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Anti-Cytokine Therapies in T1D: Concepts and Strategies

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Abstract

Therapeutic targeting of proinflammatory cytokines is clinically beneficial in several autoimmune disorders. Several of these cytokines are directly implicated in the pathogenesis of type 1 diabetes, suggesting opportunities for design of clinical trials in type 1 diabetes that incorporate selective cytokine blockade as a component of preventative or interventional immunotherapy. The rationale and status of inhibitory therapy directed against IL-1, TNF, IL-12, IL-23, and IL-6 are discussed, towards a goal of using cytokine inhibition as a therapeutic platform to establish an in vivo milieu suitable for modulating the immune response in T1D.

Keywords

Immunotherapy; autoimmunity; regulatory T cells; anti-inflammatory

1.0 Overview

Clinical trials with targeted immunotherapy directed towards lymphocytes in intervention trials, using anti-CD3 (teplizumab or otelixizumab), anti-CD20 (rituximab), or CTLA4-Ig (abatacept), resulted in transient maintenance of insulin-secretory function in some type 1 diabetes (T1D) subjects. However, the overall picture is not a positive one, because the majority of treated subjects reverted to a pattern of progressive beta cell loss after treatment [18;22;35;38], indicating the recurrence of pathogenic autoreactivity. Recurrence of pathogenic lymphocytes has also been seen in studies of T1D subjects who receive pancreas transplants, in which autoreactive effector lymphocytes have been shown to survive in spite of aggressive immunosuppressive therapy, and in some cases expand in tandem with recurrence of T1D in the transplanted organ graft [54]. These accumulated clinical observations need to be addressed in the next generation intervention trials in T1D, as they indicate a compelling need to address not only the specific effector populations, but also the host environment that nurtures and directs the recurrent immune response after initial therapy.

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Potential strategies for achieving a more durable immunological effect can be categorized as:

- **i.** Enhancing lymphodepletion or immunomodulation, i.e., making some of the current therapies more effective through synergistic mechanisms of action;
- **ii.** Boosting or replacing dominant regulatory elements in the immune system that will create a sustainable balanced immunological profile after effector cell depletion or modulation therapy;
- **iii.** Changing the tissue microenvironment to alter the likelihood that recurrent effector pathways will be activated;
- **iv.** Creating a cytoprotective milieu supporting beta cell survival and repair.

In this brief review, we will discuss an approach towards achieving these strategies through manipulation of specific cytokine pathways, with examples focused on blockade of IL-1, TNF, IL-12, IL-23, and IL-6.

2.0 Cytokine Therapies

2.1 Anti-interleukin 1 (anti-IL-1)

The family of interleukin-1 (IL-1) proteins consists of five main groups of agonists, partial agonists, and antagonists, some of which have arisen from gene duplication within the IL-1 gene cluster on chromosome 2 [10] (Table 1). These molecules are evolutionarily highly conserved and constitute key mediators of innate immunity in primitive organisms, dating back to starfish. The subgroup-agonists activate distinct receptors but with wide overlap in the use of the IL-1 receptor accessory protein (IL-1RAcP) as co-receptor, opening up the potential for the therapeutic targeting of IL-1, IL-36, and IL-33 action by the same anti-IL-1RAcP biologic.

In addition to the complex and branched control on IL-1 signalling provided by the existence of receptor antagonists and decoy receptors, the action of the IL-1 family of proteins is subjected to pronounced transcriptional, translational, and posttranslational regulation. All nucleated cells investigated so far have been found capable of expressing IL-1 family proteins upon appropriate stimulation, including the pancreatic beta cell. Monocyte-derived and dendritic antigen-presenting cells (APCs) are the most potent IL-1 producers in response to a wide variety of stimuli. Of note, the production of mature IL-1 depends upon a two-signal sequence: signal I is induced by several activators of the canonical NFκB signalling pathway, such as 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-, ERK MAP kinase-, and ROS-dependent pathways. However, proIL-1 is biologically inert and needs to be processed by caspase-1 cleavage.

