TRANSFORMING GROWTH FACTOR BETA-BASED THERAPIES, A POTENTIAL MODULATOR OF THE IMMUNE RESPONSE IN TYPE 1 DIABETES?

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Disclosure: The author has declared no potential conflicts of interest.
Received: 31.07.15 **Accepted:** 17.09.15
Citation: EMJ Diabet. 2015;3[1]:96-102.

ABSTRACT

Immunobiological interventions are proving to be an exciting new area for mobilising the immune response towards certain tumours. In contrast, classical immunotherapeutic interventions aimed at dampening the autoimmune response to host tissue have been less successful; this is particularly evident for Type 1 diabetes (T1D). In part, the failure to control autoimmunity in T1D relates to the complexity of the immune response to β cells. To resolve this dilemma, immunologists are turning to immunobiological agents that were initially deemed too high risk for therapeutic use due to their potential to inadvertently promote autoimmunity or induce deleterious side effects. Two of these immunobiological mediators under consideration are transforming growth factor β (TGFβ) and tolerogenic dendritic cells (DCs), both of which have shown robust control of the anti-islet response in animal models of T1D, the latter also recently documented to be acceptable for trialling in patients with T1D. In this review, both the challenges of translating immunobiological therapies discovered in animal models of T1D to man and the potential of TGFβ and tolerogenic DCs in the T1D setting will be discussed.

Keywords: Dendritic cells (DCs), $CD8^+$ T cells, transforming growth factor β (TGFβ).

PATHOLOGY OF TYPE 1 DIABETES: KEY PATHWAYS TO TARGET

Our knowledge of the immunological pathways that contribute to the breakdown of the immune system's tolerance for insulin-producing β cells is enabling the key cells and/or pathways to be targeted by therapeutic interventions. Animal models of Type 1 diabetes (T1D) have enabled delineation of the step-by-step process that leads to T1D development, several of which have been recapitulated in man.[1] T1D is a chronic condition that involves the cooperative interaction of the non-antigen-specific innate arm[2] of the immune system with the antigen-specific adaptive arm.[3,4] The B and T cells comprising the adaptive immune system have antigen-specific receptors on their surfaces, each cell expressing a unique receptor specific for a defined antigen, such as those present on pathogens, for example. Prior to the release of competent B and T cells from their developmental niches into the peripheral circulation, cells that bear receptors for host antigens are destroyed. However, this process is not absolute and B and T cells with autoreactive receptors are present in the bloodstream of both animals and man. In animal models of T1D, extensive infiltration of islets by immune cells precedes β cell destruction. This cellular infiltrate generates a *de novo* lymph node-like structure with defined B cell and T cell areas.[5] This islet environment is enriched with a vast array of pro and anti-inflammatory molecules: tumour necrosis factor α (TNFα)[6] and interferon γ[7] are the predominant pro-inflammatory molecules, and interleukin (IL)-4[8] and IL-10 are the predominant anti-inflammatory molecules. The induction of the T1D process occurs in the pancreatic lymph node (PLN), where migratory antigen presenting cells (APCs), most likely dendritic cells (DCs), bearing islet peptides in association with T1D-associated

major histocompatibility complex (MHC) molecules and costimulatory molecules, interact with islet-specific $CD4^+$ and $CD8^+$ T cells, inducing T cell activation.[9] Activated T cells differentiate into specialised subsets, with their effector functions being defined by the cytokines they produce. Activated T cells leave the PLN and migrate along a chemokine gradient to the islets, where a second round of activation occurs following interaction between T cells and APCs *in situ*. The culmination of the dynamic islet environment is the transformation of $CD8^+$ T cells into cytotoxic T lymphocytes (CTLs) and memory cells,[10] both of which bind to MHC class I-bearing β cells, triggering the release of the $CD8^+$ T cells' cytotoxic granule contents and ultimately inducing β cell death by apoptosis.[11] This step-by-step pathway for β cell destruction has led to several therapeutic strategies that target the activation, differentiation, migration, and survival of APCs and T cells, or the apoptotic process in β cells.[12] Although some immunobiological approaches have shown promise in man,[13,14] indefinite resolution of T1D in patients or prevention of T1D progression in individuals with a high risk of developing T1D has been ineffective. It is clear that more robust immunobiological therapies are necessary to tackle T1D. In this review, two new emerging therapies, the transient and localised introduction of transforming growth factor β (TGFβ) into islets and the use of tolerogenic DCs, will be discussed.

