M/F	None	NS
DK (8)	TDT	253 type 1 diabetic families*	M/F	*IL6-174C*	0.04
	TDT	165 trios	F	*IL6-174C*	0.0002
	TDT	168 trios	M	None	NS
NL (16)	TDT	206 trios	M/F	None	NA

C-C, case-control study; TDT, transmission disequilibrium testing, a family-based design. *150 type 1 diabetic sibpair families and 103 type 1 diabetic trio families.

(www.informatics.jax.org; Mouse Genome Informatics [MGI]: 96559) close to the NOD susceptibility locus *Idd15* (MGI: 99417), and *IL6* can therefore be considered a positional candidate gene.

We (8) and others (15,16) have evaluated the association of the *IL6-174G>C* SNP with type 1 diabetes with widely differing results (Table 1). Because all family-based studies (despite significant power) failed to confirm the initial observation of association in the U.K. case-control cohort (15), this association is most likely "spurious" (Table 1). The power of the Dutch study to confirm the observation of association in Danish patients of both sexes (8) is ~35%. Thus, the observation in the Danish type 1 diabetes families is favored as the most reliable (Table 1). We found highly significant linkage and association in females exclusively, with *IL6-174C* transmission rates of 63 and 66%, respectively (8). Sex-stratified analysis was not investigated (or at least not reported) in the U.K. and Dutch studies (Table 1).

In families with one *IL6-174CC* homozygous and one *IL6-174GC* heterozygous parent, a striking effect of the transmitted allele from the heterozygous parent was observed in female offspring in that *IL6-174C* and *IL6-174G* conferred risk and protection, respectively (8).

Studies have independently demonstrated that *IL6-174CC* females have younger age at onset than females with the *IL6-174G* allele and all males studied (8,17).

The *IL6-174G* allele has been found to cause higher *IL6* promoter activity in reporter assay studies (10) as well as serum IL-6 levels (10,18). The view of the *IL6-174G* allele as a "high producer–high promoter activity allele" has however been challenged (8,9,19,20 and studies referred to herein). Together, these studies imply that the *IL6-174G>C* SNP may well be "functional," but factors such as the *IL6* promoter haplotype, the age of the individual, the stimulus, the cell type, sex, the presence of sex steroids, and most likely several other factors may affect the activity of the two promoter variants.

Interestingly, in reporter assay studies of the two *IL6-174G>C* promoter variants, phorbol myristate acetate (PMA) failed to stimulate the *IL6-174G* promoter, and the stimulated activity of the *IL6-174C* promoter exceeded that of the *IL6-174G* promoter by ~70% in the absence of 17β-estradiol (8). The inability of PMA to stimulate the *IL6-174G* promoter was reverted by 17β-estradiol preincubation, whereas the PMA-stimulated activity of the *IL6-174C* promoter variant was unaffected by 17β-estradiol. This suggests that in 17β-estradiol–free settings (as in the period before female puberty), the *IL6-174C* promoter activity exceeds that of the *IL6-174G* promoter, whereas after the menarche, the stimulated activities of two promoter variants are identical in females. We currently have no explanation for the fact that the estradiol-free effect on the *IL6-174G>C* promoters is not seen in males; other sex-specific differences may dominate. The SNP maps to a negative regulatory domain in the *IL6* promoter (10). This site, however, has not been directly implicated in the 17β-estradiol regulation of *IL6* promoter activity, which is most likely mediated through a direct binding of nuclear factor (NF)-IL6 and NF-κB to the human estrogen receptor (21). Interestingly, in vitro exposure of lymphocytes and monocytes to PMA stimulates and inhibits *IL6* expression, respectively, indicating cell type–dependent regulation (8). To fully appreciate the functional impact of 17β-estradiol on the activity of the two *IL6* promoter variants, experiments investigating the impact of sex steroids and more physiological stimuli should be pursued in specific cell types such as dendritic cells, macrophages, T-cell (subsets), endothelial cells, and β-cells.

In conclusion, studies on the association between IL-6 gene variants and type 1 diabetes have been contradictory. High-powered population-based transmission disequilibrium testing studies show no or weak overall linkage, and stratification analysis has demonstrated a strong linkage with *IL6-174CC* that confers risk for early onset of type 1 diabetes in females. Functional analysis has shown a high IL-6 promoter activity, which is negated by 17β-estradiol. Taken together, these data suggest that *IL6-174CC* confers risk in young prepubertal females via high IL-6 synthesis. Whether the mechanism is induction of insulin resistance, inhibition of β-cell function, or both remains to be demonstrated.

Genetic studies of *IL6* and *IL6R* in type 2 diabetes. The *IL6* locus has not been linked to type 2 diabetes in genome-wide scans for type 2 diabetes susceptibility loci, whereas the *IL6R* gene maps to a region of repeated linkage to type 2 diabetes (11,12). Here, we focus on studies of the association between genetic variants in *IL6* and *IL6R* and type 2 diabetes and to studies evaluating *IL6* and *IL6R* variants in relation to insulin resistance (11,12,19,22–29) (Table 2).