Inactive pro-caspase 1 is activated by cleavage induced by signal II, which is conferred via a multiprotein complex named the inflammasome, a group of intracellular receptors of danger-associated molecular patterns (DAMPs) [30]. How the inflammasome is activated and which ligands bind to the ligand-sensing, leucine-rich domain is incompletely understood, but diverse extracellular stimuli, such as ATP, nutrients and metabolic factors, and non-degradable particulates (cholesterol or uric acid crystals, amyloid), which elicit a process of frustrated phagocytosis seem to converge on the generation of reactive oxygen species that lead to dissociation of the thioredoxin inhibitory protein TXNIP from thioredoxin. TXNIP has been proposed to activate the inflammasome [36;58]; alternatively ATP-stimulated potassium efflux via purinergic receptors may be sensed by the

inflammasome as activating signal. The expression of the inflammasome components is also influenced by signal I.

In contrast to the detailed insights into the regulation of IL-1 expression and processing, little is known about how IL-1, which lacks a leader sequence for secretion, is exported out of IL-1-producing cells and how this process is regulated. However, the better understood, intricate regulation of IL-1 offers multiple possible targets for intervention.

As most cells synthesise IL-1, virtually all cells studied hitherto 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 co-stimulation and inhibition of regulatory T-cell function [11]. IL-1 operates at the top of 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, and is in turn regulated by multiple other cytokines. IL-1 signals mainly via the NFκB and MAPK pathways but also via small G proteins and other pathways only partially understood. The cellular effects involve changes in gene expression and protein activity to assist cell and host defense, tissue repair, and remodelling, as well as cellular stress and destruction via endoplasmic reticulum and mitochondrial stress pathways.

The many ligands and receptors of the IL-1 family offer a wide portfolio of opportunities for intervention [9]. Apart from recombinant IL-1RA, soluble IL-1TI or II receptor and an IL-1TI R-IL-RAcP fusion protein (the so-called IL-1 trap), several antibody-based antagonists are manufactured and marketed, including anti-IL-1αβ, anti-IL-1TI R, and IL-1RAcP antibodies.

Apart from its immunoregulatory properties, IL-1 has long been known to exert profound inhibitory, cytostatic, pro-necrotic, and pro-apoptotic effects on the pancreatic beta cell [29]. IL-1 is expressed early in the insulitis infiltrate and may be a circulating biomarker of T1D risk. However, whereas anti-IL-1 antagonism has shown efficacy in preclinical models of T2D and reduces glycemia via improved beta cell function in T2D patients [7;12;25], anti-IL-1 strategies or genetic ablation of IL-1 or receptor have shown modest or no protective efficacy in animal models of T1D [29]. In contrast, IL-1 antagonists strongly synergize with suboptimal anti-CD3 monoclonal antibody (mAb) therapy to accelerate and promote reversal of overt diabetes in the nonobese diabetic (NOD) mouse [1].

Only one small unblinded non-randomised study of IL-1 antagonism with IL-1RA (anakinra) in 15 recent-onset T1D children has been published [47], showing reduced insulin requirements and insulin-adjusted glycated haemoglobin compared with two historical control groups, an effect that was not confirmed in two yet unpublished randomized placebo-controlled trials.

Thus, although there is solid preclinical rationale for IL-1 as an interventional target in T1D, results have been disappointing so far. 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 [1].

2.2 Anti-tumor necrosis factor (anti-TNF)

There are nineteen known members of the TNF family, which bind to specific receptors with limited cross-binding [48] (Table 2). The prototypic member of this family is TNFα. In contrast to IL-1, proTNFα contains a leader sequence, but the proTNFα is inserted into cell and plasma membranes as a homo-trimeric complex (membrane-bound TNF), which can be shed by the action of a membrane metalloprotease, TNFα-converting enzyme (TACE), or

ADAM17. Shedding probably occurs both from membranes in the *trans*-Golgi and from the plasma membrane. How shedding is regulated is incompletely understood, but it is known to be subject to inhibition by tissue inhibitor of metalloproteinase 3 (TIMP-3).

