Differentiation, Expansion, and Homeostasis of Autoreactive T Cells in Type 1 Diabetes Mellitus

Paolo Monti, PhD, Anne-Kristin Heninger, BSc, and Ezio Bonifacio, PhD

Corresponding author
Paolo Monti, PhD
Laboratory for Pre-clinical Approaches to Stem Cell Therapy, Center for Regenerative Therapies Dresden, Tatzberg 47/49, 01307 Dresden, Germany.
E-mail: Paolo.monti@crt-dresden.de

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Autoreactive T cells play a major role in the pathogenesis of type 1 diabetes mellitus (T1DM) and are considered a major target of immunomodulatory strategies aimed at preventing or delaying the disease onset. However, the T-cell response against insulin-producing β cells is still poorly understood. T cells potentially able to recognize and destroy β cells are present in most individuals, but only in a few do they differentiate into pathogenic effectors. Recent and novel findings in T-cell biology on the dynamics of T-cell activation and memory maintenance are shedding new light on the general mechanisms of the T-cell response. In this article, we discuss how new discoveries about T-cell differentiation, expansion, and homeostasis could help to clarify mechanisms of autoimmunity that lead to T1DM.

Introduction

Type 1 diabetes mellitus (T1DM) results from a chronic destruction of insulin-producing β cells presumably mediated by autoimmunity. It is now widely accepted that such an autoimmune process is predominantly T-cell mediated, associated with the activation, differentiation, and expansion of CD4 and CD8 T cells recognizing islet autoantigens, and aided by autoantigen-specific B cells. Much evidence supports this hypothesis. The strongest genetic susceptibility is associated with major histocompatibility complex (MHC) class II alleles and the disease can be alleviated with immunosuppressive drugs directed specifically against T cells. Autoreactive T cells specific for β-cell–associated antigens have been detected in patients with type 1 diabetes [1]. The three major antigens targeted by autoantibodies—insulin, glutamic acid decarboxylase 65 (GAD65), and islet tyrosine phosphatase 2 (IA-2)—contain epitopes recognized by T cells [2].

Unlike autoantibodies that proved to be excellent markers of preclinical type 1 diabetes, T-cell responses to islet cell antigens have been inconsistent in their diabetes specificity and are not uncommonly present in subjects without any other sign of autoimmunity. The presence of autoantigen-responsive T cells in healthy subjects indicates that a defect of negative selection in the thymus is probably not the only determinant in the pathogenesis of T1DM. Recently, regulation of autoreactivity in the periphery as well as understanding how naïve T cells exiting the thymus can turn into pathogenic effectors have been prime areas of research. In this article, we focus on abnormalities of T-cell homeostasis that may be responsible for differentiation and expansion of autoreactive T-cell clones in patients with T1DM that normally remain quiescent in healthy subjects, and discuss the novel therapeutic potential that arises from this area of research.

Differentiation of Autoreactive T Cells

With respect to autoantigen-responsive T cells, an important difference between healthy subjects and patients with T1DM is the stage of differentiation within the autoreactive T-cell pool. After early studies reported increased GAD65 and (pro)insulin-specific T-cell responses in patients with T1DM compared with healthy individuals [3–5], it became increasingly difficult to reproducibly show diabetes specificity to β-cell antigen-responsive T cells. Several studies demonstrated that T-cell responses against T1DM-associated autoantigens could be readily measured in patients with T1DM and subjects without any other sign of autoimmunity [6]. These findings compelled T1DM immunologists to look into qualitative rather than quantitative differences in the autoimmune T-cell repertoire in patients and healthy subjects. The investigations indicate that autoreactive T cells in patients with T1DM

can be distinguished by characteristics that are typical of cells that have already encountered their cognate antigen. These characteristics include proliferation to lower antigen concentration, a relative independence of antigen-specific proliferation in the absence of costimulatory signals [7], and the presence of specific late activation markers [8]. Direct evidence of the in vivo priming of autoreactive T cells was the measurement of T-cell responses in separated naïve ($CD45RO^-$) and memory ($CD45RO^+$) T cells. GAD65 and (pro)insulin-responsive T cells in healthy individuals were exclusively found in the naïve T-cell compartment, whereas in patients and in subjects with preclinical type 1 diabetes, T cells responding to GAD65 and (pro)insulin were found in the naïve and memory T-cell compartments [9•]. Furthermore, analysis of telomere length as a marker of proliferative history of T cells showed that the autoantigen-responsive memory T cells in patients had undergone extensive proliferation in vivo to generate a memory autoimmune T-cell repertoire.