***IL6* and type 2 diabetes.** Because the *IL6-174G>C* SNP has been investigated in all studies and is in linkage disequilibrium with the other *IL6* promoter polymorphisms, we will focus on the SNP. The *IL6-174G* allele is associated with type 2 diabetes in the majority of studies investigating this SNP in populations informative for the SNP (Table 2). Surprisingly, an independent association was not found in the largest of these studies (24). However, the *IL6-174G* allele was part of the haplotype that is associated with type 2 diabetes (Table 2). Moreover, the haplotype was comprised of a rare composite genotype, AGC/GCG, conferring modest (odds ratio ~1.7) but highly significant risk to type 2 diabetes (24). The *IL6-174G* allele is strongly associated with risk (odds ratio with each

TABLE 2
Genetic studies of *IL6* and *IL6R* in type 2 diabetes and insulin resistance

Association	Population (ref. no.)	Gene	SNP	Associated allele/ genotype	Comment	Power*
Type 2 diabetes	Native Americans (22)	*IL6*	*−174G>C*	G/GG	Pimas are GG heterozygous	1
Type 2 diabetes	Spanish Caucasians (22)	*IL6*	*−174G>C*	G/GG		1
Type 2 diabetes	U.K. Caucasians (23)	*IL6*	*−174G>C*	G	Only males included	3
Type 2 diabetes	Germans (19)	*IL6*	*−174G>C*	G	Association only significant in males and lean subjects	2
Type 2 diabetes		*IL6*	*−597G>A*	G		2
Type 2 diabetes	Danes (24)	*IL6*	*−174G>C*	None		3
Type 2 diabetes		*IL6*	*−572G>C*	GG	SNP not in Hardy-Weinberg equilibrium in control group	3
Type 2 diabetes		*IL6*	*−597G>A*	None		3
Type 2 diabetes		*IL6*	Haplotype	GCG AGC/GCG	Haplotype of *−597*, *−572*, and *−174* SNPs, trend (P = 0.06) of association for *GCG*	3
Conversion from impaired glucose tolerance to type 2 diabetes	Finns (25)	*IL6*	*−174G>C*	CC	Only in combination with *TNFA-308* A allele carriers	2
Prospective risk of developing type 2 diabetes	Germans (26)	*IL6*	*−174G>C*	CC	In individuals with BMI >28 kg/m^2	2
Type 2 diabetes	Pimas (27)	*IL6R*	*rs8192284*	None		1
Type 2 diabetes	Utah Caucasians (11)	*IL6R*	*rs8192284*	Asp358	Trend (P = 0.053)	1
Type 2 diabetes	African Americans (11)	*IL6R*	*rs8192284*	None		1
Type 2 diabetes	Danes (12)	*IL6R*	*rs8192284*	Asp358		3
Type 2 diabetes	African Americans (11)	*IL6R*	*rs2229238*	385Ile		
High insulin sensitivity	Catalonians (28)	*IL6*	*−174G>C*	CC		1
Low insulin sensitivity	Finns (29)	*IL6*	*−174G>C*	CC		3
Low insulin sensitivity	Germans (19)	*IL6*	*−174G>C*	None		2
Low insulin sensitivity	Danes (24)	*IL6*	*−174G>C*	G		3

*The reports were ranked based on the number of investigated individuals and thus the power of the study: 1 = low, 2 = intermediate, or 3 = high power. Only studies evaluating insulin resistance using minimal model measures or the euglycemic-hyperinsulinemic clamp were included, since studies using less precise determinations of insulin resistance may be flawed. Studies investigating obesity alone contain no comments. Not all genetic variants investigated are commented on if considered noninformative in the context or no association was established.

additional G allele ~18) of type 2 diabetes in non-Pima Native Americans (22).

The two studies of prospective risk of converting from impaired glucose tolerance to type 2 diabetes in Germans and Finns, respectively (Table 2), are in striking contrast to the cross-sectional genetic studies. There is no simple genetic explanation for the observed discrepancies.

In summary, studies investigating the association between the IL-6 gene variants and type 2 diabetes have been contradictory. Of note, no overall association was found between *−174G>C* and type 2 diabetes in high-powered studies (24). *−174C* was associated with prospective risk of developing type 2 diabetes in subsets (19,29). Whether this association can be explained by high *IL6* promoter activity causing insulin resistance/β-cell failure in combination with other type 2 diabetes–predisposing genes remains to be clarified. *−174G* was associated with features of the metabolic syndrome in glucose-tolerant subjects (24). It is unclear if this association can be explained by low *IL6* promoter activity in analogy to *IL6*$^{-/-}$ mice that develop maturity-onset obesity probably due to CNS effects on appetite and regulation of energy expenditure (19,23,24 and below).

