Reprint from The Review of DIABETIC STUDIES Vol 9 No 4 2012 Special Issue Immunology and Treatment of T1D

The Review of DIABETIC STUDIES

REVIEW

Chapter III.5

Interleukin-1 Antagonists and Other Cytokine Blockade Strategies for Type 1 Diabetes

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Manuscript submitted December 16, 2012; resubmitted February 7, 2013; accepted February 8, 2013

■ Abstract

Proinflammatory cytokines stimulate adaptive immunity and attenuate T cell regulation and tolerance induction. They also profoundly impair β-cell function, proliferation, and viability, activities of similar importance in the context of type 1 diabetes (T1D). Detailed knowledge of the molecular mechanisms of β-cell toxicity has been gathered within the last 2-3 decades. However, the efficacy of individual proinflammatory cytokine blockade in animal models of T1D has been inconsistent and generally modest, except in the context of islet transplantation. This suggests that the timing of the cytokine blockade relative to anti-β-cell immune activation is critical, and that combination therapy may be required. In randomized, placebo-controlled, clinical trials of limited power, TNF-α (but not IL-1) blockade has yielded moderate but significant improvements in glycemia, insulin requirement, and β-cell function. The safety experience with anti-cytokine biologics is still very limited in T1D. However, combinations with other biologics, at doses of adaptive and innate immune inhibitors/modulators that are suboptimal or ineffective in themselves, may generate synergies of true therapeutic benefit and safety in T1D. Critical and balanced appraisal of the preclinical and clinical evidence of efficacy and safety of anti-immune, anti-inflammatory, and anti-dysmetabolic therapeutics should thus guide future studies to move closer to novel treatments, targeting the underlying causes of β-cell failure and destruction in T1D.

Keywords: type 1 diabetes · beta-cell · interleukin · immune intervention · biologics · insulin secretion · TNF · tumor necrosis factor · anakinra · rilonacept · etanercept · NF-kappaB

1. Introduction

The current treatment of type 1 diabetes mellitus (T1D) consists of hormonal substitution and adjunct therapies to sustain life, improve life quality, and reduce the risk of acute and long-term diabetic complications. These symptomatic interventions have been constantly refined and supported by improved measures to monitor glycemia and other biomarkers of treatment quality as well as by patient education and empowerment. However, therapies that aim to cure or prevent T1D are still not part of standard clinical care. Although the prognosis of the disease is improving, T1D is still associated with considerable excess morbidity and mortality [1], borne mainly by patients incapable of meeting the demanding treatment goals. Replacement of functional β-cell mass with islet transplantation is hampered by poor graft survival in spite of immune-suppressive medication ([2] and RDS chapter V [3]), and segmental pancreatic grafting is restricted to recipients of renal transplants because of the risks of immune-suppression. As detailed in this Special Edition of *The Review of Diabetic Studies* (RDS chapter III and IV), clinical trials that aim to induce or maintain β-cell function after diagnosis have either demonstrated no effects or merely transient effects or are associated with unacceptable side effects.

 DOI 10.1900/RDS.2012.9.338

Abbreviations:
ADAM17 – a disintegrin and metalloproteinase domain 17
APC - antigen-presenting cell
ATP – adenosine triphosphate
BMI – body mass index
C-peptide – connecting peptide
DAMP – danger-associated molecular pattern
ER – endoplasmic reticulum
ERK - extracellular-signal-regulated kinase
FADD – Fas-associated death domain protein
IgG1 – immunoglobulin G1
IL – interleukin
IL-1RAcP – IL-1 receptor accessory protein
IL-1RTI – IL-1 type 1 receptor
LPS – lipopolysaccharides
MAP – mitogen-activated protein
MAPK – MAP kinase
MHC – major histocompatibility complex
MMCP – mixed meal stimulated C-peptide
mRNA – messenger ribonucleic acid
MyD88 – myeloid differentiation protein 88
NF-κB – nuclear factor kappa B
NIH – National Institutes of Health
NOD – non-obese diabetic
RDS – Review of Diabetic Studies
ROS - reactive oxygen species
STAT-1 – signal transducer and activator of transcription family member 1
T1D – type 1 diabetes
T2D – type 2 diabetes
TACE – TNFα-converting enzyme
TNFα – tumor necrosis factor alpha
TRADD – TNF receptor type 1-associated death domain
TRAF2 – TNF receptor associated factor protein family member 2
TXNIP – thioredoxin-interacting protein

This generally frustrating backlog in progress is thus related to the unavailability of effective and safe therapies to target the disease mechanisms that determine β-cell failure and destruction *in situ* or in grafted replacements. One reason for this backlog is that the key pathways of the disease mechanisms are not yet fully understood.