Implications of the Memory Phenotype on Antigen-stimulated T-cell Assays

Autoantibody measurements have had the benefit of robust standardization programs [10,11] and harmonization to develop common assays. Detection and quantification of autoreactive T cells is performed using assays with substantially diverse formats, each with their pros and cons. That diabetes-relevant T cells are already antigen experienced is important in choosing assays and interpreting data. For example, enzyme-linked immunosorbent spot (ELISPOT) assays are used to measure the precursor frequency of antigen-specific T cells by measuring effector cytokine production after a short stimulation period. Naïve T cells respond to antigen by producing interleukin (IL)-2; after several days of stimulation, they start to secrete effector cytokines, such as interferon-γ and IL-4. In contrast, memory T cells produce effector cytokines within hours after activation. Thus, the ELISPOT assay, usually performed after 48 hours of stimulation, should preferentially detect activation of memory T cells, which should theoretically be confined to patients with T1DM and at-risk subjects [12,13]. That these cells do not divide within 48 hours makes it possible to measure precursor frequency (Monti, Unpublished data).

A different technique to detect T-cell reactivity is to measure proliferation of T cells in response to antigens. This is performed by labeling T cells with a fluorescent probe (eg, carboxyfluorescein succinimidyl ester [CFSE]), which is subsequently diluted upon each cell division, therefore allowing proliferation to be detected by flow cytometry [14]. Alternatively, proliferation can be measured by incorporation of radiolabeled thymidine after 6 days of culture. One of the advantages of the CFSE dilution assay is the possibility of studying multiple parameters, such as cytokine profiles, activation markers, and the differentiation state of a single cell. In addition, naïve and memory T-cell populations can be separated and studied independently. Another benefit of this proliferation assay is the possibility to also use MHC multimers to directly detect autoantigen epitope-specific autoreactive T cells [15], which can subsequently be isolated and characterized further. MHC multimers can detect rare antigen-specific T cells; however, they require knowledge of target peptide epitopes and individual HLA alleles and, thus, can be used only on a limited number of patients.

Implications of a Memory Phenotype on Immunomodulation

The presence of a memory autoreactive T-cell repertoire may have a considerable impact on immunomodulation strategies aimed at preventing or delaying T1DM onset. It has been demonstrated that $CD4^+CD25^+$ regulatory T (Treg) cells are very efficient in controlling proliferation of naïve T cells but are much less effective on memory T cells [16]. Also, most immunosuppressive drugs that target T-cell proliferation by inhibiting the IL-2/IL-2 receptor (IL-2R) pathway (eg, calcineurin inhibitors, rapamycin) are more efficient in controlling a naïve rather than a memory T-cell response [17]. Overall, a memory autoimmune repertoire presents a barrier to immunomodulation and, therefore, immune intervention should have the highest chance of success when the autoreactive repertoire still has a naïve phenotype. The efficacy of many therapies that mainly affect immunomodulation on naïve T cells must therefore be dependent on pathogenetic mechanisms associated with the conversion of naïve autoreactive T cells into memory effector T cells. The relative contribution of naïve T cells to the autoimmune destruction in an ongoing autoimmune disease is a crucial question when assessing the potential efficacy of immunomodulating therapies. Paradoxically, therapies that are most effective on naïve T cells may be more successful in aggressive autoimmunity with large recruitment of naïve T cells to inflammatory sites, rather than in chronic immunity with more slowly turning over memory T cells.

Expansion of Autoreactive T Cells

The classic model of T-cell expansion/contraction typical of the immune response to acute infections may not be applicable to T1DM. For example, during acute viral infections, T cells are activated by high-affinity antigens that in turn result in the upregulation of the IL-2R α chain (CD25) and production of IL-2. The IL-2/IL-2R autocrine pathway drives the expansion phase of the clonal population. A contraction phase characterized by massive apoptotic cell death terminates the immune response upon antigen clearance. Although the kinetics of the T-cell response against

