Clinical & Experimental Immunology
The Journal of Translational Immunology
doi:10.1111/cei.12819
Clinical and Experimental Immunology
TYPE-1 DIABETES MINI-SERIES: PATHOGENESIS AND THERAPY
REVIEW ARTICLE

Of the multiple mechanisms leading to type 1 diabetes, T cell receptor revision may play a prominent role (is type 1 diabetes more than a single disease?)

D. H. Wagner
Department of Medicine, Department of Neurology, Webb-Waring Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Accepted for publication 31 May 2016
Correspondence: D. H. Wagner, Department of Medicine, Department of Neurology, Webb-Waring Center, University of Colorado Anschutz Medical Campus, 12850 East Montview Boulevard, Aurora, CO 80045, USA.
E-mail: david.wagner@ucdenver.edu

Summary

A single determinant factor for autoimmunity does not exist; disease development probably involves contributions from genetics, the environment and immune dysfunction. Type 1 diabetes is no exception. Genomewide-associated studies (GWAS) analysis in T1D has proved disappointing in revealing contributors to disease prediction; the only reliable marker has been human leucocyte antigen (HLA). Specific HLAs include DR3/DR4/DQ2/DQ8, for example. Because HLA molecules present antigen to T cells, it is reasonable that certain HLA molecules have a higher affinity to present self-antigen. Recent studies have shown that additional polymorphisms in HLA that are restricted to autoimmune conditions are further contributory. A caveat is that not all individuals with the appropriate 'pro-autoimmune' HLA develop an autoimmune disease. Another crucial component is autoaggressive T cells. Finding a biomarker to discriminate autoaggressive T cells has been elusive. However, a subset of CD4 helper cells that express the CD40 receptor have been described as becoming pathogenic. An interesting function of CD40 on T cells is to induce the recombination-activating gene (RAG)1/RAG2 T cell receptor recombination machinery. This observation is contrary to immunology paradigms that changes in TCR molecules cannot take place outside the thymic microenvironment. Alteration in TCR, called TCR revision, not only occurs, but may help to account for the development of autoaggressive T cells. Another interesting facet is that type 1 diabetes (T1D) may be more than a single disease; that is, multiple cellular components contribute uniquely, but result ultimately in the same clinical outcome, T1D. This review considers the process of T cell maturation and how that could favor auto-aggressive T cell development in T1D. The potential contribution of TCR revision to autoimmunity is also considered.

Keywords: autoimmune diabetes, immune dysfunction, TCR revision

Introduction

Type 1 diabetes (T1D), unlike its far more prevalent counterpart, type 2 diabetes, is a classic autoimmune disease characterized by immune cell attacks of pancreatic islets resulting in hyperglycaemia subsequent to insulin loss. Islets are composed of five cell types that produce hormones and enzymes associated with digestion and nutrient uptake. Beta cells constitute approximately 80% of islets and produce insulin; alpha cells produce glucagon, delta cells produce somatostatin-D, gamma cells produce pancreatic polypeptide and epsilon cells produce ghrelin, the hunger hormone [1].

During diabetogenesis immune cells infiltrate islets creating an inflammatory condition called insulitis. This was first described more than 100 years ago in a 6-year-old girl who died from complications of ketoacidosis [2]. The first cells to infiltrate islets are probably neutrophils [3], followed by antigen-presenting cells (APC), including macrophages and dendritic cells (DC) [4,5]. In fact, merocytic DC that mediate cross-presentation of islet antigens to $CD8^+$ and $CD4^+$ cells in pancreatic lymph nodes could be instrumental in initiating disease [6,7]. Eventually, lymphocytes including $CD4^+$ and $CD8^+$, as well as B cells, infiltrate the islets and are

presumed to be the primary players in loss of insulin. The cellular phenotypes in animal models have been determined, and some of the cellular phenotypes in human islets are known. However, the aetiology of T1D remains a mystery: why do cells traffic to the islets? Why do cells infiltrate the islets? By what mechanism do these invaders cause loss of insulin?