The purpose of this review is to reappraise the role of inflammatory cytokines as mediators of β-cell demise and targets of therapy. This concept was proposed a quarter of a century ago [4], has been accepted as common wisdom, and entered into international textbooks on pathophysiology and endocrinology, based mainly on circumstantial evidence from *in vitro* and animal models. With the appearance of the first clinical trials testing the feasibility, safety, and efficacy of anti-cytokine biologics in T1D, it is timely to reanalyze the relevance of the preclinical evidence for the understanding and management of human disease. It is also an appropriate time to introduce cautionary notes on the temptation to discard the concept on the basis of incomplete clinical evidence, and to recall that decades of research have supported the role of inflammatory cytokines as important mediators of β-cell damage in T1D.

Since several recent papers have summarized at length the preclinical evidence for the inflammatory concept of β-cell damage [5-8], the purpose of the present paper is to review the rationale for anti-cytokine biologics, and to discuss their strengths and weaknesses. This review also aims to provide possible interpretations of the outcomes of the relatively few clinical trials with these therapeutics that have been published to date. The aspiration of the review is to provide a more faceted view of the field to stimulate the further preclinical research needed to fill in the many gaps and questions in our understanding of the role of cytokines in the disease mechanisms leading to T1D and islet graft failure, and to guide new clinical trials.

2. Cytokines and type 1 diabetes: no lack of candidate targets

Cytokines are intercellular protein mediators of infectious, inflammatory, metabolic, traumatic, and even psychologic stress. Most cells can be induced to produce and respond to cytokines that exert auto-, para-, and endocrine functions. Cytokines are important mediators of tissue adaptation, repair, and remodeling in response to stress, but in chronic stress, they may also cause tissue destruction and scarring. When considering the central functions of cytokines and chemokines in innate and adaptive immunity and in cell migration, communication, defense, and damage, it is not surprising that most of the known cytokines and chemokines have been suggested to partake in the pathogenesis of T1D. There are also redundancies in this complex and intertwined network of inflammatory mediators, which may enable the network to substitute for single cytokines or chemokines targeted by specific biologics [9].

For many rheumatologic and autoinflammatory diseases, anti-tumor necrosis factor (TNF) α and anti-interleukin (IL) 1 biologics have proven effective in trials conducted over the last decades, and are now registered as first line therapies for many of these disorders [10, 11]. In contrast to the situation regarding these autoimmune and inflammatory conditions, clinical testing of cytokine antagonists in T1D is in its infancy, with few reported studies and even fewer ongoing trials, as can be judged by the NIH register of clinical trials,

