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Harnessing CD8$^+$ T-cell exhaustion to treat type 1 diabetes

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Abstract

Although immune interventions have shown great promise in type 1 diabetes clinical trials, none is yet in routine clinical use or able to achieve insulin independence in patients. In addition to this, the principles of type 1 diabetes treatment remain essentially unchanged since the isolation of insulin, almost a century ago. Type 1 diabetes is characterised by insulin deficiency due to destruction of insulin-producing beta cells mediated by autoreactive T cells. Therapies that target beta-cell antigen specific T cells are needed to prevent type 1 diabetes. $CD8^+$ T-cell exhaustion is an emerging area of research in chronic infection, cancer immunotherapy, and more recently, autoimmunity. Recent data suggest that exhausted T cell populations are associated with improved markers of type 1 diabetes. T-cell exhaustion is both characterised and mediated by inhibitory receptors. This review aims to identify which inhibitory receptors may prove useful to induce T-cell exhaustion to treat type 1 diabetes and identify limitations and gaps in the current literature.

Introduction

Type 1 diabetes mellitus (T1D) is due to destruction of insulin-producing beta cells in the pancreatic islets of Langerhans that causes insulin deficiency and hyperglycaemia. This is primarily mediated by T cells specific for islet autoantigens[1]. Globally, T1D is an emerging public health burden and its onset is most common amongst individuals <15 years old. Unfortunately, there are no disease-modifying prevention or intervention strategies for halting T1D progression in routine clinical use. Patients are dependent on exogenous insulin administration to maintain glucose homeostasis and this has been the case since it was first used in humans 100 years ago. Yet despite the evidence establishing the importance of glycaemic control in reducing complications, lifelong adherence to daily management regimes is both difficult for the individual and has suboptimal outcomes. Therefore, developing methods of conserving or reinstating endogenous insulin output constitutes the ultimate objective of T1D research. An ideal cure for diabetes would involve the arrest and potential reversal of the destruction of the beta-cell mass without prominent side effects, resulting in long-term independence from exogenous insulin.

Loss of T-cell self-tolerance in T1D

The aetiology of T1D, much like other autoimmune diseases, remains to be fully elucidated. The prevailing consensus is that the genetic, epigenetic and environmental factors in conjunction with

an initiation event(s) result in the successive breakdown of central and peripheral tolerance mechanisms, thus enabling disease onset[1].

Disease progression is contingent on cytotoxic $CD8^+$ T cell lytic interactions triggered by endogenous peptide displayed in MHC class I on the beta-cell surface[2]. In reaching this end-state, successive failures in self-regulatory checkpoints are required to both achieve and perpetuate the immunopathology. Loss of self-tolerance in autoreactive T cells occurs in two distinct phases: escape from thymic deletion in central tolerance, and aberrant activation in peripheral tolerance.

Central tolerance

Typically, T-cell development in the thymus ensures that only T cells that are appropriately unresponsive to self can migrate into the periphery. However, central tolerance does not completely eliminate autoreactive T cells[3] that can be detected in the peripheral circulation of T1D and healthy individuals[4]. In T1D, impaired central tolerance is due to intrinsic defects in antigen presentation to potentially autoreactive thymocytes. Reduced presentation by MHC types that convey high risk for diabetes, variable levels of thymic expression of islet-specific autoantigens especially proinsulin, post translationally modified and hybrid peptides and alternative splicing may create antigens present in the periphery but not in the thymus and impair negative selection leading to increased islet autoreactivity[5].

Peripheral tolerance

There is evidence to suggest that circulating islet-autoreactive clones can exist in both healthy and T1D subjects but there is preferential activation of these in the diseased state[6]. This indicates that mechanisms in peripheral tolerance are compromised in T1D pathogenesis.