Development of type 1 diabetes (T1D) requires a series of unfortunate events involving alignment of genetic, environmental and immunological contributors. This argument, in fact, can be made for any autoimmune disease. Developmental parameters for all autoimmune diseases are similar in many ways, although the symptoms and clinical outcomes vary dramatically. It has become clear that no individual contributor can cause diabetes, but each contributor acting in concert establishes danger and ultimately causes disease. Interestingly, rates vary greatly, depending upon geography. For example, incidence is high in Finland and Sardinia at approximately one in 250 [8]; in the United States the incidence is currently one in 300 by 18 years of age [9]; and in Canada, Australia and New Zealand the incidence is much lower, at one per 1750. In China and Venezuela the incidence is approximately one in 100 000. A 2012 study of T1D trends in Europe during a 20-year period ending in 2008 reported that 22 of 23 centres in 19 countries showed significant incidence increases, specifically reporting a 3·4% annual increase over the entire period [10]. Another study reports that incidence in Europe saw a yearly increase rate of 2·8% from 1990 to 1998, increasing to 3·2% and then 3·9% in 2010 [11]. The increases are being reported in the very young and those with moderate genetic susceptibility [11]. More and more environmental factors are believed to be contributory, but major determinants have yet to be defined.

Genetics

Surprisingly, while type 2 diabetes is reaching epidemic levels in the United States and other developed countries, T1D incidence is increasing similarly. Epidemiological patterns indicate that the worldwide incidence has increased by 2–5% during the last 20 years [9]. T1D occurs in familial clusters, yet examination of monozygotic twins shows, surprisingly, that concordance is less than 40% [12]. The mouse model of T1D, the non-obese diabetic or NOD mouse, develops intrinsically the same disease characteristics as human T1D, including lymphocytic infiltrates in islets, loss of insulin secretion and hyperglycaemia. NOD colonies are bred to maintain high genetic susceptibility loci multi-generationally to ensure disease, yet within any given NOD colony disease incidence ranges from 50 to 90% in females and 20 to 50% in males [13–15]. Male and female NOD mice are genetically identical, thus gender bias, perhaps hormonal-driven, would appear to contribute to disease. Studies show that early-life microbial exposures determine sex hormone levels that modify progression to autoimmunity in NOD mice [16,17]. Early studies in NOD mice indicate that hormonal imprinting in neonates had an influence on diabetes [18]. The situation for human disease is different; gender bias does not occur in human disease, and female to male disease ratios are virtually 1 : 1 [19,20].

GWAS studies

A concerted effort to address genetic contributions to T1D has been attempted using genomewide analytical studies (GWAS). In T1D, greater than 40 susceptibility loci have been identified thus far [21]. The most significant indicator is the human leucocyte antigen (HLA). HLA haplotypes vary within diseases such that a specific HLA haplotype associates with a particular autoimmune disease. Many of the associated haplotypes are class II; HLA DRB1*1501 (referred to as DR2 or DR15) and DQB1*0601 (DQ6) associate with multiple sclerosis [22], DRB1*0401 (DR4) and DRB1*0101 (DR1) or DQB1*0302 (DQ8) associate with rheumatoid arthritis [23,24], etc. The T1D-associated molecules include DRB1*0301 (DR3), DR4, DQB1*0201 (DQ2) and DQ8 [25]. Class I alleles in addition to, and independently of, class II alleles associate with T1D. For example A*2402, A*0201, B*1801 and C*0501 were considered predisposing; and A*1101, A*3201, A*6601, B*0702, B*4403, B*3502, C*1601 and C*0401 appear to be protective [26]. An intriguing observation was that while DQ2 and DQ8 are linked independently to T1D development, when subjects carry both DQ2 and DQ8 alleles the disease incidence increases drastically [25]. Further study demonstrates that a transposition of the DQ2 α chain to associate with the DQ8 β chain occurs, creating a unique HLA-DQ referred to as trans-DQ8 [25]. Thus autoimmune conditions reflect the presence of unique polymorphisms in MHC genes that further promote disease.