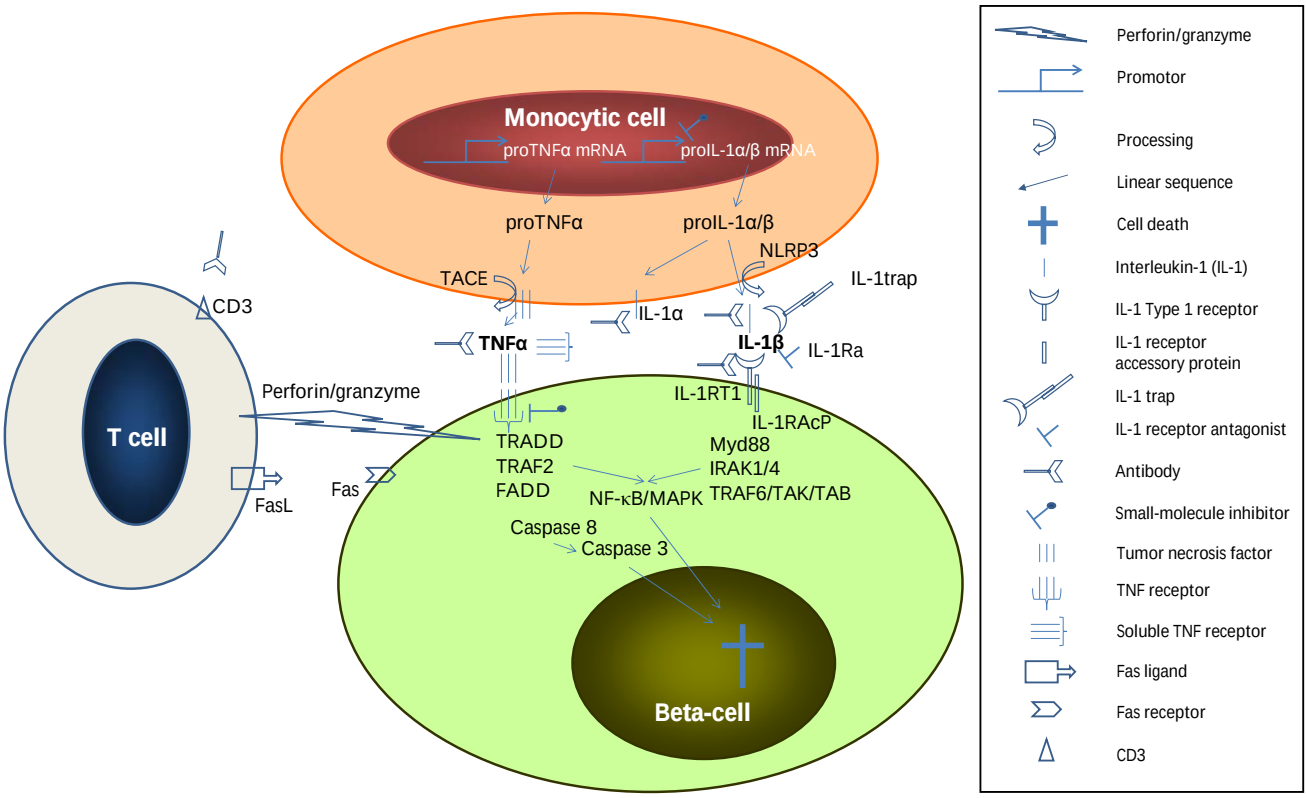

Figure 1. Expression and action of IL-1 and TNF - targets of singular or combinatorial interventions in type 1 diabetes. Beta-cell destruction is the result of the interaction between activated T cells and the pro-inflammatory environment established by insulitis. The immune and inflammatory effector mechanisms are amenable as drug targets. First, anti-T-cell therapies, such as antibodies directed towards the common T cell surface antigen cluster-of-differentiation (CD) 3, has been shown to preserve transiently β-cells mass in patients with recent-onset type 1 diabetes. In particular, TNF and IL-1 are expressed as pro-cytokines by islet-infiltrating monocytic cells such as dendritic cells and macrophages. After processing by TNFα-converting enzyme (TACE) or the NLRP3 inflammasome, respectively, TNFα and IL-1 are released into the islet microenvironment. β-cells express surface receptors for IL-1 and TNFα, which also induces the expression of β-cell Fas, thereby sensitizing the β-cell to the cytotoxic armamentarium of activated T cells. Furthermore, TNFα and IL-1 activate pro-apoptotic signals via their specific receptors, the downstream pathways of which converge on NF-κB and mitogen-activated protein kinase pathways that elicit the mitochondrial (intrinsic) death program. Although only partly effective or even ineffective when applied as monotherapy, interventions aimed at antagonizing TNFα and IL-1 may potently synergize or accelerate the action of anti-T-cell therapy [50]. The symbols indicate the levels of intervention.

www.clinicaltrials.gov. The hesitation to embark on clinical studies is most likely related to the experiences regarding recruitment, feasibility, and limited efficacy from the only anti-cytokine trials currently completed, which were conducted with TNFα and IL-1 antagonism in patients with new-onset T1D [12-14], as further outlined below.