***IL6R* and type 2 diabetes.** Genetic variants in the *IL6R* have been evaluated in four independent populations. The main focus has been two nonsynonymous exon 9 SNPs: *IL6R+48867C>A* (*rs8192284*) and *IL6R+48947G>T* (*rs2228146*) leading to an Asp358Ala and a Val385Ile polymorphism, respectively, in the mature *IL6Rα* (Table 2) (11,12,27). The latter SNP—only reported in individuals of African ancestry (11)—and other *IL6R* variants evaluated in relation to type 2 diabetes (11,27) are not discussed further.

The common Asp358 variant was associated with type 2 diabetes in Danes, and it conferred a modest (odds ratio ~1.3) but significant risk to type 2 diabetes in this population (12). This observation is supported by the finding of a trend for association of this variant in Utah Caucasians of Northern European ancestry (Table 2) (11). The Asp358Ala polymorphism was not found to associate with type 2 diabetes in Pimas (27) and African Americans (11), but the power of these studies was low (Table 2).

Thus, there is some evidence pointing to a role of the IL-6Rα Asp358 polymorphism in conferring risk to type 2 diabetes, at least in individuals of European ancestry, but the pathogenetic mechanism is not known. Obesity and/or

TABLE 3
Does IL-6 play a role in β-cell destruction and apoptosis?

Setting	For an impact of IL-6 on β-cells (ref. no.)	Against an impact of IL-6 on β-cells (ref. no.)
In vitro		IL-6 alone is not cytotoxic to rat and human islets/β-cells (31–35).
	IL-6 potentiates IL-1–induced NO synthesis in rat islets (34).	IL-6 does not potentiate cytokine-induced NO synthesis in human islets (33).
		IL-6 and dexamethasone induces Reg gene expression in RIN-m5F cells (36).
		IL-6 protects mouse pancreatic islets and β-cells from inflammatory cytokine-induced cell death and functional impairment both in vitro and in vivo (37).
In vivo human autopsies	The HIP/PAP is expressed in islets of a new-onset type 1 diabetic patient and is released from healthy cadaveric human islets upon IL-6 treatment. HIP/PAP acts as a T-cell autoantigen in NOD mice (38).	IL-6 islet expression does not correlate with insulitis/β-cell destruction in humans (35).
Rodent insulitis	IL-6 islet expression correlates with insulitis/β-cell destruction in NOD mice (35), and islet IL-6 expression is higher in NOD females than in males (39).	
Il6-Tg under the control of the insulin promoter	β-Cell–specific overexpression of *Il6* promotes islet inflammation in the NOD mouse (40) and in a non–diabetes-prone mouse strain (41).	IL-6 delays overt diabetes development in NOD mice (40) and the nondiabetic strain does not develop diabetes (41).
Il6-Tg		*Il6-Tg* non–diabetes-prone mice do not develop autoimmune diabetes (8).
Double *Il6-Il6Rα-Tg*		Double *Il6-Il6Rα-Tg* non–diabetes-prone mice do not develop autoimmune diabetes (42).

HIP/PAP, hepatocarcinoma-intestine-pancreas/pancreatic-associated protein; Tg, transgenic.

insulin resistance do not seem to be the explanation; the Ala358 variant is associated with increased BMI in Pimas (27) but not in Europeans and African Americans (11,12). Further, the Ala358 polymorphism does not affect insulin sensitivity in Caucasians of European ancestry (11,12).

Point mutations involving codon 358 do not affect expression of the membrane-bound IL-6Rα, but the Asp358 polymorphism may affect the levels of sIL-6Rα as the IL-6Rα is shedded by proteolytic cleavage in the Gln357/Asp358 juncture (13). Substitution of aspartate with glycine at amino acid position 358 reduces shedding to ~25%, and Ala357/Ala358 double-point mutations attenuates shedding by 66% in vitro. Thus, carriers of the Ala358 allele may have reduced circulating levels of sIL-6Rα due to impaired cleavage, and this may in turn result in reduced IL-6 signaling and diminished risk of type 2 diabetes, although the mechanism by which impaired IL-6 signaling diminishes risk of type 2 diabetes is still unknown.

THE IMPACT OF IL-6 ON ISLET INFLAMMATION AND β-CELL APOPTOSIS

A common feature of the two types of diabetes is a relative or absolute lack of insulin production to maintain normal glucose homeostasis. In type 1 diabetes, there is a total or almost total destruction of the β-cells (5), whereas type 2 diabetes is characterized by progressive β-cell failure and by a relatively reduced β-cell mass due to increased apoptosis (30).

Does IL-6 contribute to β-cell destruction in type 1 diabetes? The role of IL-6 in human and rodent type 1 diabetes is debated, and direct evidence for a deleterious role of IL-6 in the pathogenesis of type 1 diabetes arising from human studies is lacking. The action of IL-6 in type 1 diabetes pathogenesis may in principle be exerted at the level of the target β-cell, and/or at immune regulation, and/or at the hypothalamic-pituitary-adrenal axis. Several investigations of the putative impact of IL-6 on type 1 diabetes have been reported (8,31–42) (Table 3).

