Immune modulation and monitoring of cell therapy in inflammatory disorders

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Investigating regulatory action of tolerogenic dendritic cells

Chapter 4.1

Inducing tissue specific tolerance in autoimmune disease with tolerogenic dendritic cells

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Abstract

For decades, immune suppression has been the conventional treatment in rheumatoid arthritis. Yet, such therapy does not necessarily cope with the cause of the disease, while general immune suppression causes collateral damage. Curing the disease requires an approach targeting the cause. Tissue specific immune modulation may restore tolerance in patients with autoimmune diseases such as RA, but desires knowledge on relevant target autoantigens. In this review, we present the case of type 1 diabetes as prototype autoimmune disease with established autoantigens to set the stage for tissue specific immune modulation in RA using tolerogenic dendritic cells pulsed with autoantigens. This approach enables the induction of autoantigen specific regulatory T cells that exert their tissue specific action through a combination of linked suppression and infectious tolerance, introducing a legacy of targeted and localised immune regulation in the proximity of the lesion. We present this case as an opportunity of disease intervention therapy in RA. Indeed, several trials are in progress in RA employing various types of tolerogenic DCs. With knowledge on the mode of action, nature of the therapeutic autoantigen and excluding confounding effects of concomitant immune suppressive therapy, this strategy may provide novel immune intervention therapy that may also be exploited to prevent RA in high-risk subjects.

Keywords: Tolerogenic dendritic cells, Rheumatoid Arthritis, Type 1 diabetes, vitamin D, regulatory T cells, Loss of self-tolerance, Post-translational modification

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease which primarily affects the synovial joints. Nowadays disease modifying anti-rheumatic drugs (DMARDs) are commonly used in the clinic as therapy for disease management. Unfortunately, considerable numbers of patients do not achieve remission or (fully) respond to such

treatment. Moreover, therapy with DMARDS or biologic immune suppressive drugs also entail undesirable effects such as infections. (1, 2) This all emphasizes the need for novel intervention therapies and ideally approaches that prevent or even cure RA. Knowledge in the field of RA is increasing, yet the precise aetiology and relevant autoantigens are not completely unravelled. The first aim is to understand the mechanism underlying loss of self-tolerance and disease maintenance. We will discuss the latest findings in autoimmune diseases and the trend towards tissue specific immune modulation with tolerogenic dendritic cells.

Loss of self-tolerance

Loss of self-tolerance is critical in the pathogenesis of autoimmune diseases such as RA. It delineates a state where the maintenance and control of autoreactive T and B cells is disrupted through breaching central or peripheral tolerance. Dendritic cells (DCs) play an important role in maintaining peripheral tolerance as well as inducing an immune response. (3) Thymic medullary DCs present self-antigens to T cells, thereby mediating positive and negative selection to (high affinity) autoantigens expressed in the thymus. Native self-peptides binding with low affinity to MHC molecules remain invisible or secluded and barely contribute to shaping of the thymic T cell repertoire. (4) In addition, the transcription factor autoimmune regulator (AIRE) regulates the thymic expression of antigens present in the periphery, and limited expression of tissue specific antigens in the thymus could contribute to incomplete negative selection. (5) Consequently, the presence of autoreactive T cells in the circulation recognizing low-affinity or non-thymically expressed self-peptides is unavoidable. Peripheral tolerance checkpoints outside the thymus are therefore necessary to secure self-tolerance. (6)

Escape mechanisms revealed in Type I diabetes

In type 1 diabetes (T1D) patients, T cells specific for intermediate to low-affinity islet specific peptides were found, whereas these T cells were rarely found in control

subjects. (7, 8) Higher availability of low-affinity peptide epitopes in the periphery may be sufficient to activate T cells. While autoreactive T cells are often assumed reacting with high affinity binding peptides, we showed that low- affinity peptides should also be taken into consideration in the context of autoimmunity and lack of self-tolerance. Another finding in T1D points to an alternative escape mechanism of central tolerance, i.e. post-transcriptional modification of tissue specific proteins, hence potentially affecting thymic negative selection. Here, expression of splice variants of an islet-specific gene, IGRP, differed between thymus versus pancreatic beta cells that was associated with lack of T-cell tolerance. (9, 10) Finally, the finding that CD8 T cells, barely in contact with MHC due to very low-affinity binding still have destructive abilities (11) puts forward the need for efficient peripheral regulation of autoimmune response to resists the destructive responses.