3. TNFα antagonism

3.1 Biology of TNFα

The TNF family consists of nineteen members [15] binding to specific receptors with limited cross-activity. The prototypic member of this fam-

ily is TNFα. In contrast to IL-1, proTNFα contains a leader sequence, but is inserted into cell and plasma membranes as a homotrimeric complex (membrane-bound TNF), and can be detached by the action of a membrane metalloprotease, TNFα-converting enzyme (TACE/ADAM17) (**Figure 1**). TNFα is produced mainly by macrophages, NK cells, $CD4^+$ T cells, endothelial cells, and adipocytes in response to lipopolysaccharides (LPS), other bacterial products, IL-1, and other cytokines.

The homotrimeric TNF receptor recruits intracellular adaptor proteins such as TNF receptor type 1-associated death domain (TRADD), Fas-associated death domain protein (FADD), and TNF receptor-associated factor 2 (TRAF2) (**Figure 1**). Via the death domains of FADD, caspase 8 is activated, triggering the effector caspase of apoptosis, caspase 3. TRAF2 elicits the activation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK), redundant to IL-1 signaling (see below).

3.2 TNFa antagonists

Biologic TNFα antagonists include monoclonal antibodies raised against recombinant human TNFα (infliximab, adalimumab, and others) and a recombinant fusion protein between the soluble type 2/p75 TNFα receptor and the constant region of human immunoglobulin G1 (IgG1), termed etanercept [11] (**Figure 1**). In contrast to the IL-1 family, the TNF family does not include natural TNF receptor antagonists. A synthetic single-chain antibody TNF receptor antagonist [16] and a class of membrane-permeable small-molecule TNF receptor antagonists (triazoloquinoxalines), which act by interfering with the assembly of the intracellular TNF, but not with IL-1 receptor signaling complex [17] (**Figure 1**), have been synthesized, but not translated into clinical use.

Neutralizing TNFα antagonists are now standard therapy in rheumatology [11]. They are the first biologics of choice because they lead to more rapid pain relief than IL-1 blockers, and have been shown to be equally efficient in terms of the hard endpoint joint space narrowing [18]. Opportunistic infections, reactivation of tuberculosis, and fungal diseases are not uncommon adverse effects of TNFα blockade and warrant pretreatment screening and close monitoring [11]. TNFα antagonists improve insulin sensitivity in some but not all rheumatologic studies [19-21]. However, they have failed to do so in healthy lean, obese, and type 2 diabetes (T2D) subjects [22-26].

3.3 Type 1 diabetes

TNFα synergizes with IL-1 in causing β-cell apoptosis *in vitro* [27] (**Figure 1**). The effects of TNFα or TNFα antagonism in T1D animal models have been conflicting. Although local expression of TNFα in the pancreatic islets under the rat insulin promoter accelerates T1D by inducing a florid islet inflammatory reaction, TNFα or TNFα blockade may both protect and aggravate diabetes development depending upon dose and timing [5].

Only one clinical study of blocking TNFα in recent-onset T1D has been conducted, a 24-week double-blind, placebo-controlled, phase 2a clinical trial [12]. Four hundred eligible patients aged 3-18 years were identified in a 5-year study period, but only 18 subjects with a mean age of 12.5 could be randomized to etanercept, 17 of whom completed the follow-up. The etanercept-treated patients achieved a statistically significant 0.2% lower glycated hemoglobin between 8 and 24 weeks of follow-up. This effect persisted 12 weeks after withdrawal of etanercept. C-peptide rose in 6 of 9 etanercept-treated versus 1 of 8 placebo-treated subjects. Mean C-peptide was significantly higher and insulin requirement significantly lower in the etanercept arm. There were no evident safety concerns in this small sample of subjects.

Larger studies are needed to confirm these encouraging results, but the slow recruitment and the large proportion of eligible individuals, who cannot be randomized because they fail to give consent after being informed about the study, questions the feasibility of using this approach.