Mechanisms of peripheral tolerance regulate naïve autoreactive T cells[7, 8]. Many autoreactive thymic emigrants undergo clonal deletion or are rendered anergic. This is achieved through low avidity interactions with tolerogenic DCs that drive the proliferative response to apoptosis or inactivation. In autoimmunity, avidity maturation of CTLs recognising islet antigens has been shown to circumvent this[9]. For autoreactive T cells that do become activated, $CD4^+$ $CD25^+$ $FoxP3^+$ natural regulatory T cells (nTreg) cells are the key regulators to avoid pathogenicity.

$CD8^+$ T cells in T1D pathogenesis

Autoreactive $CD8^+$ T cells are the principal drivers of beta-cell destruction in both humans and the non-obese diabetic (NOD) mouse model of T1D, with effector $CD4^+$ T cells also having an important role. Deficiency in either $CD4^+$ or $CD8^+$ T cell populations protects mice from insulitis and diabetes[2]. Naïve T-cell activation occurs in the pancreatic lymph node following encounter with islet-derived antigens[10]. $CD4^+$ T cells provide T-cell help, license dendritic cells and prevent the deletion of $CD8^+$ T cells[11]. Following activation in the pancreatic lymph node, T cells migrate to the pancreatic islets *via* the circulation. Access to the non-inflamed islet is restricted to activated T cells specific for beta-cell antigens[12]. Once T cells have entered the islets, the islet environment becomes increasingly proinflammatory and vulnerable to further immune infiltration[13].

Insulitis describes the progressive infiltration of the islets by immune cells. $CD8^+$ T cells are the most abundant leukocytes, followed by macrophages and a comparatively smaller $CD4^+$ T-cell population[14]. Infiltration is marked by an increase in MHC class I expression in diseased islets[15]. Tetramer-staining of pancreatic sections collected from T1D patients demonstrated that $CD8^+$ T cells within affected islets are autoantigen-specific, recognising pre-proinsulin, insulin, IGRP, GAD65, pre-proislet amyloid protein and islet cell antigen 512[16]. Islet reactive $CD8^+$ T cells have also been demonstrated using staining with multimers *ex vivo* in the peripheral circulation of T1D and healthy subjects and *in situ* in the pancreas of T1D subjects[4].

$CD8^+$ T cells recognise peptide antigens which are presented by MHC class I proteins. The importance of this interaction has been demonstrated in NOD mice lacking MHC class I molecules on beta cells or lacking $CD8^+$ T cells in which no insulitis develops[2]. In humans, increased levels of islet MHC class I expression is a consistently observed hallmark of the disease[15, 16]. The dominant method of beta-cell destruction utilised by $CD8^+$ T cells is the release of perforin and granzymes from secreted granules[17]. These combined findings highlight the importance of targeting $CD8^+$ T cells in any therapeutic approach.

$CD8^+$ T-cell exhaustion

T-cell exhaustion is an altered differentiation state of T cells that is normally associated with chronic antigen presentation and/or inflammation[18]. It manifests itself as a progressive loss of effector function, sustained upregulation of various coinhibitory receptors, changed expression and utilisation of key transcription factors and metabolic abnormalities[18-20] (Figure 1). Exhausted T cells differ from memory T cells in that they do not possess antigen-independent homeostatic responsiveness[18, 21]. Exhausted $CD8^+$ T cells progressively lose the ability to produce interleukin-

2 (IL-2), to efficiently kill target cells *ex vivo* and to produce pro-inflammatory cytokines including TNF-α and IFNγ[22].

$CD8^+$ T-cell activation

To better describe T-cell exhaustion, it is necessary to compare T-cell differentiation following acute infection/vaccination to that in chronic infection/cancer (Figure 2). In both cases, following antigen recognition and driven by transcriptional regulation, naïve $CD8^+$ T cells differentiate into short lived effector T cells and memory precursor effector cells[23, 24]. These cells produce cytokines and chemokines, acquire the ability to kill target cells and have migratory potential. Following antigen clearance in acute infection/vaccination, the majority of effector T cells die, and a subset of these effector $CD8^+$ T cells differentiate into long-lived memory T cells, driven by antigen stimulation in the presence of the cytokines IL-7, IL-15 and/or IL-21 and the transcription factors T-bet and eomesodermin (Eomes)[23]. While initially similar, the differentiation process in chronic situations begins to deviate from that in acute settings. Because precursor exhausted T cells are unable to fully clear antigen, they undergo an early exhaustion differentiation process in which T cells stably adjust their proliferative and effector capacity to a lower level and this phenotype is optimised to cause minimal tissue damage while still mediating a critical level of pathogen or tumour control. As the presence of antigen continues, early exhausted T cells further differentiate into terminal exhausted T cells which are eventually deleted[25].