Post-translational modification as a mechanism of escaping central tolerance

Post-translational modification of proteins in tissues may produce peptides with high affinity to MHC and enhances the risk of priming autoreactive T cells. Assuming that such modified antigens are not expressed in the thymus, the repertoire of T cells is not adjusted to tolerate these antigens. In RA, citrullinated antigens appeared an important group of autoantigens to which an autoimmune response is directed, as anti-citrullinated protein antibodies (ACPA) are present in about 70% of RA patients. (12) Citrullinated proteins are post-translationally modified through peptidyl arginine deiminase (PAD) which converts arginine to citrulline. (table 1) Citrullination occurs physiologically under several conditions, including inflammation. (13) Smoking is proposed to promote this form of post-translational modification and is an environmental risk factors for developing RA, especially for individuals with susceptible HLA-DRB1 alleles. (14) RA shows a strong association with MHC molecules, in particular HLA-DRB1-shared epitopes (SE), which also brings forward the important role of antigen presenting cells (APCs) in shaping and controlling autoimmune responses. (15) Remarkably, HLA-DRB1 SE-molecules can display a high

affinity for citrullinated antigens. Indeed, RA-predisposing SE alleles act as immune response genes in the ACPA response, because they influence both the magnitude and the specificity of this RA-specific antibody response, whereas protection from ACPA is associated with protective HLA-DR13. (16, 17) When presented by inflammatory APCs neo-epitopes generated from citrullinated proteins are proposed to elicit an immune response that results in joint inflammation. Indeed, citrullinated proteins are found abundantly in inflamed RA joints, but are not specific to RA. (6) It is still unclear how a response to generally presented citrullinated proteins leads to localized joint inflammation. More recently in RA patients, antibodies against differential modified carbamylated proteins (anti-CarP antibodies) have been found. Here, the amino acid lysine is converted to homocitrulline by a chemical reaction. Anti-CarP antibodies form a distinct antibody cluster without overlap in recognition with citrullinated proteins. (18)

Posttranslational modification of autoantigens as a mechanism to generate novel auto antigens has been described in other inflammatory disorders and autoimmune diseases. For example, modified islet autoantigens are involved in autoimmune T1D. Autoreactive T cells against modified preproinsulin peptide have been found in T1D patients. (19) In this study, the enzyme tissue transglutaminase, also involved in celiac disease, alters preproinsulin by deamidation, creating negatively charged peptides which binds with high affinity to the risk molecules HLA-DQ8 in T1D. In RA, citrullinated vimentin peptides have been identified as T cell epitopes in HLA-DR4-positive patients. (20) To conclude, post-translational modification of antigens constitutes an important process involved in autoimmune diseases through generating high-affinity peptides in inflammation, which have not yet been presented to the immune system.

Peripheral tolerance deficiencies in autoimmune diseases

It is established that regulatory T cells (Tregs) are essential for maintaining peripheral tolerance and to prevent autoimmune diseases. (21) Defective Treg function is found in patients with autoimmune diseases. (22-25) However, effector T cells resistant to regulation have also been reported, implicating several mechanisms leading to impaired regulation. (26) Tregs from peripheral blood in RA patients are functionally different than in healthy controls, failing to regulate pro-inflammatory cytokines released by effector T cells. (27) Another study demonstrated both increased percentages and functionality of Tregs in RA synovial fluid, compared to peripheral blood. Here, the inadequate immune regulation seemed due to the impaired susceptibility of effector T cells. Even though some studies show effector T cells to be less susceptible to Treg suppressive functions, increasing Treg numbers does enhance regulation. (28) A therapeutic approach expanding Tregs involved in autoimmune regulation may restore tolerance in patients.

The role of DCs in maintaining peripheral tolerance and inducing immune response

Being the ultimate controllers of the immune response, we should take into account the essential involvement of DCs in maintaining peripheral tolerance. Immature DCs residing in peripheral tissues contribute to tolerogenicity and avoidance of destructive T cell autoreactivity through the induction of anergy and T cells secreting immunomodulatory cytokines (adaptive Tregs). On the other hand, these DCs mature upon sensing various danger signals in inflammatory milieus to activate naïve T cells thus losing their regulatory competences. Murine studies have demonstrated the central role of DCs in breaching self-tolerance and initiating RA. (29) Two signals are required to activate T cells: presentation of antigen in MHC-peptide complex and activating co-stimulatory molecules. Additionally, cellular adhesion molecules and pro-inflammatory cytokines support effector T cell activation. (30) DCs process and present yet unknown antigens to inflammatory T-cells that activate B-cells to produce ACPA (figure 1). Also, other cell types such as B cells might trigger autoimmunity since