THE CHALLENGE OF TRANSLATING THERAPIES FROM MOUSE TO MAN

Immunotherapies selected for investigation as potential modulators of an autoimmune response in man are usually based on their efficacy in animal models of the human autoimmune condition. This is particularly prevalent in autoimmune diseases such as T1D in which the tissue under immunological assault is largely inaccessible for investigation in man. The non-obese diabetic (NOD) mouse[15] and BioBreeding rat[16] are the two most common murine models used for immunopathology studies of T1D, due to similarities in the genetic, environmental, and immunological mechanisms that are believed to contribute to T1D in man. For example, many genetic loci linked to T1D in man and mice encode immunoregulatory proteins,[17,18] six of which are shared between mouse and man. Furthermore, T cells with known diabetogenic activity have shared specificities in both mouse and man.[1] It is somewhat disappointing, therefore, that many immune intervention strategies that show efficacy in murine models do not translate well to man, although it should be noted that all therapies that do show partial efficacy in man were discovered via murine investigations.[12] There are several potential reasons for the poor translational rate for therapies between mouse and man: the lack of randomised, double-blind studies in animals leading to potential bias in interpretation of data; the broad spectrum of patients recruited into clinical trials; and the lack of robust biomarkers that identify the earliest stages of the T1D process in man, which is a time period when most therapies are efficacious in murine models. To tackle the former concern, guidelines on therapeutic trials in mice have been revised in recent years and it is a requirement by certain funding bodies that experimental design for murine studies align with clinical trials in man.[19] For the latter two points, stratification of data from completed clinical trials has revealed that some therapies previously thought to be ineffective actually have positive outcomes in subsets of T1D patients. More problematic are the differences between the murine and human immune systems. Although there is a high degree of homology between the human and murine genomes, there are distinct phenotypic and functional differences in both the adaptive and innate immune systems between the two species.[20] This concern has pushed the next generation of animal models to create 'humanised mice' in which human haematopoietic stem cells,[21] peripheral blood mononuclear cells,[22] or islet-specific $CD8^+$ T cell clones from T1D patients[23] are engrafted into murine strains devoid of the IL-2 receptor common gamma chain (IL-2Rγ), a molecule important for the development of B cells, T cells, and natural killer cells.[24,25] Several strains of humanised mice have been developed in which the IL-2Rγ mutation is paired with deficiency in the recombinase-activating gene (which is important for the formation of B cell and T cell receptors), mutations resulting in severe combined immunodeficiency, and/or transgenic expression of T1D-relevant human MHC haplotypes.[26] Such humanised mice have enabled selection of particular therapies that could show the most promise in man.[27] Nevertheless, to date, engraftment of the desired human cell populations is variable depending on the humanised mouse used, and no humanised mouse perfectly recapitulates the human immune system due, in part, to the molecules expressed or produced, for example by murine stroma cells incapable of

inducing the appropriate developmental/survival signals for establishment of a complex human immune system. Addressing this caveat is currently being hotly pursued,[28] and it will be interesting to see how newer strains of humanised mice recapitulate a fully functional human immune system.