Differentiation process of $CD8^+$ T cells towards exhaustion

A number of signals drive exhaustion. The most important is persistent antigen exposure, with antigen amount being more important than antigen strength[26]. Other factors that are thought to contribute to exhaustion include limited $CD4^+$ T-cell help[27], minimal costimulation during T-cell priming, reduced common gamma chain cytokine signalling[28, 29], and the tissue microenvironment (e.g. hypoxia, nutrients, pH)[18, 30].

Precursors of exhausted $CD8^+$ T cells have been shown to possess memory potential[23, 25] but fail to acquire the ability to self-renew and mount robust recall responses. Virus specific exhausted $CD8^+$ T cells from mice with established chronic infection were unable to become memory $CD8^+$ T cells when removed from the infection. Even though a subset of exhausted T cells proliferated after transfer into naive mice (removal of antigen exposure), the proliferated T-cells maintained the exhausted phenotype they acquired during the initial exposure to chronic infection[31].

Moreover, the durability of reinvigoration of exhausted T cells with PD-1 blockade is limited due to epigenetic stability of exhausted T cells[32]. Conversely, memory $CD8^+$ T cells that are primed during acute viral infection can be driven to exhaustion if transferred to a mouse with chronic viral infection[25].

Both exhausted and memory T cells have been noted to arise from the same pool of KLRG-1^{lo} and $CD127^{hi}$ effector cells[23, 25, 30]. High expression of the transcription factors TOX and TOX2 drive $CD8^+$ T cells down the exhaustion pathway instead of becoming memory precursors[33-35]. In this early form of exhaustion, the majority of cells are Tcf-1^+, and display high expression of T-bet, intermediate expression of PD-1, and low expression of Eomes (T-bet^{hi} PD-1^{int} $Eomes^{lo}$)[23, 30]. This pool of cells still possesses moderate proliferative capability and potential to produce effector cytokines, but exhibits limited cytotoxicity[30].

In response to persistent antigen stimulation, progenitor exhausted $CD8^+$ T cells proliferate and give rise to terminal exhausted progeny[36]. Terminally exhausted cells differ from the progenitor pool in a number of ways. The majority are Tcf-1^-, and exhibit low expression of T-bet, high expression of PD-1, and high expression of Eomes (T-bet^{lo} PD-1^{hi} $Eomes^{hi}$)[23, 30]. Unlike their progenitors, these cells display poor proliferative capacity, less production of cytokines, but better cytotoxicity compared to their progenitors[30, 36].

The presence of both T-box transcription factors, T-bet and Eomes, has been shown to be critical for the formation of early and terminal exhausted T cells[23, 36]. Temporal deletion of T-bet resulted in accelerated decay in the progenitor population indicating its importance in maintaining the progenitor pool of exhausted T cells[36]. Furthermore, temporal loss of Eomes resulted in a significant reduction in the number of terminal exhausted T cells, thus suggesting that Eomes plays a critical role in initiating or sustaining the terminal pool of cells[36].

Another notable difference between the progenitor and terminal exhausted T cells is in their response to PD-L1 blockade[30, 37]. Upon treatment with anti-PD-L1 antibody, the progenitor pool of cells showed significantly improved responsiveness compared to untreated cells[37]. In contrast, there was little improvement in the responsiveness of the terminal exhausted T cells following PD-L1 blockade[37].