DC-less mice are able to break self-tolerance and develop fatal autoimmunity. (31) For therapeutic purposes, it would be useful to prevent full maturation of peripheral DCs. Since this cannot easily be achieved in vivo, an alternative approach is to generate tolerogenic DCs with a stable semi-mature phenotype that present antigen in tolerogenic setting with inhibitory co-stimuli, MHC-peptide complex and anti-inflammatory cytokines such as IL-10 and TGF-β.

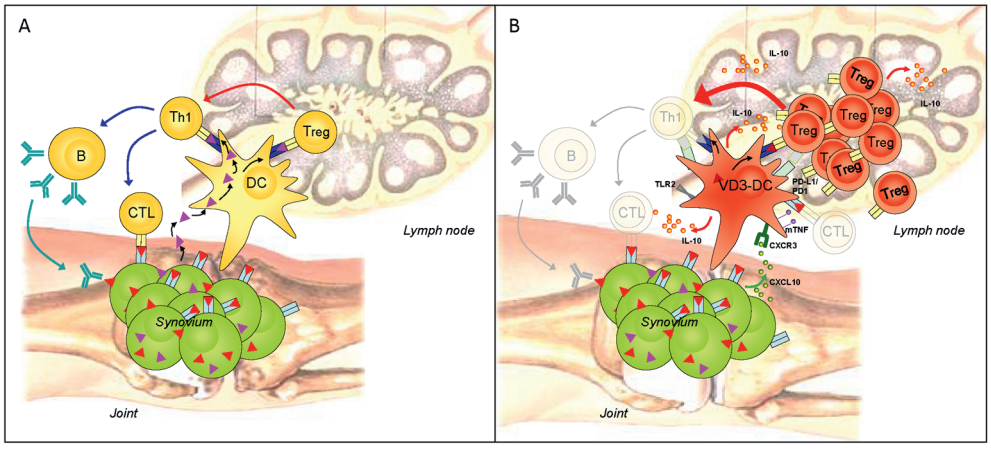

Figure 1. Tissue specific therapy in RA. A) Uncontrolled joint inflammation causes tissue destruction. Dendritic cells process and present damaged synoviocytes and present autoantigens to inflammatory T cells that activate B-cells to produce ACPA. B) In therapy, tolerogenic dendritic cells prime tissue specific regulatory T cells (Tregs) mediated by mTNF. Tregs suppress autoreactive T- and B- cells, selectively inhibiting the autoimmune response in the joint. PD-L1 is involved in inducing apoptosis of effector T cells (CTL and Th1). CXCR3 expressed on the surface of tolDCs facilitates migration to the inflamed joints producing CXCL10.

Joining fields of rheumatoid arthritis and type 1 diabetes

Type 1 diabetes is a prototype tissue specific autoimmune disease sharing interesting features with RA. Yet, several differences are notable in the pathogenesis (table 1). The involvement of adaptive immune responses and presence of autoantibodies in patients with autoimmune diseases has been well established. ACPA show a strong association with RA and predict progression of joint damage in patients, supporting a predominant (pathogenic) role for autoantibodies in the disease pathogenesis. (32) In contrary, the relevance of antibodies in T1D appears less evident. Autoantibodies are not required for diabetes induction as shown in a patient with X-linked agammaglobulinemia, who developed T1D. (33) Clinical remission of type 1 diabetes in patients involved in clinical immune intervention trials rarely associate with changes in islet autoantibodies. (34) It is, however, not yet excluded that autoantibodies could contribute to control of the inflammatory response in the pancreas, which is getting out of hand, and thus might be “smoke” rather than “fire” in T1D. Alternatively, islet autoantibodies are a bystander product of the autoimmunity. Second, while the role of CD8 T cells in RA is not evident, CD8 T cells are key players in T1D and destroy beta cells in the islets of Langerhans. Presence of beta cell-specific CD8 T cells has been demonstrated in human islets. (35) Instead, the CD4 T cells are central in mediating RA and B cell activation, particularly Th type 1 and more recently discovered Th type 17. (14) The contribution of different effectors in RA compared to T1D implies diversity in autoimmune pathogenesis. Still, similar regulatory processes can be involved since Tregs affect miscellaneous cells around APCs such as Th1, B and CD8 T cells. This immune regulation can be defective in autoimmune diseases, allowing development of reactivity to self-antigens (Chapter loss of self-tolerance).