TNFα is produced by many cells, including macrophages, NK cells, CD4 T cells, endothelial cells, and adipocytes, in response to LPS and other bacterial products, and IL-1 and other cytokines. TNFα binds to the homo-trimeric TNF receptor, which recruits intracellular adaptor proteins, such as TRADD, FADD, and TRAF2. Via the death domains of FADD, caspase 8 is activated which in turn triggers the effector caspase of apoptosis, caspase 3. TRAF2 elicits NFκB and MAPK activation, redundant to IL-1 signalling.

TNF was discovered as a tumor apoptosis- and cachexia-inducing cytokine, and, like IL-1, is a potent mediator of the acute phase response and septic shock. In addition, like IL-1, TNF is a chemokine and an adipocytokine secreted from adipose tissue and involved in the pathogenesis of insulin resistance and the metabolic syndrome. Although TNF alone has little direct effect on the pancreatic beta cell, it synergizes potently with IL-1 and IFNγ in inducing beta cell dysfunction and apoptosis [28].

The effects of TNF or TNF antagonism in T1D animal models have been conflicting. Although expression of TNF locally in the pancreatic islets under the rat insulin promoter accelerates T1D by inducing a florid islet inflammatory reaction, TNF or TNF blockade may both protect and aggravate diabetes development depending upon dose and timing [27].

Only one clinical study of blocking TNF in recent-onset T1D has been conducted. This was a 24-week, double-blind, placebo-controlled phase 2a clinical trial [32]. Out of almost 400 eligible patients aged 3–18 and identified in a 5-year study period, it was possible to randomize only 18 subjects with a mean age of 12.5 to the recombinant, soluble, neutralizing TNF receptor-IgG fusion protein etanercept; 17 completed the follow-up protocol, and the study was terminated due to slow recruitment. Etanercept-treated patients achieved a 0.2% lower glycated hemoglobin between 8 and 24 weeks of follow-up; this difference was statistically significant and persisted 12 weeks after withdrawal. C-peptide rose in 6/9 etanercept-treated versus 1/8 placebo-treated subjects, and the mean C-peptide was significantly higher in the etanercept arm. The improved beta cell function translated into a significantly lower insulin requirement in the active arm. There were no evident safety concerns in this small sample of subjects. Larger studies are needed to confirm these encouraging results. However, slow recruitment, despite a large proportion of eligible subjects (not randomized because information about the study resulted in lack of consent) questions the feasibility of using this approach.

2.3 Anti-IL-12/23

IL-12 and IL-23 are secreted by dendritic cells, macrophages, and monocytes and support the maturation and maintenance of proinflammatory effector T cells. Their primary roles appear to be involved in the induction of committed T cell lineages, IL-12 for Th1 and IL-23 for Th17 cells, acting on naïve, immature, and/or “plastic” uncommitted T cells in concert with stimuli that include antigen exposure and other cytokines. Because IL-12 and IL-23 are heterodimers that share the common p40 subunit, inhibition of p40 is an attractive target to interfere with both Th1 and Th17 development and function.

Ustekinumab is a human IgG1κ monoclonal antibody that binds with high affinity and specificity to the shared p40 subunit in both IL-12 and IL-23, blocking signaling by inhibiting the interactions of these cytokines with their receptors [4;14]. Ustekinumab has shown efficacy in several human autoimmune diseases, including psoriasis, psoriatic arthritis, and Crohn’s disease [8;26;37;43;56] for the treatment of adult patients (18 years or

older) with moderate to severe plaque psoriasis. Analysis of tissue from the affected target in those diseases (e.g., skin in psoriasis) has documented the expected mechanism of action for this therapy, with significantly diminished Th1 and Th17 activity after systemic administration of drug [46].