Inhibitory receptors and negative regulatory pathways

Elevated and sustained expression of inhibitory receptors is a key hallmark of $CD8^+$ T-cell exhaustion[23, 30]. While normally expressed by functional effector T cells during activation, multiple inhibitory receptors are progressively acquired and persistently expressed as T-cell exhaustion progresses. The major inhibitor receptors are PD-1 and CTLA-4, while others include LAG-3, CD244 (2B4), CD160, TIM-3 and others. The pattern of inhibitory receptor coexpression dictates the level of T-cell dysfunction due to exhaustion[38].

Programmed Cell Death 1 (PD-1) is transiently expressed during the initial activation of T cells but is upregulated in a sustained manner on exhausted T cells[23, 39, 40]. PD-1 engages with its ligands, PD-L1 or PD-L2, which are expressed widely in nonlymphoid tissues and upregulated by pro-inflammatory cytokines such as IFNγ. PD-1 is important for reducing T-cell activation in the periphery to minimise the damage to tissue during a local immune response. Ligation of PD-1 results in negative regulation of the PI3K/AKT and RAS signalling pathways[40-42]. This, in turn, leads to inhibition of TCR-mediated lymphocyte proliferation and effector cytokine production[43]. PD-1 expression dominates the hierarchical expression of inhibitory receptors in exhausted T cells[44, 45].

CTLA-4 is a receptor protein that competitively binds the B7 ligands (CD80 and CD86) with higher affinity than CD28[46-48]. Normally, engagement of the co-stimulatory protein CD28 releases positive signals that impact T-cell activation, expansion, and differentiation[23]. Following T-cell receptor engagement, CTLA-4 is upregulated and it counteracts these signals, inhibiting T-cell activation and initial T-cell priming in secondary lymphoid organs[23, 40].

Following activation, CTLA-4 is also constitutively expressed on Tregs as it is a target of Foxp3, an important Treg transcription factor[40]. Therefore, aside from dampening T-cell activation and priming, CTLA-4 is also thought to play an important role in augmenting Treg-mediated immune suppression[40].

T-cell immunoreceptor with Ig and ITIM domains (TIGIT) negatively regulates T-cell function by competitively binding CD155, a ligand shared by CD226[49, 50]. Binding between TIGIT and CD155 results in increased IL-10 production and decreased IL-12 production by dendritic cells (DCs), leading to suppression of T-cell activation and promotion of T-cell exhaustion[23, 51].

Lymphocyte activated gene-3 (LAG-3) inhibits T-cell activation and effector function[40]. It is thought to negatively regulate cell cycle progression and other cellular functions[23]. While it is normally expressed transiently following T-cell activation, LAG-3 has been shown to be highly

expressed in exhausted T cells in both chronic infection and cancer[23, 40]. T-cell immunoglobulin- and mucin-containing protein 3 (TIM-3) is significantly upregulated in exhausted T cells and is a marker of severe exhaustion in chronic LCMV, HCV, and HIV infections[23]. TIM-3 is normally co-expressed with PD-1[23].

Stability of T-cell exhaustion

$CD8^+$ T-cell exhaustion is a differentiation process where exhaustion-associated features are maintained by stable epigenetic modifications even if the antigen levels are reduced or cleared[25, 31, 32]. These stable epigenetic modifications restrict T-cell expansion and clonal diversity during PD-1 blockade[52]. In addition to epigenetic programs, specific transcription factors, TOX and NR4A have been identified as critical regulators of formation and maintenance of the exhaustion program[33-35, 53, 54]. Exhausted T cells do not form in the absence of TOX. TOX is initially induced by calcineurin and NFAT2 but later becomes calcineurin-independent and is sustained in exhausted T cells. TOX drives T-cell exhaustion by mediating transcriptional and epigenetic changes in T cells[33].