An in vitro–independent cytotoxic effect of IL-6 on β-cells has not been demonstrated, and one study indicated a protective effect in mouse islets and in a mouse β-cell line (Table 3). IL-6 may synergize with other proinflammatory cytokines in islets of some species (34). IL-6 can be produced by β-cells in vivo (43), but the implications of this phenomenon in β-cell destruction or protection remains to be elucidated. (T-cell activation? Autocrine synergy with proinflammatory cytokines?)

IL-6 is present in infiltrated islets both before and during immune infiltration in NOD mice. The observation of higher islet IL-6 expression in NOD females than in males supports a deleterious effect of IL-6 (Table 3). In humans, there is little evidence for a role of IL-6 from human autopsies of recent-onset type 1 diabetic patients (Table 3). Taken together, the in vivo data on spontaneous autoimmune diabetes in NOD mice suggest that IL-6 may play a pathogenetic role at the level of islet inflammation close to the target β-cell, but IL-6 alone is unable to induce or promote β-cell destruction; additional factors are needed, such as other proinflammatory cytokines.

β-cell specific overexpression of IL-6 promotes islet inflammation but is insufficient to precipitate diabetes in nondiabetic strains and in fact delays overt diabetes development in NOD mice (Table 3). Hence, additional factors are apparently needed for precipitation of diabe-

TABLE 4
In vitro IL-6 effects on islet insulin production

Ref. no.	Species	IL-6 concentration	Time of incubation (h)	Medium insulin accumulation	Insulin release (glucose concentration [mmol/l])
32	Rat	500–2,000 ng/ml	48	↑	→ (1.67), ↓ (16.7)
		5,000 ng/ml	48	→	→ (1.67), ↓ (16.7)
31	Rat	0.1 pmol/l to 1.0 nmol/l	0.5	NA	→ (8)
		0.1 pmol/l	24	NA	→ (2), → (20)
		10 pmol/l	24	NA	→ (2), ↓ (20)
		1 nmol/l	24	NA	↑ (2), ↓ (20)
34	Rat	10 nmol/l	2	→	↓ (16.7)
47	Mouse	500–5,000 units/ml	24–72	NA	↑ (NA)
37	Mouse	400 ng/ml	24	NA	→ (2), → (20)
33	Human	125 ng/ml	48	→	→ (1.67), → (16.7)

NA, not available. →, unchanged; ↑, increased; ↓, decreased.

tes. These studies argue against a direct deleterious effect of IL-6 alone in vivo and are supported by the observations from studies of universal overexpression of the *Il6* gene in *Il6-Tg* mice and in double *Il6-Il6Rα-Tg* mice (Table 3). Surprisingly, studies of *Il6* knockout NOD mice and studies of anti–IL-6 treatment in spontaneously diabetic mouse strains have not been reported.

In conclusion, there is little evidence for an essential biological role of IL-6 in type 1 diabetes, although IL-6 may potentiate other pathogenetic mechanisms.

IL-6 and β-cell apoptosis in type 1 and type 2 diabetes. Apoptosis is the main cause of cell death in type 1 diabetes (4) and of reduced β-cell mass in type 2 diabetes (30). In vitro studies of islets and β-cells have not demonstrated an apoptotic effect of IL-6 alone (Table 3). A possible in vitro antiapoptotic effect of IL-6 on islets and a β-cell line exposed to inflammatory cytokines was even suggested, and IL-6 increased transcriptional activities of genes encoding mainly proapoptotic, although also some antiapoptotic, molecules in islets and β-cells (37). Increased apoptosis was not observed in transgenic mice expressing IL-6 in islets (40,41). Hence, there is no evidence for IL-6 as a required factor in β-cell apoptosis in vivo or ex vivo.

LOW-GRADE INFLAMMATION AND IL-6 AS FACTORS IN TYPE 2 DIABETES PATHOGENESIS

Low-grade inflammation has been shown to precede and be a risk factor of future development of type 2 diabetes, and lifestyle modifications and medical treatment lowering the inflammatory state reduce risk of future development of type 2 diabetes (2,3), suggesting that inflammation may play a role in the pathogenesis of type 2 diabetes. The mechanism(s) by which low-grade inflammation is involved in precipitating type 2 diabetes is not conclusively clarified.

Elevated levels of IL-6 predict future risk of type 2 diabetes development (44–46). C-reactive protein, however, seems to be a stronger predictor than IL-6 in two of the studies investigating both parameters (45,46). These studies show association but not causation. The association between IL-6 and progression to type 2 diabetes development may merely reflect an attempt to counterregulate low-grade inflammation induced by other inflammatory mediators.