A remarkable similarity of RA and T1D is the association with particular HLA class II alleles. Individuals with HLA-DRB1 SE are at highest risk for developing RA, while in T1D the risk alleles are HLA-DQ2 and HLA-DQ8. The existence of protective HLA alleles

in RA and T1D (HLA-DRB1 containing DERAA and HLA-DQ-6, respectively) is also intriguing, however the mechanisms by which they protect in autoimmune diseases are not yet understood. (36-38)

Table 1. Common features and differences in the immune pathogenesis of RA and T1D.

Similarities		*RA*	*T1D*
=	**Post-translational modification**	PAD converting arginine to citrulline Chemical reaction converting lysine to homocitrulline	Tissue-transglutaminase inducing deamidation of proinsulin
=	**Impaired immune regulation**	Defective Treg function and susceptibility Teff for regulation	Defective Treg function and susceptibility Teff for regulation
=	**Genetic protection**	HLA DRB1 containing DERAA	HLA DQ6
=	**Genetic risk predisposition**	HLA DRB1 SE	HLA DQ 2 and 8

Differences		*RA*	*T1D*
≠	**Relevant autoantigens**	Citrullinated antigens	Beta-cell specific antigens
≠	**Environmental factors**	Smoking	Unresolved
≠	**Trigger for loss of self-tolerance**	Smoking, PTM	Unknown
≠	**Role B and T-cells**	Predominantly B cells and CD4 T cells	Predominantly CD8 T cells
≠	**Role autoantibodies**	Most likely pathogenic Predict progression of joint destruction in RA	Not pathogenic

Heterogeneity of disease population

Disease heterogeneity has to be taken into account when considering therapy application to patients. Both RA and T1D involve heterogeneous populations: the clinical course can vary between patients, being that some patients show a mild disease course and others suffer fulminant autoimmune destruction resulting in rapid physical deterioration. Disparity in autoimmune pathogenesis has as consequence that patients do not respond uniformly to therapy. It is therefore difficult to predict effectiveness of a specific therapy in individual patients. Identifying immune signature associated with clinical benefit through biomarkers and distinguishing subpopulations, will aid assessment of new therapies and progressing towards personalized therapy. Islet transplantation in T1D has guided the discovery of several immune correlates of disease progression and intervention. Several accomplishments of immune monitoring have been achieved in T1D islet transplantation. For example, the presence of autoreactive CD8 and CD4 T cells before transplantation have a high association with graft failure and can predict clinical outcome after transplantation. (39) In RA, ACPA can be used to sub-classify patients in ACPA positive and negative. These subgroups display major differences genetic risk predisposition, remission rates and response to treatment (1) Therefore, ACPA may act as a proper biomarker for a substantial group of RA patients, but it is necessary to define common features of ACPA negative patients which remains difficult. Anti-CarP antibodies are present in about 45% of RA patients and may be an additional entity to further classify RA patients. (18, 32) The presence of varying autoantibodies suggests divergence of involved autoantigens in individual patients. Keeping in mind disease heterogeneity, especially when selecting target autoantigens is imperative for tissue specific immune modulation.

Towards tissue specific immune modulation

Current treatments in RA and T1D are very dissimilar (table 2). RA therapy is mainly focussed on restraining joint inflammation with immune suppressors to avoid pain

and further damage. In T1D, we remain at insulin replacement treatment to control glucose balance, since autoimmune destruction of beta cells in the pancreas obliterates endogenous insulin production. Yet no therapy is used to intervene in the islet autoimmunity causing the disease. Using different approaches, the overarching goal symptomatic control in RA and T1D therapy is essentially the same. Therapy with tolerogenic semi-mature DCs, addressing the disordered balance in the immune system could be applicable in several autoimmune diseases and unites the fields of RA and T1D.

Table 2. Treatment RA and T1D.