4. Interleukin-1 antagonism

4.1 Biology of IL-1

The IL-1 family of proteins includes four main groups of evolutionary highly conserved agonists, partial agonists, and antagonists; some of which have arisen from gene duplication within the IL-1 gene cluster on chromosome 2 [28]. The subgroup-agonists are central mediators of innate immunity, and activate distinct receptors, but with wide overlap in the use of the IL-1 receptor accessory protein (IL-1RAcP, **Figure 1**) as co-receptor. This common use of the IL-1RAcP enables therapeutic targeting of the action of several IL-1 family agonists such as IL-1, IL-36, and IL-33 by the same anti-IL-1RAcP biologic.

The main IL-1 activities are conferred by IL-1β, which is liberated from producing cells via pathways as yet unclear, and IL-1α, which is either

anchored in the plasma membrane (Figure 1) or translocated to the nucleus as a transcriptional co-regulator. IL-1β and IL-1α both bind to the same ectodomain of the IL-1 type 1 receptor (IL-1RTI) monomer, which then recruits IL-1RAcP to induce signaling (**Figure 1**). IL-1 receptor antagonist is a natural competitive inhibitor of IL-1β and IL-1α. It binds to the receptor and has no agonist activity.

IL-1 signaling is tightly regulated by the existence of the receptor antagonist and the truncated IL-1 type 2 decoy-receptor (IL-1RTII). It is also subject to pronounced transcriptional, translational, and posttranslational regulation. All nucleated cells so far investigated have been found capable of expressing IL-1 family proteins upon appropriate stimulation, including the pancreatic β-cell. Monocyte-derived and dendritic antigen-presenting cells (APCs) are the most potent producers of IL-1 in response to a wide variety of stimuli.

Similarly to TNFα, IL-1 is expressed as a pre-protein that requires processing to become biologically active [28] (Figure 1). Thus, the production of mature IL-1 depends upon a two-signal sequence:

1. Signal I is induced by several activators of the canonical NF-κB signaling pathway, including TLR ligands, metabolic factors, and cytokines that engage receptors recruiting the intracellular MyD88 docking protein. Signal I is required to drive proIL-1 mRNA transcription and translation, and this signal is amplified by glucose-induced calcium-, extracellular-signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase-, and reactive oxygen species (ROS)-dependent pathways. However proIL-1β is biologically inert, and needs to be processed by caspase-1 cleavage.
2. Inactive pro-caspase 1 is activated by cleavage induced by signal II, which is conferred via a multiprotein complex termed the inflammasome, a group of intracellular sensors of danger-associated molecular patterns (DAMPs) coupled to caspase-1 and caspase-5 [29].

How the inflammasome is activated, and which ligands bind to the ligand-sensing leucine-rich domain, is only incompletely understood. However, diverse extracellular stimuli such as ATP, nutrients (including glucose and free fatty acids), and non-degradable particulates (cholesterol or uric acid crystals, amyloid), which elicit a process of frustrated phagocytosis, seem to induce the generation of ROS, leading to the dissociation of the thioredoxin inhibitory protein TXNIP from thioredoxin [30-36]. TXNIP has been proposed to activate the inflammasome [30, 34, 35], but this is intensely debated. Alternatively, ATP-stimulated potassium efflux via purinergic receptors may be sensed by the inflammasome as activating signal [29]. The expression of the inflammasome components is also influenced by signal I. In contrast to the detailed insight into the regulation of IL-1β expression and processing, little is known about how IL-1β, which lacks a leader sequence for secretion, is exported out of IL-1-producing cells and how this process is regulated.

As most cells synthesize IL-1, virtually all cells studied to date express IL-1R and respond to IL-1. The main action of IL-1 is to drive the acute-phase response of inflammation and stress, but IL-1 has multiple additional neuronal, endocrine, metabolic, and immune effects, including effector T cell costimulation and inhibition of regulatory T cell function [37]. IL-1 operates high in the cytokine and chemokine hierarchy, and drives the expression of multiple proinflammatory and anti-inflammatory cytokines and chemokines, including the expression of IL-1 itself. It is also regulated by multiple other cytokines. IL-1 signals mainly via the NF-κB and MAPK pathways (**Figure 1**). The cellular effects involve changes in gene expression and protein activity to assist cell and host defence, tissue repair, and remodeling, as well as cellular stress and destruction via endoplasmic reticulum (ER) and mitochondrial stress pathways.