GWAS has generated numerous other indicators including, but not limited to, interleukin (IL)-2R, cytotoxic T lymphocyte antigen (CTLA)-4, IL-27, IL-2, IL-10, signal transducer and activator of transcription (STAT)-4, C-C chemokine receptor type 5 (CCR5) and the lymphocyte marker, CD69 [27]. Typically, CD69 has been identified as a lymphocyte 'activation' marker, but recent studies show that CD69 plays an important decisional role in lymph node migration, cell retention and memory formation [28]. It was determined that CD69 and sphingosine-1-phosphate receptor 1 (S1PR) expressions are intertwined inversely. S1PR expression is required for lymphocyte egress from secondary lymphoid tissues and thymus [29], and CD69 expression increases as S1PR expression decreases [28,29]. GWAS has been performed on a number of autoimmune diseases in addition to T1D, including lupus, multiple sclerosis, rheumatoid arthritis, coeliac disease and Crohn's colitis [30]. After all these studies, thus

far the only discriminating factor for disease prediction is the HLA haplotype, which is not absolute. The hope was that GWAS would discover a single gene or gene cluster that would predict likelihood to develop T1D in much the way that BRCA has done for breast cancer. This has not happened. Given that no clearly predictive genes have yet been discovered, one review summarized the question of 'Have GWASs been a failure?' with a qualified 'yes' [31]. The best conclusion from the information provided by GWAS is that autoimmune diseases require immune dysfunction.

Immune contribution

The reason that HLA is linked with autoimmunity is probably because of its function, the presentation of antigen(s) to T cells. As such, HLA molecules dictate immune response outcomes. HLA class I (HLA-A, B and C) molecules are expressed on almost all cell types while HLA class II molecules are restricted to what are known as professional APC, including B cells, macrophages and DCs [32], and in humans, activated T cells express HLA class II [33]. Once professional APCs encounter exogenous antigens, classically a non-viral antigen, the antigen is taken up via phagocytosis involving membrane engulfment or by receptor mediated uptake; for example, a B cell receptor binding an antigen and the receptor/antigen becoming internalized through membrane invagination. Once internalized the antigen associates with an endosome to be broken down to constituent parts, peptide fragments, nucleotide fragments and lipids, etc. Peptides associate chemically with HLA to be transported to the cell surface for external presentation. HLA molecules are polymorphic, and those individuals unfortunate enough to express HLA haplotypes that can present self-antigens preferentially are clearly much more at risk of developing disease. In addition it is possible that the autoimmune background generates unique autoimmune-favouring HLA haplotypes, such as trans-DQ8 in T1D [25,34]. The physics of antigen presentation leading to a T cell activation event that leads further to inflammation requires that the antigen/HLA association be sufficient to stimulate appropriate T cells. Thus, appropriate T cells carrying T cell receptor (TCR) molecules that can respond to self-antigens must be available, adding the next ingredient in autoimmunity. Another layer of complexity that could contribute to autoimmunity is post-translational modification (PTM) of proteins that may generate novel self-antigens. As much as 90% of proteins produced by mammals undergo some form of post-translational modification [35]. Modifications include glycosylation, phosphorylation, citrullination, acetylation, peroxidation and deamination, etc. [35]. Each of these processes occur in the periphery, thus creating an environment for generation of novel antigens; in other words, proteins and peptides that have not been seen in the thymus by developing T cells. Further complicating this mechanism, it is also possible that autoimmune backgrounds have altered PTM mechanisms compared to non-autoimmune backgrounds. For example, protein glycosylation patterns were different when the protein was isolated from an autoimmune background than when the same protein was examined from a non-autoimmune background [36,37]. A recently described mechanism for creation of novel epitopes is the hybrid insulin peptides (HIP) model, which is acutely appropriate for T1D. Insulin peptides have been considered potential self-antigens in T1D for several years [38]. The HIP model suggests that during type 1 diabetes insulin peptides hybridize with other proteins, creating a unique set of epitopes that may be capable of activating autoaggressive T cells [39].