IL-6 AND INSULIN PRODUCTION AND SECRETION

In addition to a decreased β-cell mass due to increased apoptosis, as described above, insulin production and secretion are impaired in type 2 diabetes (6). In vitro studies investigating the impact of IL-6 treatment on pancreatic islets have not demonstrated a consistent effect of IL-6 on insulin production and release, but most studies show that IL-6 inhibits glucose-stimulated insulin secretion from rodent islets (31–34,37,47) (Table 4). β-Cell function in human islets is not independently affected by IL-6 (33).

Impaired β-cell function in *Il6Tg* mice has not been reported, and $Il6^{-/-}$ mice have identical fasting insulin levels when compared with pair-fed $Il6^{+/+}$ littermates (48), arguing against an independent in vivo effect of IL-6 on β-cell function. The initial report of disturbed glucose metabolism and obesity in the $Il6^{-/-}$ mouse, suggesting a protective role of IL-6 in obesity and disturbed glucose metabolism, did not investigate insulin production, and the control group was not pair-fed (49).

In healthy males challenged with IL-6 (50,51), there is no significant effects on serum insulin levels compared with control subjects. Thus, an acute increase in IL-6 does not alone seem to affect β-cell function in humans. This finding is supported by studies in rodents (52).

Despite the inconsistency of the in vitro data, these studies do not exclude that IL-6 may affect insulin release under certain conditions such as high IL-6 levels, high glucose concentrations, or in synergy with other inflammatory mediators. Studies investigating the effect of long-term low-grade inflammation (i.e., slightly increased IL-6 serum levels) on β-cell function are warranted for the evaluation of the role of IL-6 both in β-cell function and apoptosis proneness.

ROLE OF IL-6 IN INSULIN RESISTANCE

The role of IL-6 in insulin resistance is controversial (53). In an attempt to clarify the current standing, we will evaluate the role of IL-6 on insulin action in the three main cell types involved in peripheral insulin resistance and glucose homeostasis, i.e., adipocytes, skeletal muscle cells, and hepatocytes.

Does IL-6 cause insulin resistance in adipocytes? IL-6 is expressed in and released from both the subcutaneous and the omental adipose tissues (54,55), with a two- to threefold higher in vitro release of IL-6 from omental compared with subcutaneous adipocytes in vitro (55). The

TABLE 5
In vitro effects of IL-6 on insulin resistance in adipocyte cell lines

Effect of IL-6 on insulin sensitivity (ref. no.)	Negative effect on insulin sensitivity
Short-term IL-6 treatment enhances glucose transport, and the effect is additive to insulin-stimulated glucose transport (59).*	No
IL-6 decreases IRS-1 protein expression and insulin-stimulated tyrosine phosphorylation and reduces insulin-stimulated glucose transport (60).*	Yes
IL-6 decreases protein expression of the IR-β subunit and IRS-1 and inhibits insulin-induced activation of IR-β, protein kinase B, and ERK-1/2. It further suppresses insulin-induced lipogenesis and glucose transport by reducing expression of *GLUT4* (61).*†	Yes
IL-6 induces the expression of SOCS-3 (61,62).*†	Yes
IL-6 decreases adiponectin gene expression and secretion in a dose- and time-dependent manner (63).*	Yes

Investigated cell lines: *3T3-L1 adipocytes, †3T3-F442A adipocytes. Protein kinase B is also known as Akt. IR, insulin receptor.

in vivo release of IL-6 from fat contributes as much as 35% to the basal circulating levels (54) and may at least in part explain the positive correlation between serum levels of IL-6 and obesity (56,57). In rodents, obesity is associated with macrophage accumulation (denoted "adipositis") in adipose tissue (58), and these macrophages release inflammatory mediators and molecules promoting inflammation. *IL6* is expressed in macrophages but more so in adipocytes (58). *IL6* expression is thought to be stimulated in a paracrine fashion by proinflammatory mediators released in the tissue, but whether IL-6 acts as a pro- or anti-inflammatory molecule in fat is unclear.

Does chronic or acute IL-6 exposure cause insulin resistance in adipocytes and, if so, by which mechanism? There is a striking paucity of human and animal studies investigating the effect of acute and long-term IL-6 exposure on insulin signaling in adipocytes. In vitro studies on adipocyte cell lines (59–63) are summarized in Table 5. Collectively, the studies in Table 5 strongly point to a role of IL-6 as causing insulin resistance in adipocyte cell lines. These observations need confirmation in primary adipocytes.

IL-6 infusion in a physiological concentration increases subcutaneous adipose tissue glucose uptake in humans (64), arguing against IL-6 as an insulin resistance–inducing agent in adipocytes (59), but independent confirmation is warranted.

It is of interest to note that SOCS-3 expression is increased in adipocyte cell lines exposed to IL-6 in vitro (61,62) and in adipose tissue of obese insulin-resistant nondiabetic individuals and that SOCS-3 expression is strongly correlated with IL-6 expression in vivo (65). SOCS-3 is also induced in adipocytes upon insulin stimulation and functions as a negative regulator of insulin signaling by binding to and ubiquitinating insulin receptor substrate (IRS)-1 and -2, thereby targeting these important signaling intermediates for proteasomal degradation (66).