4.2 IL-1 antagonists

The many ligands and receptors of the IL-1 family offer various opportunities for intervention [38]:

- Recombinant IL-1Ra (anakinra)
- Soluble IL-1TI or II receptor
- IL-1RTI-IL-RAcP fusion protein (the so-called IL-1 trap, rilonacept)
- Several antibody-based antagonists, including anti-IL-1β antibody (e.g. canakinumab, gevokizumab), anti-IL-1α/β antibody, anti-IL-1RTI (AMGEN 108), and IL-1RAcP antibodies
- A small-molecule IL-1 synthesis modulator has been announced [39] (**Figure 1**)
- As for TNF, non-competitive small-molecule and even peptide IL-1 receptor antagonists have been described [40-42], but not entered into clinical use

Anti-IL-1 biologics are registered for a group of autoinflammatory diseases caused by gain-of-function mutations or aberrant regulation in the inflammasome, leading to intermittent or constitutively unregulated IL-1 production, also termed intrinsic and extrinsic inflammasomopathies, respectively [43]. The spectrum of clinical manifestations in this group of heterogeneous diseases ranges from severe sterile wide-spread serositis, arthralgias and death caused by amyloidosis, to mild fevers and rashes, and these disorders are cured within days by the administration of IL-1 antagonists [10]. IL-1 antagonists are also in routine clinical use for rheumatoid arthritis [10], and have been reported to improve glycemia and β-cell function in T2D [39, 44-46].

Anakinra causes a transient reaction at the injection site in between 30-50% of treated cases which is not seen with the IL-1 trap (rilonacept), an IL-1 inhibitor, or anti-IL-1 antibodies. IL-1 blockade is rarely associated with severe infectious adverse effects. With a 1.8% increase in the rate of serious infection in anakinra-treated patients vs. 0.6% in placebo-treated patients in blinded randomized studies, the adverse effects did not reach statistical significance.

4.3 Type 1 diabetes

Apart from its immuno-regulatory properties, IL-1 has long been known to exert profound inhibitory, cytostatic, pro-necrotic and pro-apoptotic effects on the pancreatic β-cell [6, 47, 48]. IL-1 is expressed early in the insulitis infiltrate, and may be regarded as a circulating biomarker of T1D risk. Anti-IL-1 strategies or genetic ablation of IL-1 or IL-1RTI have shown moderate or no protective efficacy in animal models of T1D [49]. In contrast to this disappointing result, IL-1 antagonists strongly synergize with suboptimal anti-CD3 monoclonal antibody therapy to accelerate and promote reversal of overt diabetes in the NOD mouse [50].

In an open-label, non-randomized study of IL-1 antagonism with IL-1Ra (anakinra) in 15 recent-onset T1D children, insulin requirements and insulin-adjusted glycated hemoglobin were reduced compared with two historical control groups [13]. In an uncontrolled study, one week of once-daily anakinra therapy reduced insulin resistance assessed by euglycemic hyperinsulinemic clamp, as well as insulin requirements and home monitored plasma glucose, in 14 patients with a mean BMI of 31 kg/m^2 and a duration of T1D of more than 5 years [51]. The residual β-cell function in these patients, and the effects on C-peptide secretion, are not reported in this meeting abstract. Thus, it is unclear whether the effects on insulin sensitivity are secondary to improved residual β-cell function.

Two investigator-initiated randomized, placebo-controlled, phase 2a trials have been conducted in T1D of recent-onset, one with IL-1Ra (anakinra) and one with IL-1β antibody (canakinumab) [14]. Each trial enrolled patients with standardized mixed meal stimulated C-peptide (MMCP) at baseline > 0.2 nM, and in both the primary endpoint was the 2-hour area-under-curve baseline-adjusted MMCP. Over five months, more rapidly than planned, the canakinumab trial enrolled 69 out of 105 subjects screened for eligibility, aged 6 to 45 years, within 14.3 weeks of diagnosis, to receive 2 mg/kg at a maximum of 300 mg IL-1β antibody or placebo s.c. monthly for 12 months by 2:1 randomization. Ninety-six percent of the randomized subjects completed the study. Compliance was excellent with more than 95% of planned injections being administered.