Rationale for use of ustekinumab in T1D is largely based on the attractive mechanistic rationale of blockade of both Th1 and Th17 pathways, since evidence from analysis of lymphocyte phenotypes in patients supports potential roles for each. In addition, since interruption of IL-12/23 signaling cascades interferes with production of downstream inflammatory mediators, such as IFNγ and IL-6, the milieu for supporting Treg activity is improved. Most of the evidence implicating the Th1 and Th17 pathways in T1D is necessarily indirect, derived from analysis of lymphocytes and cytokine profiles from peripheral blood. Islet antigen-specific T cell lines and clones are readily expanded in cultures of peripheral blood lymphocytes from T1D subjects and produce multiple cytokines, notably IFNγ. More recent reports indicate that peripheral blood CD4 cells from new-onset T1D subjects also produce IL-17 in response to activation or autoantigen stimulation, and interestingly IL-17 enhances beta cell apoptosis induced by other inflammatory cytokines, such as IFNγ and IL-1β [2;20]. This has led to the proposal that signaling by early inflammatory mediators secreted by Th1 cells and macrophages renders β cells susceptible to IL-17-mediated apoptosis [2]. This view suggests a fairly comprehensive picture in which inflammatory signals from innate immune cells (macrophages and dendritic cells), an autoreactive cytolytic response driven by adaptive Teff cells (Th1 and Th17), and an inadequate or dysfunctional protective response from Treg cells combine into a pathogenic program dependent on the IL-12/23 signaling pathways.

Studies in murine models are problematic for prediction of therapeutic efficacy for ustekinumab. In the NOD mouse the dominant Teff cells appear to be IFNγ-producing Th1 cells. The role of Th17 cells in the NOD model is controversial. While some studies have shown a role for IL-17 and IL-21 in the development of T1D in the NOD mouse [15;49], others have suggested that the role of Th17 is indirect, possibly by conversion to Th1 cells [3;21]. The role of IL-12 blockade is similarly controversial in the NOD mouse—and contradictory. There have been suggestions that therapeutic efficacy may be dependent on the age of the mice, the stage of disease, and the type of disease model [17;34;40;41;51–53;55;57].

2.4 Anti-IL-6

IL-6 is an abundant proinflammatory cytokine associated with immunity and autoimmunity, produced by many different cell types. It is often characterized as “downstream” of IL-1, but in fact is involved in multiple, different stages of immune response and is a uniquely attractive therapeutic target. IL-6 plays an important role in the communication between the innate and adaptive immune systems, functioning to influence the development and action of both pathogenic and regulatory T cells. One particularly intriguing role for IL-6 is a pivotal position in Th17 and Treg commitment, in which IL-6 combines with TGFβ to promote the Th17 lineage, whereas, in the absence of IL-6, TGFβ contributes to Treg development [5;23]. In addition, IL-6 is a potent effector cytokine, with multiple direct tissue effects that promote proinflammatory cascades [33;39]. Thus, IL-6 blockade may promote both anti-inflammatory and pro-regulatory mechanisms simultaneously in a tissue environment under active autoimmune attack.

Studies in murine autoimmunity suggest that Treg function is disabled by inflammatory cytokines in the local microenvironment, which drive Teff lineages and inhibit Foxp3. For example, IL-6 induces methylation of the *Foxp3* gene enhancer in nTreg cells, leading to down-regulation of Foxp3 expression and resulting in the generation of pathogenic “ex-

Foxp3" cells which have been shown to induce diabetes within 8–11 days after transfer into NOD *Rag2*−/− mice [24;59]. This type of reversibility, or plasticity, of T cells is one of the major concerns with current T cell immunotherapies, in that beneficial effects may only be transient if cytokines, such as IL-6, act as a local barrier to the establishment of durable regulatory profiles.