Similarities		*RA*	*T1D*
=	**Disease management as current goal of therapy**	DMARD – control joint inflammation	Insulin – control glycemia
=	**Disease heterogeneity**	ACPA positive/negative	

Differences		*RA*	*T1D*
≠	**Use immune suppressive drugs**	Conventional	Not applied as treatment
≠	**Role of anti-TNF**	Beneficial	Ambiguous
≠	**Access to lesion/ imaging**	Possible	Problematic
≠	**Immune monitoring**	-	Diab-Q-kit, Cytokine analysis (ELISA), Proliferation assay (LST)
≠	**Proof of safety human vaccination autoantigens**	Not yet determined	Safety proven (PI C19-A3)
≠	**Definition clinical efficacy**	Radiographic imaging	C-peptide level

When tolerance to self-antigens is lost, restoring the balance of the immune system to counteract autoimmune inflammation might be a solution. Even though understanding disease mechanisms in RA provided targeted immune therapies such as anti-TNFα, these therapies still act as non-specific immune suppressors with the risk of infections. To induce tissue specific immune regulation, there are two requirements: (1) targeting of tissue specific antigens by using (2) anti-inflammatory adjuvants. With the concept that immature and semi-mature DCs have the ability to direct the immune system to a tolerant state, the development of in vitro tolerogenic DCs (tolDCs) has been explored for clinical application in autoimmunity.

Generation of tolDCs in vitro

Various methods generating different types of tolDCs in vitro were studied. Rapamycin, dexamethasone, vitamin A, vitamin D, IL-10 and growth factors such as G-CSF, VEGF, VIP and numerous others are known to induce tolDCs. These tolDCs resemble semi-mature DCs and require shared features, these include anti-inflammatory cytokine profiles (low IL-12 and high IL-10), resistance to maturation and induction of specific T-cell profiles. Stability of the tolerogenic phenotype of DCs and resistance to maturation is of crucial importance as tolDCs will be injected in human and flaring up of autoimmunity would be detrimental. TolDCs modulated with $1,25(OH)_2$ vitamin D_3 (VitD3) alone or combined with dexamethasone preserve a stable regulatory phenotype in vivo upon restimulation with LPS, CD40-L and inflammatory cytokines (IL-6, TNF, IL-β and PGE2). (30) Compared to VitD3 or dexamethasone alone, a combined treatment enhanced modulation regarding surface marker expression, inhibition of pro-inflammatory cytokine production, and decrease of T cell stimulatory capacity. (30, 40) Combined successive treatment with vitD3-dex is for these reasons an attractive modulating therapy to induce tolDCs.

TolDCs mechanisms of action

Tolerogenic DCs function through the induction and stimulation of antigen specific Tregs. Important for this process are programmed death ligand 1 (PD-L1) and membrane-bound TNF (41, 42). Tolerogenic DCs are capable of deleting T cells in an antigen-dependent manner and with co-ligation of PD-L1. (43) Cytotoxic CD8 T cells counter this effect by eliminating tolDCs. (44) Membrane bound TNF, however, is involved in induction of Tregs and blocking of this molecule with adalimumab prevented generation of Tregs and their suppression of proliferation of CD4 T cells. (42)

The induced Tregs (iTregs) are expected to suppress autoreactive B and T cells and dampen the inflammatory reaction (Figure 1) and are, therefore, capable of targeting pathogenical response in both T1D and RA. Upon cognate interaction with DCs, iTregs stimulate the expression of regulatory receptors (ICOS-L and B7-H3) thereby altering the pro-inflammatory DC phenotype to anti-inflammatory, a mechanism refered to called 'infectious tolerance'. These modulated DCs can further prime IL-10 producing Tregs of different specificities co-presented with the iTreg antigen. (45) Therefore, the suppressive effect of tolDCs is not limited to the specificity of the selected pulsed peptide, but spreads to other epitopes expressed in the proximity of the pro-inflammatory DC by linked suppression. Effector T cells with a different specificity than the iTregs are suppressed simultaneously, provided that their corresponding antigen is presented by the same antigen presenting cell. (45) Finally, tolDC express CXCR3 on the surface, which facilitates the migration to the inflammatory lesion producing CXCL10. (46, 47) Taken together, therapy with pulsed tolDCs offers targeted and localised immune regulation in vivo, via induction of tissue specific Tregs in the proximity of the lesion through infectious tolerance and linked suppression. These features render tolDCs ideal as intervention therapy for RA, possibly without a need for additional immune suppressive drugs.