Over 30 months, much more slowly than planned, the anakinra trial recruited 69 out of 90 subjects screened for eligibility, aged 18 to 35 years, within 12 weeks of diabetes symptoms, to receive daily s.c. injections of 100 mg of IL-1Ra or placebo for 9 months by 1:1 randomization. Seventy-four percent of the randomized subjects completed the study. Compliance was assessed by counting returned empty vials (around 80%) and by the measurement of IL-1Ra levels at follow-up visits. IL-1Ra levels were significantly elevated over endogenous levels, but lower than anticipated based on the dose, although this was difficult to assess as the time of day of the visits varied in relation to the injection time-point.

Apart from the increased frequency of injection reactions in the anakinra trial both anakinra and canakinumab were safe; in particular there was no increase in severe infections. Neither study met the primary endpoint. There was a slightly and non-significantly higher MMCP at 1 month in the canakinumab-treated subjects, which did not translate into a lower insulin requirement. Predefined subgroup analyses of outcome stratified by several baseline variables revealed significantly lower MMCP at 1 year in the canakinumab-treated subjects with the lowest baseline tertile. In the anakinra trial, intermediary baseline MMCP was significantly associated with higher MMCP at 9 months. It should be noted that these subgroup outcomes should be considered with caution because of the low number of individuals in the strata and the nature of secondary analysis. The results can at most be considered as a hypothesis

for validation in future trials. Anakinra blocks IL-1 signaling, elicited by both soluble IL-1β and *trans*-signaling by IL-1α bound to the surface membrane of T cells, monocytes, and possibly even pancreatic β-cells. Therefore, the differences in the putatively protective effects between anakinra and canakinumab in these subgroup analyses could be due to unopposed IL-1α action redundant to that of IL-1β in the canakinumab trial.

It is important to determine the reasons for the negative outcome beyond the obvious possibility that blocking IL-1 action after the occurrence of overt T1D does not affect β-cell function, an interpretation supported by the similar lack of efficacy in hyperglycemic NOD mice [50]. Firstly, the timing of the intervention could be wrong, i.e. the onset of treatment is too late relative to the pathogenetic process and the degree of β-cell destruction. The inflammatory lesion is more intense and transient in patients with recent-onset T1D than in long-standing T2D, suggesting that the therapeutic window for IL-1 blockade is smaller in T1D than in T2D. The modest but significant efficacy observed in prevention studies in animal models suggests that IL-1 blockade should be instituted in the prediabetic phase. This notion is supported by recent human studies [52, 53], pointing to the peak in monocyte IL-1 production in the late prediabetic period and around the time of diagnosis, which rapidly normalizes after 1 month. Thus, patients with a diabetes duration of 2-3 months, as recruited to these trials, may already have passed the optimal window for therapeutic intervention.

Secondly, dosing may have been insufficient. The doses chosen were those approved for the treatment of rheumatologic disorders, but it cannot be excluded that higher doses may be required to prevent the end-stage insulitis lesion in the pancreatic islets. Thirdly, the decline in C-peptide was less than expected [54]. The intensification of insulin therapy, shown to preserve β-cell function [54, 55] via immune effects of insulin and by direct antiapoptotic effects independent of glycemia, may have masked an effect on the rate of the C-peptide decline in these trials, and may have required longer treatment and follow-up to be revealed. Fourthly, the efficacy of IL-1 antagonism may be glucose-dependent. The promising results in the etanercept trial and the non-masked pilot study of anakinra in T1D were obtained in patients with much higher baseline glycemia [12, 13]. A similar dependence of the positive effect on the metabolic derangement has been suggested in trials in T2D, where glucose and free fatty acids have been identified as drivers of islet IL-1 mRNA expression and processing via the inflammasome *in vitro* [56]. This notion is also supported by the observation that the peak of monocyte IL-1 production is normalized within 1 month after diagnosis of T1D [52].