β cells is complex and not fully understood, it is likely that this response is raised against low to medium affinity antigen epitopes and that these T cells remain chronically activated after contraction. This notion is based on our understanding of autoreactive clones that exit the thymus, and the fact that the preclinical phase of T1DM can last years and decades [18]. During this preclinical phase, it is thought that the T-cell response undergoes a progressive enrichment of autoreactive clones responding to different antigenic determinants as a result of epitope spreading. Progression to disease, like that reported for autoantibodies [19], may be acute or follow a relapsing/remitting pattern, as was already described for other autoimmune diseases such as experimental autoimmune encephalomyelitis [20]. According to the characteristics of a chronic immune response, the T-cell response in T1DM should be sustained by a slow turnover of the autoreactive T-cell pool. As such, alternative pathways to the IL-2/IL-2R axis are under investigation.

The IL-7/IL-7R Axis in Autoimmunity

A possible mechanism for autoreactive T-cell expansion involves the homeostatic cytokine IL-7. IL-7 has a fundamental role in T-cell survival and acts as a potent mitogen for T cells at supraphysiologic concentrations [21,22]. Increased serum concentration of IL-7 has been observed in patients with reduced T-cell counts in several pathologic (eg, viral infections such as measles and HIV) and iatrogenic (eg, chemotherapy, radiotherapy, and immunosuppression) conditions. Exposure of the immune system to IL-7 triggers homeostatic proliferation of memory autoreactive T cells [23••]. The phenomenon of IL-7–mediated homeostatic proliferation seems to promote broadening of the T-cell receptor repertoire by favoring peripheral expansion of low-medium affinity T cells specific for antigens, together with thymic output of newly generated T cells [24••,25].

The IL-7/IL-7R axis has been implicated in the pathogenesis of autoimmunity and type 1 diabetes. In the nonobese diabetic (NOD) mouse, a natural condition of lymphopenia and consequent homeostatic proliferation has been linked to the generation of the autoimmune response [26]. In patients with T1DM undergoing islet transplantation, immunosuppression results in reduction of T-cell counts and consequent increase of serum IL-7. This was shown to increase the proliferation of memory T cells in vivo and expand the circulating autoantigen-responsive memory T cells.

Further evidence for a potential role of the IL-7/IL-7R axis in the generation and expansion of autoreactive T cells comes from recent genetic studies. A genetic association with T1DM susceptibility was found for polymorphisms within the *CAPSL* gene [27]. Although the function of this gene is unknown, it is located adjacent to and in the same linkage disequilibrium block of the *IL-7Rα* gene. More detailed analysis of the 5p13 region, which contains the *CAPSL* and *IL-7Rα* genes, identified two new single nucleotide polymorphisms located in *IL7Rα* that were also associated with T1DM susceptibility [28,29•]. Moreover, a separate genome-wide association study investigating susceptibility genes for multiple sclerosis also identified a single nucleotide polymorphism within the *IL7Rα* that conferred susceptibility to multiple sclerosis [30].

Although a definitive role of the IL-7/IL-7R axis in the pathogenesis of T1DM has not been proven, IL-7 or its receptor may represent a novel candidate target for preventing T1DM. Most of the commonly available immunosuppressive drugs affect the IL-2/IL-2R pathway, which is fundamental for protection from common pathogens and therefore causing general immunosuppression. Blocking the IL-7/IL-7R axis has several advantages over blocking the IL-2/IL-2R pathway. IL-7 is not directly involved in the immune response to common infections, suggesting that immune surveillance against pathogens would not being affected. Second, IL-2 plays a major role in the maintenance of $CD4^+CD25^+$ Treg cells. $CD4^+CD25^+$ Tregs lack CD127 and their survival is IL-7 independent [31]. Therefore, downregulating IL-7 signaling would be suitable in parallel with Treg therapy. Downregulation of the IL-7/IL-7R axis can be obtained by neutralizing IL-7 or by blocking the receptor with specific antibodies. Studies in mice revealed that blocking the IL-7R causes a profound T-cell depletion, which would be undesirable [32]. In contrast, neutralizing IL-7 causes a much less severe T-cell depletion because the pathway has some redundancy and other cytokines (eg, thymic stromal lymphopoietin) can also signal through the IL-7R [33].