In addition to this role as an inhibitor of Tregs, recent studies with human T cells have suggested that IL-6 may drive an important phenotype characteristic of treatment-refractory autoimmunity: In these studies, Teff from T1D subjects were found to be resistant to suppression by Treg from healthy controls [44]. Follow-up work demonstrated that this phenotype was also present in patients with some other autoimmune diseases and that this finding correlated with hyperactivity of the IL-6 signaling pathway, suggesting that autoreactive Teff are driven by tonic IL-6 signals to be refractory to regulation (Buckner et al., in press). So although IL-6 is often regarded as a lineage determinant for T cell development, it is more than that, with functional consequences promoting the establishment and maintenance of a proinflammatory, anti-regulatory tissue environment in autoimmunity.

As noted previously, the magnitude and activity of the Th17 pathway in T1D is a focus of active investigation. It has been suggested that the increase in Th17 cells observed in patients may be due to increased production of IL-6 by T1D monocytes [6], and this could also relate to increased Th17 activity upon T cell activation [20;31] and in the pancreas of T1D subjects [16].

Tocilizumab is a recombinant humanized monoclonal antibody specific for the human IL-6 receptor (IL-6R) that binds both the membrane-bound and soluble forms of IL-6R, currently approved for use in RA and sJIA in the USA and Europe. The pediatric experience is particularly promising for practical consideration of use in T1D, since tocilizumab has been used in young children with systemic juvenile idiopathic arthritis, yielding dramatic success, with approximately 90% of children achieving an ACR50 response. Tocilizumab has also shown preliminary efficacy in pilot open label phase 1 trials or small case studies in a variety of autoimmune or inflammatory conditions, including SLE, Takayasu and giant cell arteritis, Crohn's disease, systemic sclerosis, polymyositis, relapsing polychondritis, polymyalgia rheumatica, ankylosing spondylitis, and Behcet's disease [50]. In patients with RA after 3 infusions of tocilizumab, there was a decrease in the frequency of peripheral Th1 and Th17 cells and a corresponding increase of Treg [42], correlated with significant clinical response.

There have been no clinical studies in diabetes with tocilizumab. In a small open-label study of non-diabetic RA patients, tocilizumab therapy resulted in a significant decrease in the HOMA index for insulin resistance and a significant increase in serum adiponectin levels [45]. Also of note are the results of a recent mendelian randomization analysis for a SNP in the *IL6R* gene (Asp358Ala) that phenotypically recapitulates the effects of tocilizumab, which showed reduced risks for coronary heart disease and type 2 diabetes [19]. Thus, anti-IL-6R therapy may have metabolically favorable effects relating to the metabolic properties of subjects with T1D, notably insulin resistance. On the other hand, a recent study using sorted human islet cells suggested that IL-6 may have a role in stimulating alpha cell release of GLP-1 and thereby improve beta cell function [13].

3.0 Towards combination therapy including antigen delivery

Cytokine inhibition, as described in this article, is designed to play a major role in establishing a tissue milieu and cellular microenvironment permissive for regulatory immune responses, by simultaneously interfering with proinflammatory pathways and promoting alternative cellular functions that are more homeostatic. These cytokine

pathways, however, are intrinsically malleable, designed and honed by evolution to be flexible adaptors to external and tissue stress. As a consequence, one way to view cytokine inhibition in T1D is to view therapy as a staged process, in which a regulatory platform needs to be nurtured in vivo, suitable for enabling additional immune modulation therapy to succeed. Cytokine inhibition is an important approach for achieving this platform, but since the malleable nature of cytokine pathways requires them to be reversible and adaptable, it is unlikely that cytokine inhibition alone will be able to achieve durable therapeutic success.

Adaptive immunity to antigens, on the other hand, is designed to engender durable effector memory populations that can sustain an established immunological program. In this regard, a key goal for using cytokine inhibition as a therapeutic platform is to establish an in vivo milieu suitable for directing the adaptive immune response towards a therapeutically desirable outcome. For T1D, given current knowledge about effector pathways, blockade of cytokines such as IL-1, TNF, IL-12/23, and IL-6 has the potential to help create a suitable platform that will favor regulatory adaptive responses at the expense of proinflammatory Th1 and Th17 ones. A recent example is the ability of IL-1 blockade, in itself ineffective, to synergize with suboptimal doses of anti-CD3 mAb to accelerate and potentiate the reversal of overt T1D in NOD mice. [1].