A fifth consideration is that much of the positive effect seen in the phase 1/2a studies in T2D may be due to the reversibility of the inhibitory effect on β-cell function caused by IL-1 observed *in vitro* [57]. Functional β-cell mass from the outset was strikingly similar in T1D patients (baseline fasting C-peptide 0.85 nM at an ambient blood glucose of 6.9 mM) and T2D patients (baseline fasting C-peptide 0.96 nM at an ambient blood glucose of 10.8 mM) in the phase 2a anakinra trials [14, 44]. However, when considering the much more intensive mononuclear cell infiltration in T1D vs. T2D patients, it is possible that the dose of 100 mg of anakinra o.d. was insufficient to antagonize the amounts of IL-1 secreted into the islet microenvironment in T1D, but not in the T2D patients. Finally, it is likely that blocking IL-1 alone does not preserve the β-cell function in T1D because of the redundant signaling from other proinflammatory cytokines and the concerted action of the innate and adaptive immune effector mechanisms. The effective synergy between suboptimal doses of anti-CD3 monoclonal antibody and IL-1 blockade in the NOD mouse, as mentioned above [50], is in favor of this consideration.

All these hypotheses require testing in future trials that are designed and powered to address each possible explanation. In this respect, it is important that the lessons learned from practical and logistic experience are used to guide the choice of anti-IL-1 agent. Clearly, the addition of daily anakinra injections to the multiple insulin injections, instituted recently in newly-diagnosed T1D, may be an unacceptable burden, contributing to the slow recruitment and high drop-out rate in the anakinra trial. The treatment cannot be introduced into clinical practice until more experience regarding the safety of IL-1 blockade in T1D has been obtained. In this regard, the rapid reversibility of the action of anakinra relative to IL-1 antibodies or the IL-1 trap (rilonacept) represents a safety advantage.

In summary, although there is a preclinical rationale for IL-1 as an interventional target in T1D, results from clinical trials have so far been disappointing. It is possible that timing and dosing of IL-1 antagonists are critical parameters as is the use in combination with other anti-cytokine or

anti-adaptive or innate immune cell approaches [50].

Conclusions and perspectives

There is extensive *in vitro* evidence for a pronounced impact of proinflammatory cytokines on adaptive and innate immunity, T cell regulation, and tolerance induction. These cytokines affect β-cell function, proliferation, and viability via NF-κB, STAT-1, and MAPK signaling, causing ER and mitochondrial stress and activation of the intrinsic death pathway. In striking contrast, the efficacy of individual proinflammatory cytokine blockade in animal models of T1D, with the exception of islet transplantation, has been inconsistent and generally modest, suggesting that the timing of the cytokine blockade relative to anti-β-cell immune activation is critical, or that combination therapy is required. In randomized, placebo-controlled, clinical trials of limited power, TNFα but not IL-1 blockade has yielded modest but significant improvements in glycemia, insulin requirement, and β-cell function. The safety experience with anti-cytokine biologics is still very limited in T1D.

Many questions still remain unanswered. The most important ones are the following:

- Are the marked effects of individually targeted inflammatory cytokines on β-cell function and viability an *in vitro* artefact caused by isolation of single biologic mediators dissociated from their natural network of antagonists and regulators?
- As combinations of proinflammatory cytokines are required for human but not rodent β-cell damage, is it irrational to expect effects from single cytokine blockade in animal models and human trials?
- Do we appreciate when interception of cytokine action should be optimally introduced?
- Are doses of anti-cytokine biologics universal for autoimmune, autoinflammatory, and inflammatory diseases, considering the extensive differences between target organ composition and perfusion in these disorders?
- To what extent is proinflammatory cytokine blockade able to preserve β-cell mass relative to function *in vivo*?
- What are the adjunct adaptive immune and metabolic determinants of cytokine actions, and can they be exploited therapeutically?

These and other questions need to be answered in future trials to determine whether anti-cytokine therapies will find a place in the future therapeutic armamentarium against T1D.

Disclosure: The author reports no conflict of interests.

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