Homeostasis of the Autoreactive T-cell Pool: The Stem-like T-cell Concept

Homeostasis of the T-cell compartment is the result of a complex balance between newly generated naïve T cells exiting the thymus and the turnover of lymphocytes resulting from T-cell death and peripheral homeostatic proliferation. In physiologic conditions, the number and the relative abundance of T-cell populations (naïve, memory, and Tregs) are relatively stable. Over time, factors such as a reduction in thymic output with increasing age and exposure to infections modify the relative abundance of the different T-cell populations. How the immune system maintains lifelong T-cell memory in the absence of antigen stimulation is still incompletely resolved. It was thought that T-cell memory is maintained because of the presence of long-lived memory T cells slowly turning over in response to the homeostatic cytokines IL-7 and IL-15. Naïve T cell, memory T cell, and Treg cell half-life and turnover rate have recently been characterized using in vivo stable isotope labeling (^2H_2O) and label decay studies in T cells [34•,35]. This study's

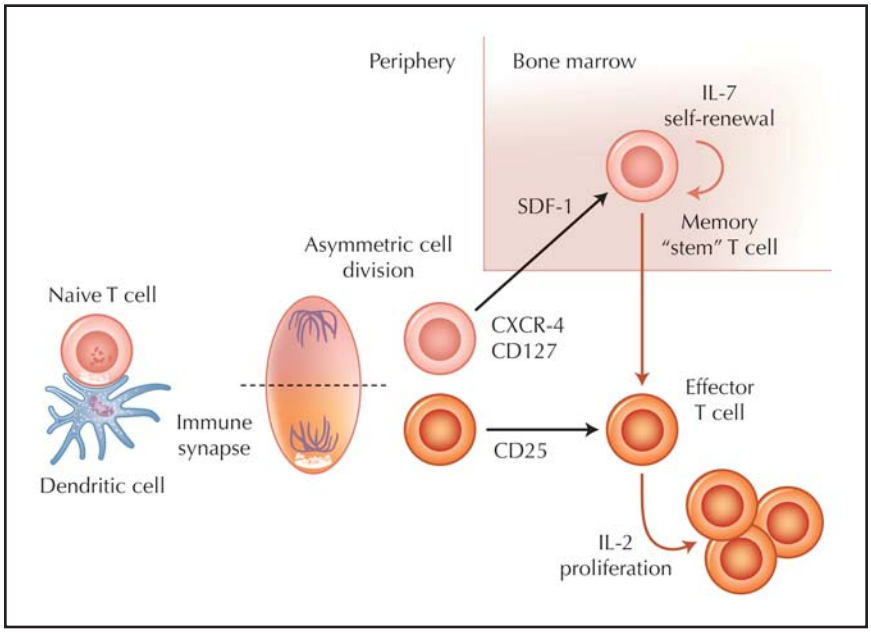

Figure 1. A hypothetical model for generation of memory "stem-cell–like" T cells. A naïve T cell forms an immune synapse with a dendritic cell during antigen presentation. The T cell is activated and undergoes mitosis in which the direction of cell division is perpendicular to the immune synapse. This results in a daughter cell that receives persistent signals from the immune synapse (which has been acquired from the transitory encounter with the dendritic cell) with full activation and subsequent differentiation into an effector T cell ($CD25^{high}$ and $CD127^{low}$). The other daughter cell without the immune synapse does not receive full activation signals, remains resting ($CD25^{low}$ and $CD127^{high}$), and arrests its differentiation to an intermediate state. This incompletely differentiated cell becomes a memory stem-cell–like T cell and migrates to the bone marrow. In the bone marrow, interleukin (IL)-7 contributes to its slow turnover for self-renewal. The memory stem-cell–like cells can enter into the differentiation pathway (eg, upon a second antigen stimulation) to T effectors and migrate to the periphery to replenish a rapidly turning over peripheral T-cell pool. SDF-1—stromal-derived factor-1.

results showed that naïve T cells are long-lived cells with half-lives of several years. Surprisingly, memory T cells are characterized by rapid turnover, with half-lives of only a few months. This raises the question, how can memory T cells turn over rapidly and at the same time avoid cellular aging and telomere shortening to provide lifelong memory? Thus, it has been hypothesized that long-term memory is maintained through a continuous repopulation of antigen-specific memory cells from a population of slowly dividing memory T-cell precursors also called "stem-cell–like" T cells [36,37••]. This pool of T cells is highly committed and can generate only T cells, but would be able to maintain long telomeres and have a capacity to self-renew.