HARNESSING THE IMMUNOSUPPRESSIVE PROPERTIES OF TGFβ FOR IMMUNE INTERVENTION IN TYPE 1 DIABETES

A defining feature of T1D in animal models of the condition is the chronic pro-inflammatory nature of the islet environment. One of the most dominant pro-inflammatory molecules present in inflamed islets, from the initial infiltration of immune cells to the final destruction of β cells, is TNFα.[6] The importance of TNFα in pushing the diabetic response was exemplified by the evidence that manipulation of intra-islet TNFα levels changed both incidence and kinetics of T1D occurrence in animal models: increasing TNFα accelerated disease progression, whereas blockade of TNFα signalling prevented disease occurrence.[6] This link between TNFα and T1D prognosis also holds true for man, with certain TNFα gene polymorphisms being associated with T1D susceptibility.[29] The chronic inflammatory environment created by TNFα presents a particular challenge in designing effective therapies, as the pro-inflammatory molecule enables several immunoregulatory pathways to be bypassed.[30] Although it would seem reasonable to assume that blockade of TNFα would be beneficial in T1D, an approach that has been successfully employed for the short-term treatment of rheumatoid arthritis,[31] long-term blockade of TNFα would likely prove detrimental for the normal function of the immune system.

Concerns regarding widespread, systemic modulation of key molecules, such as TNFα, involved in many diverse homeostatic and immunological functions led us to speculate that localised and temporal introduction of a potent immunosuppressive molecule may disable the autoimmune response but preserve normal immunity to infection. We selected human TGFβ as our immunoregulatory compound and designed a model system in which the timing and duration of TGFβ production by β cells in the islets of NOD mice was tightly controlled.[32] TGFβ is a well-known immunoregulatory molecule produced by cells of the innate and adaptive immune systems; a member of a family of signalling molecules, TGFβ not only suppresses activation of immune cells, it is also involved in the development and homeostasis of non-immunological tissues. This divergence in function is linked to the tissue-specific expression of the three receptors that TGFβ can bind to: TGFβR1, TGFβR2, and TGFβR3, the first two receptors cooperatively interacting to induce immunoregulation of the target cell. Transmission of signals through TGFβRs is governed by a series of SMA and MAD-related (SMAD) proteins; principally phosphorylated SMADs 2 and 3 that are chaperoned to the nucleus by SMAD 4. Shutdown of TGFβ signalling is achieved by increasing levels of the repressor SMADs 6 and 7 in the target cell.[33] One of the most documented properties of TGFβ is its involvement in the development and function of both natural and induced $CD4^+$ regulatory T cells (Tregs).[34] Tregs represent a unique lineage of $CD4^+$ T cells intricately equipped to dampen autoimmune responses. Many studies have documented a link between paucity in Treg numbers and/or decreased functionality contributing to autoimmunity, including T1D.[35] Furthermore, disruption of TGFβRs on islet-reactive $CD8^+$ T cells empowers their resistance to Treg-mediated suppression.[36] TGFβ would seem, therefore, a natural choice as a therapeutic molecule to control T1D. However, grave concerns surround the use of TGFβ therapy to control autoimmunity. Although the presence of TGFβ can be beneficial in the early stages of the autoreactive response, it has been shown to be detrimental following the induction of autoimmune-related complications leading to tissue dysfunction due to the fibrosis-inducing properties of TGFβ.[37] In NOD mice, for example, the constitutive transgenic production of TGFβ in islets led to severe pancreatic fibrosis and decreased the lifespan of afflicted mice. In addition, TGFβ is strongly linked to the propagation of tumours. It must be noted that this latter, unwelcome property of TGFβ is potentially linked to the type of tumour and whether the tumour is forming or metastasising.[38] In part, the detrimental properties of TGFβ are related to cross-talk between pathways that are involved in multiple steps of tissue homeostasis.[39]