Generation of rogue T cells and central tolerance

The immunological rules of inflammation are identical for foreign antigen removal response to self-antigen-driven autoaggressive responses: T cells carrying a TCR that have affinity for antigen(s) recognize and react to the presented antigen, leading to production and release of proinflammatory cytokines that establish inflammation. The process by which T cells are generated creates the possibility for development of autoaggression. Pluripotent, immature lymphocytes (designated as double-negative, DN) migrate to the thymus from the bone marrow. Developing T cells in the thymus acquire TCR molecules after recombination-activating gene (RAG)1 and RAG2 recombination proteins become induced during the DN stage of development [40], and this action takes place physically in the cortical region (Fig. 1). The architecture of the thymus is similar to that of secondary lymphoid organs, including lymph nodes, composed of a cortical and a medullary region. Once RAGs are induced, the TCR-β gene is rearranged and the resultant molecule is expressed, associating with a prefabricated α chain. This constitutes the pre-TCR [41]. By as-yet unknown mechanisms, RAG1 and RAG2 proteins are induced once again and the α locus is rearranged, removing the delta gene locus [42]. The newly rearranged α gene is transcribed, translated and the protein derived displaces the pre-α protein and associates with the β protein to create a potentially functional TCR (αβ). This process happens at least somewhat randomly within each developing T cell, therefore any given T cell is as likely to express a self-antigen reactive TCR as it is likely to express a foreign-antigen reactive TCR. At this developmental stage cells have become double-positive (DP) for expression of CD4 and CD8 and remain in the cortical region (Fig. 1). The newly expressed TCR molecules on DP cells interact with major histocompatibility complex (MHC) molecules, including HLA-A, B and C, class I molecules, or HLA-D, class II molecules that are located at the cortico–medullary junction (Fig. 1), to undergo positive selection. Cells that