How are stem-cell–like T cells generated? A deterministic mechanism to generate cell heterogeneity termed asymmetrical cell division has recently been proposed as an explanation for achieving fate diversity in daughter cells. It appears that the polarization of naïve T cells during antigen presentation by dendritic cells also coordinates the plane of cell division, which is parallel to the immune synapse. As a result of this polarization, the daughter cell proximal to the immune synapse terminally differentiates into an effector-memory cell. The other daughter cell, which is deficient in activation signals after mitosis, remains in an intermediate stage of differentiation (Fig. 1) [37••,38•]. In contrast to the model of "progressive" differentiation of naïve T cells into effectors and finally memory, the asymmetric cell division model suggests an early bifurcation in the fate of long-lasting memory T cells. An in vivo example that supports this model is the chronic immune response to persistent cytomegalovirus [39]. The remarkable number of effectors required to keep cytomegalovirus infection under control is a population of rapidly turning over T cells in circulation. This population is supported by memory cells primed early in infection with a slow dividing rate that provide "memory inflation" to the circulating memory T-cell pool.

Where are memory stem-cell–like T cells? Bone marrow is gaining new interest in the T1DM pathogenesis. As a primary lymphoid organ, bone marrow is not expected to be involved in the process of autoantigen presentation and activation and expansion of naïve and memory autoreactive T cells, which occurs in pancreatic lymph nodes. However, studies have shown that bone marrow of NOD mice contains a reservoir of autoreactive T cells, suggesting that bone marrow is a preferential homing site for at least some autoreactive clones [40•]. Cells are normally targeted to the bone marrow through the expression of the chemokine receptor CXCR-4, whose ligand CXCL-12 (or stromal-derived factor-1) is expressed at high levels in the bone marrow. Even though bone marrow is not a primary site for antigen presentation, it is rich in IL-7, which is produced by bone marrow stromal cells. The lack of antigenic stimulation and the presence of the homeostatic cytokine IL-7 could make bone marrow a preferential niche for memory-stem T cells. Relevant to this hypothesis, we recently identified a population of circulating $CD4^+$ cells that transiently upregulate CD34 upon antigen stimulation. These cells do not overexpress CD25 and have high levels of CD127 and CXCR-4 (Monti, Unpublished data). In addition, IL-7 upregulates CXCR-4 on T cells. Our hypothesis is that autoreactive T cells generated early in T1DM pathogenesis are retained in the bone marrow where they slowly proliferate in response to IL-7 without differentiating into effector cells, and thus constitute a long-lasting reservoir of autoreactive T cells that can replenish the peripheral autoreactive T-cell pool.

Similar to the IL-7/IL-7R axis, a potential role for the CXCL-12/CXCR-4 axis in T1DM pathogenesis can be postulated. Alterations of the CXCL-12/CXCR-4 axis have been demonstrated in patients with T1DM and NOD mice. In humans, polymorphisms in the *CXCL12* gene have been associated with TIDM susceptibility [41]. In NOD mice, elevated levels of CXCL-12 in the bone marrow result in an increase in T-cell recruitment to the bone marrow. Interestingly, treating NOD mice with AMD3100, an antagonist for the CXCL-12 receptor CXCR-4, mobilizes T cells from the bone marrow to the periphery, and concomitantly inhibits insulitis and delays diabetes onset [42]. These results suggest that the autoreactive T-cell reservoir in the bone marrow could play an important role in the maintenance of the peripheral autoreactive T-cell pool.

Thus, interplay between mechanisms of homeostasis via the IL-7/IL-7R axis and the CXCL-12/CXCR-4 axis could provide the adaptive immune system with long-term memory and a continuous source of antigen-specific T cells via stem-cell–like T cells. Autoreactive T cells with stem cell properties would be an important target for preventing T1DM. Identifying specific markers on such cells could lead to exhaustion and resetting of the autoimmune response to a point where therapies that are effective on naïve T cells can be effective in controlling re-emergence of autoreactivity.

Conclusions

Differentiation of autoreactive naïve precursors into memory T cells is a key step in the development of T1DM. Recent findings suggest that a pool of stem-like memory T cells slowly cycling in response to IL-7 is present in the bone marrow. These cells, able to maintain long-term memory, represent a potential novel target for immune modulation in T1DM.

Disclosure

No potential conflicts of interest relevant to this article were reported.

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