In recent studies (53,67), adipose tissue *IL6* mRNA has been shown to be elevated in insulin-resistant humans, and the elevated *IL6* mRNA levels correlated with reduced rates of insulin-stimulated glucose disposal (68). On the other hand, plasma IL-6 is related to fat mass and not to insulin responsiveness (67). However, none of the studies claiming that IL-6 levels are causative rather than merely correlated to insulin resistance provide any evidence for a role of IL-6 in insulin sensitivity (53). Studies investigating the role of IL-6 in insulin resistance in adipocytes in vitro and in vivo have mainly looked at short-term effects of high IL-6 levels. This is likely to elicit a different response (63) compared with the long-term low-grade IL-6 stimulation seen in individuals developing insulin resistance. Thus, studies of the effect of long-term low-grade IL-6 challenge of primary abdominal and omental adipocytes on insulin sensitivity in vitro and in vivo are indicated.

Does IL-6 induce insulin resistance in skeletal muscle? Skeletal muscle is the largest insulin-sensitive tissue and contributes to >90% of the insulin-stimulated glucose disposal in healthy individuals (6). Despite this, there is striking paucity of human and rodent in vivo as well as ex vivo studies evaluating the action of IL-6 on this issue.

Like adipose tissue, skeletal muscle does express IL-6 mRNA and protein at rest, but the contribution to the circulating levels is unclear. Small amounts of IL-6 are released from resting skeletal muscle in elderly but not young individuals, and IL-6 stimulates its own expression in muscle cells (53). During exercise, large quantities of IL-6 are released from muscle tissue beds (69,70), and IL-6 released from muscle tissue has been proposed to be an exercise signal (also called a "work-factor") coregulating glucose homeostasis during exercise. From a simplistic physiological point of view, it seems irrational that working muscle releases a factor that inhibits insulin signaling when the muscle needs insulin action for aerobic glucose metabolism. Thus, it is unclear whether IL-6 causes insulin resistance in skeletal muscle (Table 6) (52,65,70,71). However, the studies in male C57BL/6 mice suggest that dose and time of exposure of IL-6 may alter the effect of IL-6 on insulin sensitivity in skeletal muscle (Table 6). Interestingly, a study modeling long-term low-concentration exposure to IL-6 in individuals developing type 2 diabetes does not support that such an IL-6 challenge causes insulin resistance (71) in accordance with observations in humans (65). Finally, there is no difference in basal and insulin-stimulated *IL6* mRNA expression in insulin-sensitive and -resistant resting rodents and humans (53).

Conclusively, there is limited evidence that long-term IL-6 stimulation per se causes insulin resistance in skeletal muscle. However, the impact of IL-6 on insulin sensitivity in skeletal muscle cells may depend on concentration and duration of exposure. Therefore, further investigations are warranted.

IL-6 negatively affects insulin signaling in hepatocytes. In contrast to what has been detailed for adipocytes and skeletal muscle cells, there is far more consistency in studies investigating the effect of IL-6 on insulin signaling in hepatocytes (52,71–74) (Table 7).

The rodent in vivo and in vitro studies and the in vitro studies on the human hepatocarcinoma HepG2 cell line are strikingly consistent (Table 7); these studies indicate that IL-6 signaling in hepatocytes is mainly directed via the JAK/STAT pathway (Fig. 1) leading to STAT3 phosphorylation → *SOCS3* transcription → inhibition of *1*) insulin receptor autophosphorylation and *2*) tyrosine phosphorylation of IRS-1 and -2 → decreased glycogen storage due to decreased gluconeogenesis and increased glycogenolysis.

TABLE 6
IL-6 as an insulin resistance–inducing agent in skeletal muscle

Setting	Result (ref. no.)	Indicates IL-6 induction of insulin resistance
In vivo rodent	Five-day continuous subcutaneous infusion of hIL-6 (sixfold elevation of IL-6 ~ levels reached in obesity) does not suppress skeletal muscle insulin receptor signal transduction in mice at rest (71).	No
	Two-hour IL-6 challenge reduces insulin-stimulated glucose uptake in skeletal muscle in mice and is associated with defects in insulin-stimulated IRS-1–associated PI3K activity, increased levels of fatty acyl-CoA, and increased tyrosine phosphorylation of STAT3 in skeletal muscle (52).	Yes
In vivo human	Three-hour IL-6 (high- and low-dose) administration does not affect muscle glucose uptake at rest (70).	No
	SOCS-3 expression in human skeletal muscle is not related to insulin resistance in the presence of elevated IL-6 (65).	No

PI3K, phosphatidylinositol 3-kinase.

Collectively, theses studies provide strong evidence for the ability of IL-6 to reduce insulin sensitivity in hepatocytes by hampering insulin signaling. Whether other signaling pathways are activated and contribute to the described changes in insulin signaling is still unclear and needs further investigation.