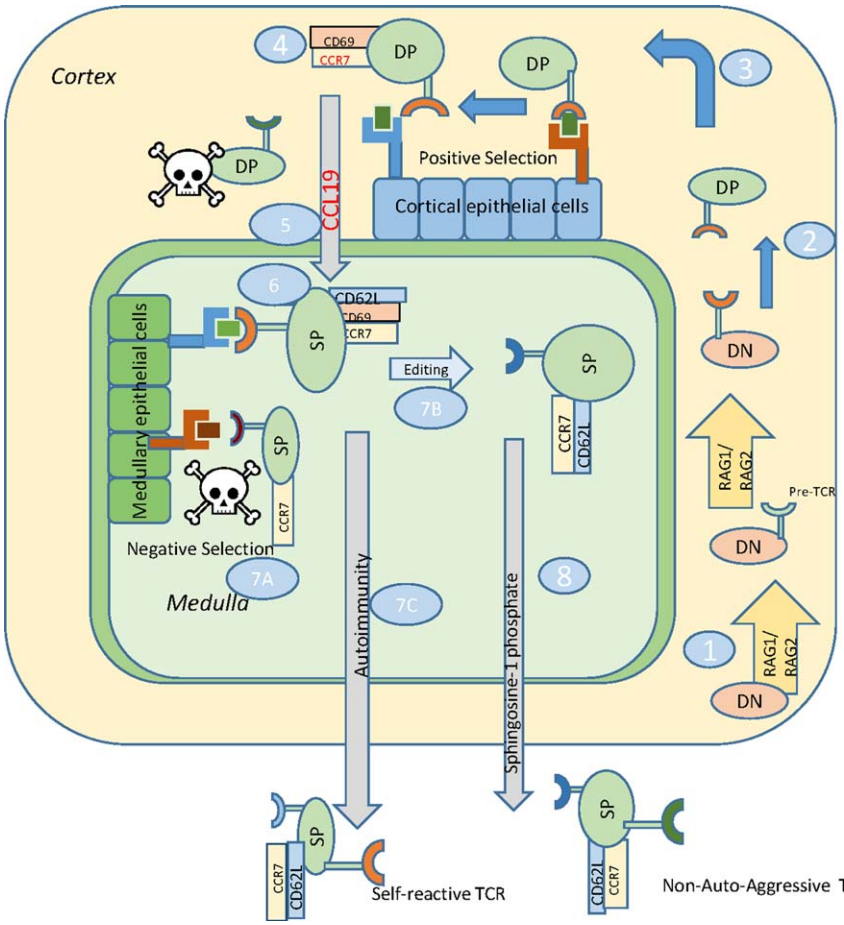

Fig. 1. Autoaggressive T cell development in the thymus. 1, Double-negative (DN) cells enter the thymus and recombination-activating gene (RAG) proteins become activated to rearrange T cell receptor (TCR) beta locus, cells express pre-TCR, RAGs become induced again to generate a mature TCR. 2, Developing cells progress to double-positive (DP) state. 3, DP cells interact with cortical epithelium to undergo positive selection in the thymic cortex. 4, Following TCR interaction with cortical thymic epithelial cells (cTEC), DP cells express CCR7 and CD69. 5, CCL19 is released from the medulla to attract DP cells based on CCR7 expression. 6, DP cells become single-positive for CD4 or CD8, interact with medullary thymic epithelial cells (mTEC) to undergo negative selection. Cells express CD62L. 7a, cells with high affinity, self-reactive TCRs are deleted; 7b, some cells undergo TCR editing to alter TCR V alpha expression; 7c, Self-reactive T cells may escape negative selection or be generated from faulty TCR editing. 8, SP cells with appropriate TCRs exit to the periphery following sphingosine-1-phosphate (S1P) concentration gradients.

interact successfully are primed for survival; cells that cannot interact with the available HLA molecules undergo death-by-neglect (Fig. 1). Positive selection assures that only T cells that are responsive to the available HLA are maintained [43,44].

Successful interactions between TCR and MHC cause the cells to express CD69 and CCR7, a chemokine receptor that interacts with CCL19 [45–48]. Chemokine receptors allow cells to migrate towards the source of chemokine, following an upslope concentration gradient. CCR7 expression allows the cells to migrate to the medulla [49]. Mice that are deficient in CCR7 or if CCL19 is blocked accumulate immature thymocytes in the cortex [48]. CD69 has long been considered a very early 'activation' marker, in that TCR engagement induces CD69 rapidly in the periphery as well as in the thymus [50]. Therefore, CD69 expression has been associated with successful positive selection [51]. While CD69 function is not understood fully, it is now known to be involved in tissue retention [28]. When CD69 was over-expressed in mice, thymocyte development and positive or negative selection were not hampered but cells accumulated in the medulla, much the same as CCR7-deficient mice, and failed to be exported from the thymus, creating systemic lymphopenia [52]. CD69 helps to retain developing cells within the cortical region but CCL19, released from the medulla, over-rides CD69, allowing cells to migrate to the medullary region [53,54]. Once in the medulla, DP cells complete maturation to the single-positive, $CD4^+$ or $CD8^+$ stage. In the medulla, cells begin to express CD62L, a selectin involved in lymph node homing [55]. While the mechanism of CD62L induction is not yet defined, expression of CD62L is associated with more mature thymocytes [56]. Once cells reach the medullary region, epithelial cells (medullary thymic epithelial cells or mTEC) located there express MHC molecules that are presumed to carry a series of self-antigens. The now positively selected thymocytes interact once again with HLA class I and class II molecules where self-antigen reactive T cells are presumably removed by activation-induced cell death if they encounter an antigen to which they have high affinity [57,58]. A transcription factor called AIRE (autoimmune regulator) regulates this process [58]. Severe autoimmunity develops in AIRE deficiency [59,60]. Humans with AIRE deficiency experience a multitude of autoimmune diseases [59]. As cells complete maturation in the medulla expression of CD69 is lost, while retaining CCR7 and CD62L

expression. These phenotypically mature cells can migrate to populate lymph nodes through CCL19 and other signals, and be retained in the node through CD62L interactions.