We hypothesised that our approach of a temporal and site-directed introduction of TGFβ into the target tissue, using β cells that have been genetically modified to express TGFβ under control of a doxycycline-regulated transcriptional switch, may dissociate the desired immunosuppressive

properties of TGFβ from the unwanted fibrotic/tumour-propagating properties of the molecule. Encouragingly, this seemed to be the case: in NOD mice transgenic for these TGFβ-modified β cells, a 1-week exposure of the islet environment to TGFβ resulted in either protection from T1D progression or a significant delay in disease development,[32] with no evidence of adverse reactions in any tissue investigated. Two features stood out from this report. Firstly, preliminary mechanistic studies determined that, despite extensive evidence that TGFβ promotes Treg behaviour, the immunosuppressive effects of TGFβ on the autoreactive response to β cells was independent of Treg cells. Secondly, the timing of delivery of TGFβ was critical for reaping the benefits of the molecule's immunosuppressive property: TGFβ specifically targeted the aggressor phase of the T1D process, as similar transient introduction of TGFβ prior to widespread β cell destruction had no impact on disease progression. Our data suggested that the TGFβ targeted the anti-islet CTL and memory response. Although overall levels of $CD8^+$ T cells were not diminished in protected islets, the phenotype and function of these aggressor $CD8^+$ T cells was altered. Although it is speculative, it is possible that TGFβ triggered a de-differentiation of CTL and memory $CD8^+$ T cells back to a pseudo-naïve status.

However promising transient and site-directed TGFβ therapy may be, challenges lie ahead in how this approach is adaptable to man. Nevertheless, the desire to design novel vehicles that enable pancreatic introduction of therapeutic molecules is an area of active research and may yield a range of potential approaches to introduce TGFβ exactly where and when it is needed in order to prevent progression of, or resolve, T1D. In the meantime, succinctly establishing the mechanisms by which transient TGFβ can modulate the key killer cells in T1D may offer greater insights into the T1D process itself. One potential subset of cells that may be sensitive to the immunosuppressive properties of TGFβ is the stem cell-like memory $CD8^+$ T cells.[40] Stem cell-like memory $CD8^+$ T cells form at the same time as CTLs and conventional memory $CD8^+$ T cells, and act as a reservoir of precursor cells that can repopulate the CTL and memory $CD8^+$ T cell compartments if they become compromised.[40] Recently, Skowera et al.[41] documented increased levels of islet-reactive stem cell-like memory $CD8^+$ T cells in T1D patients compared with control cohorts, suggesting that the increase in this cell population may serve as a novel biomarker for disease progression. In this context it is interesting to note that new preliminary data from our laboratory demonstrated that the ability of our TGFβ-based therapy to completely protect from T1D development, as opposed to significantly delaying disease occurrence, was linked to the level of islet-residing stem cell-like memory cells remaining following cessation of TGFβ signalling, which in turn correlated with the levels of intra-islet TNFα (EA Green, unpublished observations). This interplay between pro and anti-inflammatory cytokines at the level of stem cell-like memory $CD8^+$ T cells is under investigation.

TGFβ-TOLERISED DENDRITIC CELLS AND ISLET TRANSPLANTATION

T1D was initially defined as an autoimmune assault on β cells leading to their complete annihilation. Now we know that some β cells survive the initial assault by the immune system, but these cells become increasingly dysfunctional. Although treatment of T1D patients with drugs may restore the functionality of these residual β cells, it is unlikely that sufficient insulin is produced to resolve T1D. It is therefore likely that effective immunobiological therapies for T1D patients who have a substantial loss in β cell numbers will need to be combined with either additional therapeutic interventions that either induce endogenous β cell regeneration or islet transplantation. Preventing rejection of transplanted islets is particularly challenging: the high number of donors necessary for one recipient increases the risk of allogenic reactions coupled to existing anti-islet memory T cells that rapidly target the transplanted β cells for destruction.[42,43] The ability to generate large quantities of functional islets from a patient's stem cells[44] will hopefully resolve the problems of the paucity and alloreactivity of β cells, but the problem of the memory T cell response to β cells remains.