An important question, however, is whether the autoantigen exposure that naturally occurs in T1D is sufficient to push the adaptive pathways towards such a regulatory and anti-inflammatory outcome. It is quite possible that it will be necessary to provide additional antigen, a form of therapeutic vaccination, simultaneously with cytokine blockade to assure the desired adaptive immune response. The T1D field has experimented with antigen therapies using various forms of insulin and recently an alum-formulated GAD vaccine, but these trials all lacked the fundamental pro-regulatory and anti-inflammatory platform discussed here. Indeed, some of these trials used adjuvants that may have actually interfered with prospective beneficial effects, and exploratory studies on tolerogenic forms of adjuvants are badly needed. Given the success of some of the anti-cytokine therapeutics in other autoimmune diseases, and the mechanistic rationale for simultaneously blocking proinflammatory effector pathways and promoting regulation, the time is at hand to move forward with additional trials using available cytokine inhibitors in T1D and pave the way for combining these treatments with antigen delivery.

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Abbreviations

DAMPs	damage/danger-associated molecular patterns
ROS	reactive oxygen species
T1D	type 1 diabetes
T2D	type 2 diabetes
Treg	regulatory T cell(s)
Teff	effector T cell(s)
TLR	toll-like receptor
IL-1RA	IL-1 receptor antagonist

IL-1RAcP	IL-1 receptor accessory protein
TXNIP	thioredoxin inhibitory protein
LPS	lipopolysaccharide
NOD	non-obese diabetic (mice)

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Highlights

- cytokine inhibition supports regulatory immunological mechanisms
- cytokines influence immunologic determinism in tissue microenvironments
- cytokine blockade can establish a foundation for antigen-specific therapy
- several cytokine inhibitors should be evaluated in T1D clinical trials

Table 1

The interleukin-1 family

Nomenclature		Receptor	Receptor accessory protein	Decoy receptor
IL-1F1	IL-1α[+]	IL-1RI	IL-1RAcP	IL-1RII
IL-1F2	IL-1β[*]	IL-1RI	IL-1RAcP	IL-1RII
IL-1F3	*IL-1Ra*	IL-1RI		IL-1RII
IL-1F6	IL-36α	IL-1Rrp2	IL-1RAcP	
IL-1F8	IL-36β	IL-1Rrp2	IL-1RAcP	
IL-1F9	IL-36γ	IL-1Rrp2	IL-1RAcP	
IL-1F5	*IL-36Ra*	IL-1Rrp2		
IL-1F4	IL-18[*]	IL-18Rα	IL-18Rβ	
IL-1F7	*IL-37a,b*[+]	IL-18Rα	?	
IL-1F11	IL-33[*+]	ST2	IL-1RAcP	
IL-1F10	*IL-38*	?	?	

italics indicate antagonists or partial agonists

[+] also transcriptional co-factors

[*] generated from inflammasome processing by caspase-1

Table 2

The tumor necrosis factor family

Nomenclature	Receptor	Decoy receptor
membrane/soluble TNF	p55, p75 TNF-R, SFV T2	
Lymphotoxin α/β1, 2	p55, p75 TNF-R, LTβ R	
LIGHT	LTβ R, DCR2, HVEM	
CD40L	CD40	
FasL	Fas	
TRAIL	TRAIL R1-2	TRAIL 3–4
APRIL	BCMA, TAC1	
BAFF/Blyss	BAFF-R/BL-3, BCMA, TAC1	
CD27L	CD27	
CD30L	CD30	
OX40L	OX40	
4-1 BBL	4-1 B-R	
GITRL	GITR	
RANKL	RANK, OPG	
TWEAK	TWEAK-R	
EDA	EDA-R	