Target autoantigens in RA and T1D

In contrast to RA, autoantigens involved in T1D pathogenesis that may act as targets for therapy are becoming established. A good candidate for therapy is proinsulin that is specifically expressed in beta cells and thus enables targeted tissue specific therapy. Intriguingly, newly diagnosed T1D patients elicit proinflammatory T-cell responses to a naturally processed and presented peptide fragment of proinsulin, C19-A3, whereas healthy HLA matched subjects respond by protective T-cells responses (IL-10). (48) Indeed, T1D patients responding by IL-10 producing T cells develop disease manifesting approximately 7 years later than those not producing IL-10. On this premise, a clinical trial with T1D patients is launched soon using generated tolDCs from autologous monocytes pulsed with proinsulin peptide C19-A3. This peptide has already been tested in humans and proven to be safe for clinical trials. (49) Defining similar target autoantigens is necessary to present tolerance specifically in inflamed joints. A diverse, and still growing list of citrullinated autoantigens are associated with RA such as vimentin, fibrinogen, collagen type II, α-elonase, clusterin, histones and PAD-4. (12)(1) However, most of these antigens exist in tissues throughout the body, rather than being restricted to joints, which may impair the ambition of achieving localised, tissue-specific therapy. Furthermore, RA patients show variable reactivity to autoantigens, further challenging the choice of target antigens for therapeutic application. Besides improving biomarkers to tackle this issue, an alternative readily available approach is the use of a mix of antigens. Yet, since tolDC were shown to act through linked suppression, it is not necessary to know all autoantigenic targets, provided that these occur in the proximity of the therapeutic antigen of choice, or indeed, on the same APC.

Safety of tolDC therapy

In RA, two trials with tolDCs are in progress: Rheumavax using a panel of four citrullinated peptides (vimentin, fibrinogen alpha and beta chain, collagen type II), and AUTODECRA with autologous synovial fluid. (3, 50) Both trials assess safety of

tolDC administration. In these trials, tolDC are attained by blocking NFkB (Rheumavax) or with dex-vitD3 (AUTODECRA), be it that the latter protocol primary modulates through dexamethason, which may impair the capacity of tolDCs to induce antigen-specific Tregs. (41) So far, intradermal injection of tolDC's seems safe in terms of adverse effects such as allergic reactions, exacerbation of autoimmunity and proinflammatory immunity, as preliminary data of the Rheumavax trial and other trials in T1D indicate. (12, 51) Yet, confounding effects of concomitant immune suppressive drugs (especially anti-TNF and etanercept) on tolDC therapy must be investigated and excluded. For instance, membrane-bound TNF is involved in the generation of iTregs. Standard therapy in RA often targets TNF, which may interfere with tolDC efficacy. (42) Anti-TNF drugs can even precipitate new autoimmunity since TNF is important for tolerance induction. We reported a case reported the development of T1D after treating arthritis with anti-TNFα. (52)

Monitoring immunological and therapeutic efficacy

Immune correlates are a requisite to monitor pro- and anti-inflammatory immune responses in patients treated with tolDC's. Investigating suitable biomarkers for RA patients is therefore an important goal. Lessons can be learnt from T1D, where biomarkers of disease progression and therapeutic intervention are extensively studied and validated. (34) In the C19-A3 tolDC trial, immune responses are determined using three methods: 1) cytokine analysis detecting IFN-gamma and IL-10 production upon stimulation with C19-A3 (ELISPOT), 2) T cell proliferation to C19-A3 (LST) and 3) quantification of autoreactive cytotoxic T cells using quantum dot nanotechnology detecting T cells against a range of islet epitopes (Diab-Q-kit). (39) For assessing therapeutic efficacy, RA has an advantage over T1D regarding access to the lesion. Radiographic imaging can be used in RA to display joint damage, whereas T1D relies on biomarkers to estimate insulin production (circulating c-peptide).

Conclusion

We are now able to generate stable tolDCs that can be tailored to induce tissue specific tolerance. This innovation represents an attractive tool to attack the pathogenic source of both autoimmune diseases. The safety of administrating tolDC's has been demonstrated in early tolDC trials in clinical autoimmunity, while the immunological and clinical efficacy needs to be established. Combining knowledge on autoimmune diseases such as T1D and RA helps us understand the common features of autoimmune responses and how to battle these without taking a toll on the immune system controlling infections or tumors. Future research needs to reveal potent targets for tissue specific immune intervention and prevention therapy in RA as well as address the monitoring of immunological efficacy.

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