T-cell exhaustion and autoimmunity

Manipulation of T-cell exhaustion has potential as a therapeutic opportunity for control of autoimmune disease. Several lines of evidence indicate that pathways related to T-cell exhaustion play an important role in restraining T cells in autoimmune diabetes. (1) It takes months (in mice) to years (in humans) after onset of autoimmunity to develop diabetes. (2) Autoimmune diabetes is rapidly induced by blocking the PD-1 pathway in NOD mice[55]. Treatment with PD-1 pathway blockers can induce diabetes in humans with or without pre-existing autoimmunity and the diabetes rapidly develops in subjects with pre-existing autoimmunity[56-59]. Deficiency of PD-1[60] or LAG-3[61], or treatment with blocking anti-LAG-3 antibody accelerates autoimmune diabetes in NOD mice[61]. Similarly, blockade of TIM-3 has been shown to increase the clinical and pathological severity of experimental autoimmune encephalitis (EAE) in rats[62]. (3) Recent data show that T cells in the islets of NOD mice display an exhausted phenotype with high PD-1, TIM-3, TIGIT and TOX expression (see Abdelsamed *et al.*[63] and our unpublished data). (4) Transcriptomic profiling of T cells from patients with autoimmunity showed that a T-cell exhaustion signature correlated with a more benign form of autoimmune disease, indicating that mechanisms associated with T-cell exhaustion may be important in controlling autoimmunity[57].

CD-2 costimulation was able to prevent the development of an exhausted phenotype in T cells displaying traits of exhaustion (failure to express IL-7R) due to persistent stimulation with antigen[57]. Cells maintained IL-7R expression, limited PD-1 upregulation and enhanced cell survival. However, this inhibition of exhaustion by CD-2 signalling was reversed by administration of the inhibitory receptor PD-1, which indicates that a state of exhaustion can be induced – at least, *in vitro*. In the context of T1D, alefacept, an immunomodulatory drug that binds to CD2, disrupts its signalling and also depletes memory T cells, was found to preserve C-peptide secretion, reduce insulin use and hypoglycaemic events in newly diagnosed patients, even when therapy had been ceased for a year[64].

An inverse relationship exists between IL-7 receptor expression and PD-1, with PD-1 being upregulated on effector T cells infiltrating into the pancreas and in the pancreatic lymph nodes upon administration of IL-7Rα antibody[65, 66]. IL-7R blockade reverses diabetes when administered to NOD mice[65, 66].

Therapeutic exhaustion for treatment of T1D

There are broadly two potential goals of therapeutic exhaustion in the treatment of T1D. The first is the induction of exhaustion to slow T1D progression in individuals with a previously non-exhausted phenotype[67, 68]. Chronic antigen exposure may be one way to achieve this. The second is agonism of the inhibitory receptors expressed by exhausted T cells, as exhausted T cell populations are not always completely immunologically inactive[69].

Therapeutic exhaustion may prevent progression to clinical disease, given the potential for exhaustion to inhibit epitope spreading in other autoimmune conditions (such as myasthenia gravis)[70]. However, preventative therapies such as this would be dependent on early identification of patients that would benefit.

One of the key benefits of potential therapeutic exhaustion (as opposed to systemic forms of immunosuppression) is that exhaustion is antigen-specific; it occurs within populations of $CD8^+$ T cells with an affinity for the same antigen. This suggests the potential for exhaustion-based therapies to selectively dampen the immune response, without impairing functional immunity.

Hurdles to achieving therapeutic exhaustion

Currently, there are critical limitations to therapeutic exhaustion, regarding both its research and its future clinical implementation. In terms of research, therapeutic exhaustion (especially in T1D) is a relatively new area, and thus current data are limited. Early research on exhaustion was

conducted in mice, primarily in those with chronic LCMV infection. Much of the T1D data gathered recently are extrapolations from immune-related adverse events in cancer immunotherapy, NOD mice, or anecdotal patient evidence. Very little data are in the form of rigorous trials. Each of these data sources pose their own problems.