During the process of negative selection some thymocytes destined for failure have an opportunity for redemption, utilizing a process called TCR editing [61–66]. Rather than a single attempt at TCR generation, which was the prevailing paradigm for many years, self-reactive thymocytes can be induced to re-express the RAG proteins, which will once again rearrange TCR genes [64]. Again, the mechanism of RAG induction in the thymus is unknown. Receptor editing was first described in B cells, taking place during development in bone marrow [67]. TCR editing helps to account for repertoire expansion in the thymus and argues in favour of cell conservation, thus reducing the need for continual migration of bone marrow cells. The particulars of negative selection have not yet been explained fully. How are cells able to interact positively with MHC to undergo positive selection and then the same TCR again interacts with MHC to undergo negative selection? One explanation is that positional kinetics, i.e. positive selection, occurring in the cortex while negative selection occurs in the medulla, create differential signals to the developing T cell, although this hypothesis has not been proven. Another viable option is that different affinity or avidity thresholds between TCR molecules on developing T cells and HLA/MHC promote positive *versus* negative selection. Autoimmunity can certainly create permissive conditions allowing autoaggressive T cells to escape thymic negative selection known as central tolerance, which has been proposed [68,69]. One concern about central tolerance failure being the only means of generating autoaggression is that the vast majority of thymic output occurs early in life. In mammals, thymic involution, loss of thymic architecture and volume, occurs at or close to puberty. If autoaggressive T cells arise solely by escaping negative selection, then logically autoimmune disease would only onset prior to or soon after puberty. This, however, is not always the case.

In T1D the majority of disease onsets occur in juvenile subjects; however, an ever-growing population is experiencing onset during the 3rd, 4th, 5th and even 6th decade of life [70]. To account for this, either peripheral mechanisms of tolerance are in place that become dysfunctional over time, or an alternative mechanism of autoaggressive T cell development occurs. Another intriguing option is that early in life diabetes onset constitutes one type of disease, perhaps associated closely with central tolerance failure, while disease onset later in life constitutes a different type of disease. The latter case would involve mechanisms to develop autoaggressive T cells independently of thymic control. It has been presumed that TCR editing is the final point in TCR development. To the contrary, we and others demonstrated that T cells are capable of inducing RAG1 and RAG2 proteins in the periphery and, subsequent to that, alter TCR expression [71–86]. This process is known as TCR revision. While it has not yet been determined what induces RAGs in the thymus, the mechanisms of revision are the same as those for editing; the locale of the T cell, in the periphery as opposed to the thymus, has dictated the name change.

TCR revision

It has been shown that a subset of T cells, both in the thymus and in the periphery, express the CD40 molecule [36,37,71–73,87–98]. This was somewhat surprising, given that CD40 expression has long been associated with only APC. However, more extensive research demonstrated that CD40 expression is ubiquitous, being expressed on all identified APC, on neural cells including microglia, on adipocytes, on endothelial cells and on T cells, including $CD4^+$ and $CD8^+$ cells [36,37,71–73,80,88–94,99–101]. CD40-expressing CD4 cells are referred to as Th40 cells, and have been shown to become highly pathogenic in autoimmune disease models [36,37,71–73,87–95,99]. Among its functions, CD40 acts as a co-stimulus on T cells [37,87,88,91–94,99]. This indicates that alternative, and heretofore under-considered, co-stimulatory molecules occur on T cells. Identifying these molecules could reshape the understanding of T cell biology significantly.

An intriguing and surprising discovery was that CD40 engagement on Th40 cells induced the RAG1/RAG2 TCR recombination machinery [71,73]. This was the first ever demonstration in a primary T cell of a mechanism to induce RAG proteins. RAG1 and RAG2 form heterodimers that interact further with Ku proteins, DNA polymerases and helicases, etc., leading to alteration of TCR expression [71–73,91,102,103]. In the periphery, at least, CD40 interacts directly with the RAG(s) complex in the nucleus [71]. Following induction of RAGs, altered expression of TCR-α [73,104] and TCR-β [83,84,105] molecules on long-standing peripheral T cells occurs [71,74–83,85,102,106–119]. A central paradigm of immunology holds that once T cells exit the thymus TCR molecules do not undergo alteration. To the contrary, several laboratories have shown that peripheral T cells re-express RAG1 and RAG2 proteins and subsequently alter TCR expression [81,83,85,109,120,121]. Importantly, revised T cells are distinct from recent thymic emigrants [81]. There is an evolutionary advantage to TCR revision, which is to generate an expansive T cell repertoire able to respond to a universe of foreign antigens (Fig. 2). Viruses are extremely numerous, exhibiting a survival advantage by being highly mutable. Success of any organism, in this case mammals, at controlling viral infection will rely upon a highly adaptive immune system. The estimated number of individual TCR molecules achievable through RAG-mediated recombination is vast, approximately 10^{13} distinct molecules [122]. Maintaining and storing such a number of T cells would