TGFβ-based therapies may offer a solution to this problem. Based on the finding that TGFβ-modified β cells were capable of abrogating both effector and memory T cell responses to β cells, Thomas et al.[45] explored the possibility that islets containing these modified β cells may impede the immune response to β cells in the transplantation setting, where memory T cells can reignite autoimmunity against syngeneic tissue. To test this, 300–500 syngeneic islets containing either TGFβ-modified or normal β cells were transplanted under the

kidney capsule of diabetic NOD mice recipients, and graft survival studies were performed. Although transplantation with islets containing normal β cells restored normal glycaemia, this was transient and lasted <4 days. In contrast, transplantation of islets containing TGFβ-modified β cells that secreted TGFβ for up to 21 days post-transplantation resulted in significant functional graft preservation. Importantly, no fibrosis in the transplanted tissue was apparent. Two key observations in recipient mice receiving TGFβ-modified islets versus normal islets were documented: the transient production of TGFβ at the graft site impeded infiltration of grafted islets with T cells but not DCs; and in the graft-draining renal lymph node, DC activation was reduced, resulting in decreased activation of islet-specific T cells and significantly lower production of pro-inflammatory cytokines. Although promising, it was difficult to envisage how a similar approach of using islets in which modified β cells transiently produce TGFβ for a short duration post-transplantation could be used in the clinical setting. Transformation of human β cells with self-limiting viral vectors[46] expressing TGFβ could potentially be a source of TGFβ-modified donor islets in man, although the safety of such viral vectors in the clinic is a concern.

The correlation between TGFβ, DC phenotype, and graft acceptance led Thomas et al.[45] to speculate that the TGFβ-enriched environment may modify DCs *in situ*, generating tolerogenic DCs that actively suppress the effector and memory T cell response to the transplanted islets. To test the theory that TGFβ-tolerised DCs were potent suppressors of graft rejection, bone marrow-derived DCs were exposed to TGFβ *in vitro* for 24 hours and then transplanted into diabetic recipients in combination with islets containing unmodified β cells. In contrast to the previous study, this alternative TGFβ-based approach prevented islet graft destruction indefinitely in the majority of diabetic recipients. Although the exact mechanisms by which these TGFβ-tolerised DCs robustly disable the immune response to transplanted islets is yet to be elucidated, the approach of exposing DCs *in vitro* to TGFβ as opposed to introducing TGFβ *in vivo* in a transplanted graft is likely to be more amenable to the medical community as a potential translational therapy in man.

The concept of using tolerised DCs therapeutically is not new.[47] The central role of DCs in the activation of T cells has been exploited for some time to generate DC-based therapies for cancer, where a potent immune response to the tumour is desired.[48] The converse of using modified DCs to 'switch off' T cells was for some time viewed with scepticism: there were concerns as to whether a specific subtype of DC should be selected for tolerisation,[49] and also concerns regarding the stability of the tolerogenic DC profile. Nevertheless, a vast array of approaches have been shown to tolerise DCs, with the ultimate goal being to prevent activation, function, or survival of autoreactive T cells. For example, treatment of DCs with immunomodulatory compounds such as vitamin D analogues,[50] or knockdown of costimulatory molecules[51] that are essential for DC-mediated activation of T cells, have shown efficacy in generating stable and functional tolerogenic DCs (reviewed extensively in Van Brussel et al.).[52] Furthermore, a recent Phase I clinical trial using tolerogenic DCs in T1D patients proved promising; the therapy was well tolerated and deemed safe.[53] This finding has opened the door to start trialling tolerised DC therapy in T1D, although the best tolerisation strategy and the question as to whether peptide-pulsing of the tolerogenic DCs with islet antigens is necessary still needs to be resolved. Nevertheless, the potential of using tolerogenic DCs to treat autoimmune disease is an exciting immunobiological approach that is likely to evolve rapidly.

CONCLUSION

In the past decade we have made strong progress in understanding the pathogenesis of T1D. More robust murine studies and the availability of human samples[54] is enabling stronger correlation between disease pathology in mouse and man. In turn, the growing evidence more clearly points to potential routes for effective therapy. TGFβ-based immunotherapies that separate the immunoregulatory properties of the cytokine from the deleterious pathological properties may offer a new immunobiological approach to tackle T1D progression and/or islet graft rejection. Future research that more concisely delineates the relationship between TGFβ-based therapies and the anti-islet immune response will be advantageous in the selection of new immunotherapy pathways.

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