Firstly, a fundamental issue in the translation of cancer research to autoimmunity is that most exhaustion-based cancer immunotherapies antagonise inhibitory receptors that are upregulated within the exhausted T-cell phenotype. In autoimmunity, exhaustion appears inversely correlated with disease severity, *ipso facto*, inhibitory receptors are less common (and thus more difficult to agonise) in the patient populations that would most benefit from exhaustive therapy[67]. This may be because one of the definitive precipitants for exhaustion is chronic antigen exposure. By the time patients reach Stage 3 T1D (clinical diagnosis), there may be inadequate islet antigen concentration remaining to induce exhaustion in islet-specific populations of T cells. However, it is known from patients receiving exogenous insulin that their insulin-specific T cell populations have a more exhausted phenotype than other islet-specific antigens[68]. This suggests the potential for induction of exhaustion (with exogenous antigens) despite a relative lack of endogenous autoantigen. However, in subjects at high risk for diabetes, insulin administration did not delay or prevent T1D[71]. Also, it is possible that antigens could expand pre-existing effector T cells instead of inducing T-cell exhaustion[72]. It is thus important to understand other factors necessary to drive T-cell exhaustion. T-cell fate following peripheral encounter with self-antigen is dictated by the antigen level, the nature and site of exposure, T-cell avidity, activation state of the antigen presenting cells, tissue microenvironment, nature of cytokines and the response to cytokines and modulatory effects of other cells including B cells, $CD4^+$ T cell help, Treg cells, NK cells and stromal cells. Therefore, a lack of inhibitory receptors may be targeted by a dual approach; prior induction of exhausted populations may provide adequate upregulation of inhibitory receptors for subsequent agonism[67, 69].

Secondly, it is not well understood how T1D predisposing factors such as HLA and insulin genotypes may interact with exhaustion. It is well understood that these factors are much more common in people with T1D than those without[73]. How HLA subtypes interact with exhaustion is of particular interest, because TCR-MHC class I binding may affect TCR-mediated exhaustion[74].

Thirdly, due to the prohibitive danger of pancreatic biopsies in humans, much of what is known about human T1D pathophysiology around the time of disease onset has been learnt from the NOD

mouse[75]. NOD mice are a model for human T1D because of features shared with humans, such as their spontaneous development of disease which is T-cell mediated. However, there are pros and cons to studying NOD mice[75]. Studies that use NOD mice are often criticised because their histological differences to that of diabetic human pancreata[76]. Many therapies that have proven effective in NOD models of disease have been ineffective in humans[75]. Pearson *et al.* argue that preclinical trials in NOD mice offer a false comparison, as many studies in NOD mice are done aetiologically earlier in the disease process than the first presentation of T1D in humans[77]. In addition, the genetic homogeneity of NOD mice and immunological differences to humans present further challenges for clinical translation[75]. A multicentre trial by Gill *et al.* also highlighted a lack of intersite reproducibility between trials conducted in the NOD mouse model[78]. The NOD mouse remains the most likely model for pathophysiological study and many preclinical trials, but researchers need to maintain awareness of its limitations. Relatively recent methods of 'humanising' NOD mice include transplantation of relevant human genes, cells, and microbiota into NOD mice[79].

Fourthly, we need to ensure clinical validity of human trials. Some clinical trials have not been able to demonstrate an improvement in outcomes despite improvements in surrogate markers of disease. For example, teplizumab (anti-CD3 monoclonal antibody) studies have shown C peptide improvements in 'responder' populations, but without improvements in HbA1c or insulin requirements[80].

Hurdles in clinical implementation

There would also be several hurdles in the clinical implementation of therapeutic exhaustion. One such hurdle is in identifying responders to exhaustive therapies, given the heterogeneity of exhausted T-cell populations between patients[68, 80]. Patients would also have to be identified early in order to fully benefit from therapeutic exhaustion, which is difficult given that T1D is asymptomatic in its early stages, and the rapidity of progression in those who would benefit most from exhaustion therapy[68]. Screening children for high-risk HLA subtypes or islet antibodies, or targeting familial T1D, may increase the benefit of preventative exhaustive therapies[73, 81].

Secondly, evidence suggests that prolonged stimulation of inhibitory receptors is required, which may indicate a need for prolonged treatment[67]. Because T-cell exhaustion is set quite early and

memory T cells can be exhausted by chronic antigen exposure, timing of induction of exhaustion may not be important.