Why hepatocytes react so consistently to IL-6 exposure compared with adipocytes and skeletal muscle cells is an open question. It is tempting to speculate that the presence of IL-6Rα in the hepatocyte cell membrane mainly directs IL-6 signaling toward the JAK/STAT pathway, whereas soluble IL-6Rα in other cell types as adipocytes and skeletal muscle cells preferably activates the SHP-2/ERK pathway favoring cell growth, proliferation, and differentiation (Fig. 1).

IL-6 ACTION ON CEREBRAL ENERGY EXPENDITURE REGULATION: A ROLE IN TYPE 2 DIABETES PATHOGENESIS?

IL-6 also affects other organ systems of direct or indirect importance for the glucose homeostasis in type 2 diabetes including cerebral centers involved in regulation of energy expenditure and the hypothalamic-pituitary-adrenal axis (7). The context IL-6 → hypothalamic-pituitary-adrenal axis → type 2 diabetes has not attracted much interest.

Focus turned to the action of IL-6 on cerebral centers involved in regulation of energy expenditure and the subsequent risk of developing obesity and type 2 diabetes (rev. in 75) as *Il6*$^{-/-}$ mice developed mature-onset obesity, impaired glucose tolerance, and increased glucose levels (49). This observation has recently been challenged by others using pair-fed wild-type littermates (48), but although the scientific approach in the latter study is superior to the former study (49), several important inferences can be drawn from the first study and other studies investigating this field, as recently reviewed by Wallenius

TABLE 7
Studies of IL-6 on insulin signaling in hepatocytes

Setting	Result (ref. no.)	IL-6 induces insulin resistance
In vitro		
Rat hepatocytes	IL-6 inhibits insulin-stimulated glycogen deposition in primary rat hepatocytes through decreased glucose incorporation into glycogen and increased glycogen degradation (72).	Yes
Mouse hepatocytes and HepG2 cells	Acute IL-6 challenge inhibits insulin receptor signal transduction and insulin action in both mouse hepatocytes and HepG2 cells *1*) by decreasing tyrosine phosphorylation of IRS-1, *2*) by decreasing the association of the p85 subunit of PI3K with IRS-1, and *3*) by inhibiting insulin activation of Akt. Further, IL-6 inhibits insulin-induced glycogen synthesis by 75% (73).	Yes
HepG2 cells	Acute short-term IL-6 induces SOCS-3 mRNA and protein expression and is paralleled by inhibition of insulin receptor signaling as described above. Further, SOCS-3 directly inhibits insulin receptor autophosphorylation (74).	Yes
In vivo		
Mouse	Acute short-term IL-6 challenge increases hepatic SOCS-3 expression and associates with inhibition of hepatic insulin-dependent receptor autophosphorylation and IRS-1 tyrosine phosphorylation (74).	Yes
Mouse	Sixfold 5-day chronic IL-6 challenge increases STAT3 phosphorylation; reduces hepatic insulin receptor autophosphorylation by 60% and tyrosine phosphorylation of IRS-1 and -2 by 60 and 40%, respectively; and decreases refeeding-dependent glucokinase mRNA induction by ~40% (71).	Yes
Mouse	Short-term IL-6 pretreatment blunts insulin's ability to suppress hepatic glucose production and insulin-stimulated IRS-2–associated PI3K activity in liver (52).	Yes

PI3K, phosphatidylinositol 3-kinase.