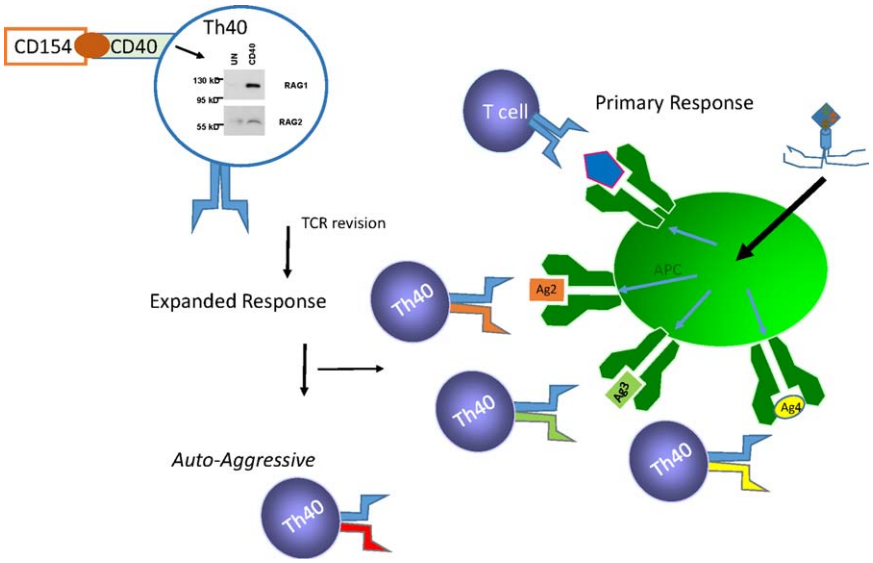

Fig. 2. T cell receptor (TCR) revision generates expanded adaptive immunity but may create autoaggressive TCRs. Th40 cells are induced through CD40 engagement to express recombination-activating genes (RAG)1 and RAG2. Because viruses have multiple antigens and a broader TCR response would facilitate viral clearance, TCR revision provides an economical means to expand the immune response. A negative consequence would be the potential to generate a non-negatively selected, autoaggressive T cell.

constitute a Herculean task. A more efficient means of adaptive immunity than generating an enormous number of individual T cells that must be stored and maintained for later use could include TCR revision. This process also could generate a localized subset of responsive T cells. For example, a viral infection at localized tissue would generate several antigens (Fig. 2). TCR revision occurring only on the alpha locus while maintaining the original beta chain could create a selectively diverse subset of T cells (Fig. 2) to facilitate antigen recognition and attack of the infection more effectively. The downside to a highly adaptive immune system is that some individuals, those with the appropriate MHC/HLA, would have an increased risk for autoimmunity. Alteration of TCR in the thymus could facilitate escape of self-reactive T cells, including cells capable of responding to improperly post-translationally modified proteins or responding to hybrid protein epitopes. For example, if TCR editing (TCR changes in the thymus are referred to as editing, while alteration of TCR expression in the periphery is called revision) occurs in the wrong compartment, thereby thwarting positional kinetics, i.e. in the medulla after negative selection takes place, then a self-reactive TCR bearing T cell could escape the thymus. Similarly, a T cell that successfully positively selects, and has low enough affinity or avidity to pass through negative selection, is then revised to possess a high-affinity self-reactive TCR cell that poses new danger potential by being able to escape into the periphery.