Thirdly, there is presently a lack of data regarding long-term follow-up. Therapeutic exhaustion is a new area of research, and little is known about exhaustion's long-term benefits on hyperglycaemia. Therapeutic exhaustion may not have long-term efficacy, as with other immunosuppressive therapies in the treatment of T1D. While studies suggest that T-cell exhaustion is a stable phenotype, it should be recognised that there is heterogeneity within exhausted T cells[36] and a subset of exhausted T cells can survive for a long time and retain the ability to mount a recall response following rechallenge. To promote the development of therapeutic approaches that harness T-cell exhaustion, it will be essential to understand the mechanisms that control maintenance of these subsets of exhausted T cells. However, given that exhausted $CD8^+$ T cells have recently been demonstrated to be favourable even in symptomatic T1D patients[68, 82], exhaustion may still improve clinical outcomes as a non-curative treatment of T1D.

Lastly, the on-target and off-target side effect profiles of therapeutic exhaustion are not yet well characterised. Given that exhaustion is a form of immunosuppression, it is conceivable that there may be malignant or infective side effects that are not yet well known[67]. This may be particularly concerning in cases where viruses are a precipitant for T1D[83, 84], as causing exhaustion during their presence may prevent viral clearance. The off-target effects of inhibitory receptor agonists on Treg cells is also not well characterised[69].

It is also important to note that while these data are current at the time of writing, this is a swiftly moving field of research.

Conclusion

T1D is an ancient disease which has been nonfatal for almost a century, thanks to insulin therapy. Despite this success, clinically approved T1D therapy has stagnated. Development of immunotherapies for T1D is a major goal of research efforts worldwide. Inducing $CD8^+$ T-cell exhaustion to prevent autoimmune destruction of pancreatic beta cells represents an array of encouraging therapeutic avenues in the treatment of T1D. Even if exhaustion fails to permanently prevent progression to symptomatic diabetes, it may still have therapeutic value in slowing beta cell loss, which has been demonstrated to have favourable outcomes in preventing the sequelae of

T1D. However, this field is relatively new, and as such it is difficult to navigate, understand, and synthesise the current literature. The continuation of research that aims to understand the role of $CD8^+$ T-cell exhaustion in T1D is needed.

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CONFLICT OF INTEREST

The authors have no conflict of interest declare.

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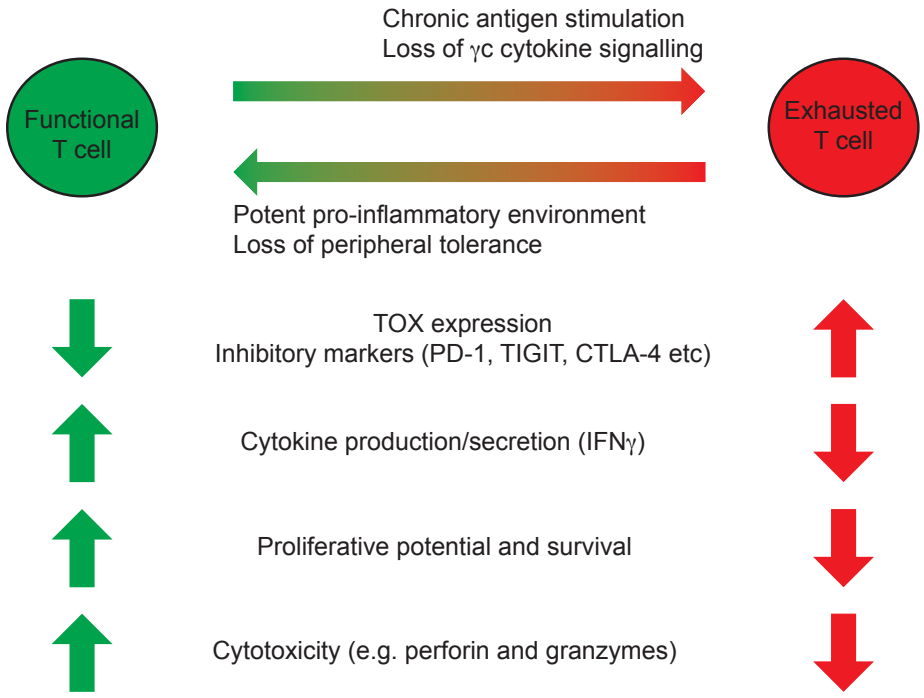
Figure legends