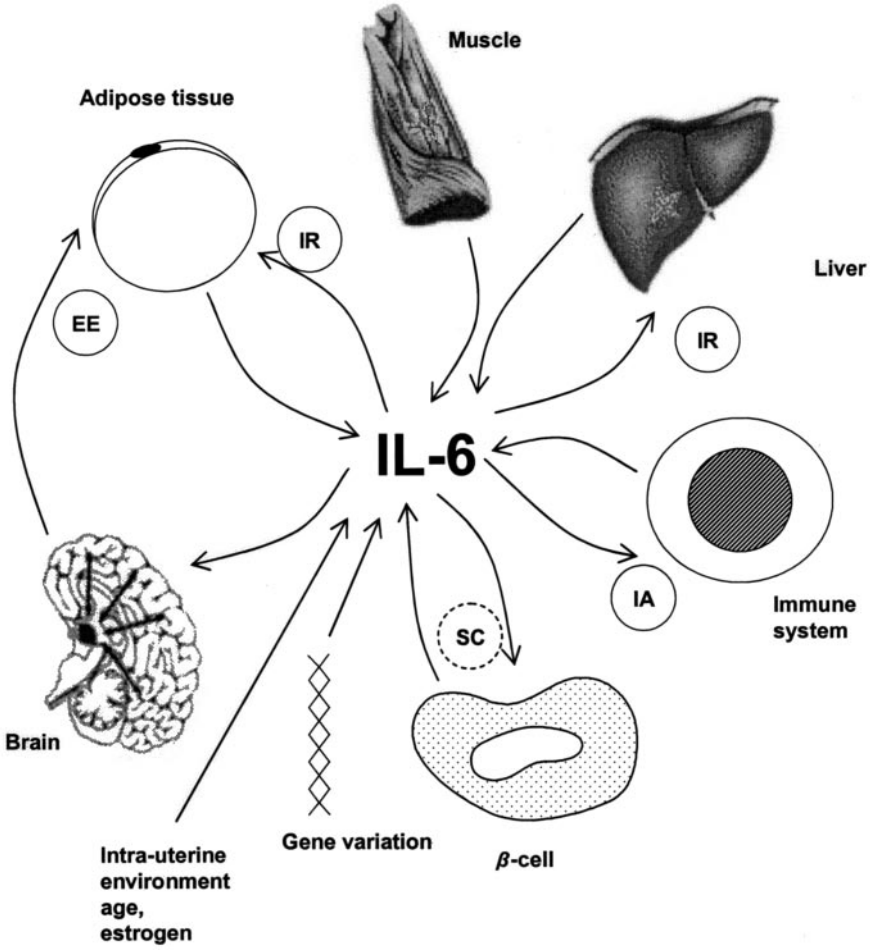

FIG. 2. Model of IL-6 actions of potential relevance for the pathogenesis of type 1 and type 2 diabetes. IL-6 is produced by many inflammatory cells, adipose tissue, working muscle, and even the β-cell. Expression is determined by genetic variation, intrauterine environment, age, and sex steroids. IL-6 induces insulin resistance in adipose tissue and liver and may synergize with proinflammatory cytokines to produce β-cell damage. IL-6 also regulates energy expenditure probably by the effect on brown adipose tissue via effects on the CNS. EE, energy expenditure; IA, immune activation; IR, insulin resistance; SC, synergy with proinflammatory cytokines.

et al. (75). Obesity in *Il6*$^{-/-}$ mice was partly reversed by long-term IL-6 replacement (49), and more strikingly intracerebroventricular, but not intraperitoneal, IL-6 injection increased energy expenditure without changing the respiratory exchange ratio, suggesting that the anti-obesity effect of IL-6 is mainly exerted at the level of the CNS. Single peripheral administration of IL-6 also increases energy expenditure in humans (75). Repeated daily intracerebroventricular injections of IL-6 for 14 days in mice and adenoviral IL-6 expression in the hypothalamus for 5 weeks in rats led to reduction in body weight and reduction of fat mass compared with control animals without significant reduction in food intake per body weight (rev. in 75). Interestingly, the rats exposed to increased hypothalamic IL-6 had increased expression of uncoupling protein-1 in brown adipose tissue. IL-6 levels in cerebrospinal fluid correlate negatively with total body fat in obese humans and the cerebrospinal fluid levels are of the same magnitude as in serum, and in some individuals even higher than in serum (75), suggesting that cerebrospinal fluid IL-6 is at least in part regulated independently of serum IL-6, possibly by local production in the brain.

In conclusion, several lines of evidence indicate that IL-6 affects centers in the CNS involved in energy regulation and expenditure and that low levels and low CNS production of IL-6 may be a mechanism contributing to the development of obesity and subsequent insulin resistance.

CONCLUSIONS

There is little evidence for an independent role of IL-6 in type 1 diabetes pathogenesis arising from in vitro studies of human and rodent islets and from in vivo rodent studies, apart from the demonstration of correlation between IL-6 expression and insulitis in NOD mice islets, although IL-6 may synergize with other inflammatory mediators to aggravate β-cell damage. Genetic studies, although controversial, have pointed to a role of IL-6 in human type 1 diabetes in females. These studies have not unraveled the mechanism(s) by which IL-6 plays a role in female type 1 diabetes pathogenesis or the site of action of IL-6.

There is evidence that circulating levels of IL-6 are elevated years before onset of type 2 diabetes, but whether this is involved in precipitating type 2 diabetes is still an open question. There is no evidence for an independent role of IL-6 in impaired β-cell function and progressive β-cell apoptosis. Chronic and acute IL-6 exposure causes impaired insulin signaling in hepatocytes in vivo and ex vivo. There is evidence mainly pointing to a role of IL-6 in causing impaired insulin signaling in adipocytes in vitro, but this remains to be demonstrated in vivo. Long-term IL-6 stimulation per se does not seem to cause insulin resistance in skeletal muscle, but further investigations are in demand to confirm this. Further, impaired IL-6 action in CNS centers involved in energy regulation may be a cause of obesity and thus insulin resistance, but this notion needs further characterization and independent confirmation. Genetic studies have provided indications pointing to a role of genetic variance in the genes encoding IL-6 and IL-6Rα on risk of developing type 2 diabetes, but further clarifications in this field are warranted.

Taken together, IL-6 may contribute to, but is probably neither necessary nor sufficient for development of type 1

and type 2 diabetes (Fig. 2). Several issues are unresolved, and many future studies of IL-6 in the pathogeneses of type 1 and type 2 diabetes are needed.

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