TCR revision opens new avenues for consideration by possibly contributing to the process of autoaggressive T cell generation, but also by providing new potential therapeutic options. Because revision is not restricted to a single event, but rather one Th40 cell can undergo two or even three revisions [71–73,107], this process could constitute an unexplored tolerance mechanism. Thus, while revision could promote the generation of a 'new' autoaggressive T cell that responds to self-antigen, additional revision might alter the autoaggressive T cells' TCR expression, making that cell tolerant of self-antigens.

T1D disease model

The initiation of T1D minimally requires (1) the generation of beta islet self-antigens (sAg); (2) the presence of appropriate MHC (HLA); and (3) T cells bearing autoaggressive TCR molecules. In prodiabetic conditions neutrophils, which are capable of generating oxidative stress and hence tissue damage, infiltrate the islets early during postnatal development [3]. In rodents and humans waves of beta cell death occur, due probably to islet reconstruction during pancreatic development [123,124], thus creating additional islet sAg sources. While this process occurs under normal conditions, it is probable that the process is exaggerated under autoimmune conditions. (Model: trauma to the islet, including neutrophil action, initiates sAg creation.) Plasmacytoid DC (pDC) and macrophages are recruited to the islet and take up sAg then migrate to pancreatic lymph nodes (LN). Th40 cell numbers in spleen and peripheral LN of young NOD mice are equivalent to non-autoimmune mice, but in pancreatic LNs Th40 cell numbers are expanded significantly as early as 3 weeks of age [72]. Pathogenicity of Th40 cells is demonstrated by their ability to transfer T1D to NOD.severe combined immunodeficient (SCID) recipients [37,72,73,90,91]. Purportedly then, Th40 cells are stimulated in the pancreatic LN and are then recruited to infiltrate islets. Because Th40 cells are capable of TCR revision, the odds of increasing sAg-reactive T cells on site would be increased dramatically. Th40 cells produce IL-17 [87,91,94] that can act as a neutrophil attractant [125,126] and produce interferon (IFN)-γ that drives diabetogenesis.

An issue in T1D is why is disease onset so disparate? Some individuals experience onset as young children or juveniles, while others do not experience onset until adulthood. Potential explanations are that all conditions for disease onset, including pre-existing autoaggressive T cells, are present in juveniles, but not in adults. We hypothesize that certain T cells, specifically Th40, alter TCR usage over time to become autoaggressive (TCR revision). While the conditions to create such cells would necessarily be determined genetically, in some individuals those T cells either do not yet exist or are regulated successfully through tolerance mechanisms that eventually fail. A central paradigm of tolerance is the control of the CD40–CD154 dyad [127,128]. Multiple studies demonstrate that controlling CD40-mediated signals is tolerogenic, including in T1D [72,90,129]. Consequently, disruption of the CD40/CD154 balance becomes pathogenic. Induced increases in CD40-bearing cells, specifically Th40 cells, would necessarily promote pathogenesis. When the balance of CD40 was broken by adoptive transfer of high numbers of Th40 cells, diabetes developed [72,73,95,97]. Such increases could be mediated by any of a number of infectious agents. A major element to explain why disease onset is so varied may focus upon the generation of autoaggressive T cells. Even with all the other disease-specific criteria, e.g. sufficient sAg, appropriate HLA for presenting antigen, etc., until autoaggressive T cells develop in sufficient numbers or are able to break tolerance, disease will not onset. If CD40 were engaged purposefully on autoaggressive T cells, at the proper time it may be possible to forestall T1D onset by altering autoaggressive TCR expression.

Acknowledgements

The author declares grant support from the following: the American Diabetes Association and NIDDK.

Disclosure

D. H. W. is Chief Scientific Officer for Op-T-Mune, Inc., Denver, CO, USA and owns stock in the company Op-T-Mune, Inc., Denver, CO. The current value of the company is less than US$10 000. D. H. W. has received funding for research but only from Federal and State Agencies, not from private companies.

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