Figure 1. Functional dichotomy of T-cell effector capacity. T-cell functionality described on a spectrum from completely functional to severely exhausted (non-functional). Different external stimuli can push activated T cells towards either state. The exhaustion phenotype is desired as a therapeutic target in autoreactive memory T cells as this will prevent reconstitution of autoimmunity.

Figure 2. (a) Transient antigen exposure (e.g. acute infection) results in differentiation of naive $CD8^+$ T cells into effector T cells, allowing them to clear antigen. Once antigen is cleared effector T cells are outlived by a smaller population of memory T cells that express common gamma chain cytokine receptors and respond to these cytokines to survive for long periods in an antigen independent manner. Memory T cells proliferate and upregulate effector machinery upon reactivation following secondary antigen exposure.

(b) In contrast, during prolonged antigen exposure as a result of different treatments (e.g. during insulin therapy, after islet transplantation or inhibitory receptor agonism early in the disease), T cells are unable to fully clear antigen and gradually develop into exhausted T cells with decreased effector function, proliferation and self-renewal capacity. The exhausted T cells express low levels of the common gamma chain cytokine receptor and depend on persistent antigen stimulation for proliferation and survival. The exhausted phenotype results from a differentiation process in which T cells stably adjust their proliferative and effector capacity to a lower level and this phenotype is optimised to cause minimal tissue damage while still mediating a critical level of antigen control. Note that the ability of exhausted T cells to mount an immune response is disproportionately small for their population size, and thus antigen can persist even longer. In the setting of persisting antigen, early exhausted T cells differentiate into terminal exhausted T cells and in some cases can eventually be deleted entirely.



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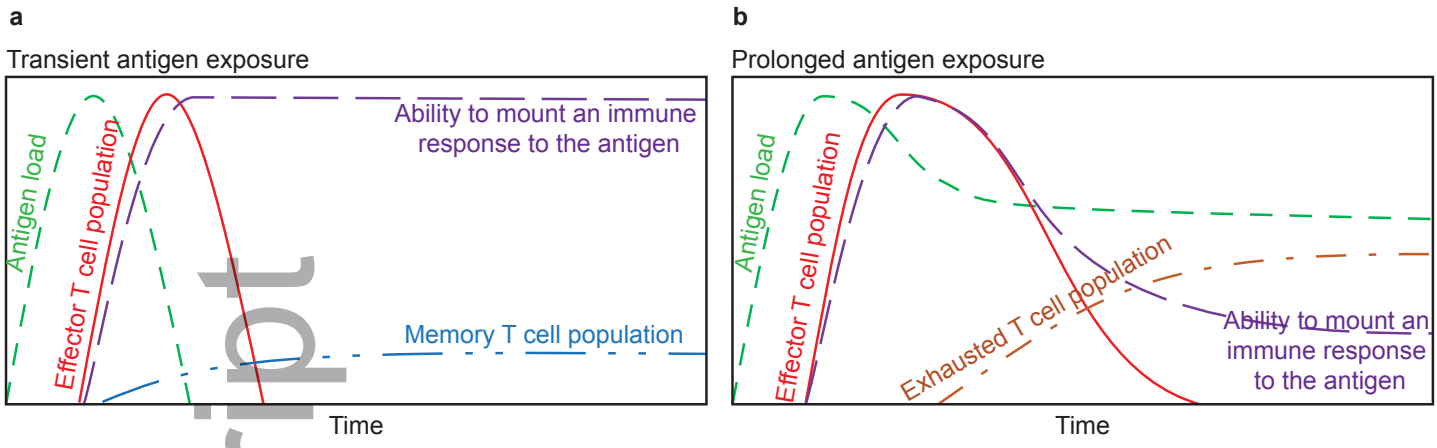

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