Editorial

New insight into type 1 diabetes development: resolving early diabetogenic CD4$^+$ T cell responses that precede seroconversion

Sonja Schallenberg[1], Karsten Kretschmer[1,2,3]

[1]Molecular and Cellular Immunology/Immune Regulation, DFG-Center for Regenerative Therapies Dresden (CRTD), Center for Molecular and Cellular Bioengeneering (CMCB), Technische Universität Dresden, Dresden, Germany; [2]Paul Langerhans Institute Dresden (PLID) of the Helmholtz Zentrum München at the University Hospital and Medical Faculty Carl Gustav Carus of TU Dresden, Dresden, Germany; [3]German Center for Diabetes Research (DZD e.V.), Ingolstädter Landstraße 1, Neuherberg, Germany

Correspondence to: Karsten Kretschmer. Molecular and Cellular Immunology/Immune Regulation, DFG-Center for Regenerative Therapies Dresden (CRTD), Center for Molecular and Cellular Bioengeneering (CMCB), Technische Universität Dresden, Dresden, Germany. Email: Karsten.Kretschmer@tu-dresden.de.

Provenance: This is a Guest Editorial commissioned by Section Editor Zhijun Han, MD (Department of Laboratory Medicine, Wuxi Second Hospital, Nanjing Medical University, Wuxi, China)

Comment on: Heninger AK, Eugster A, Kuehn D, *et al.* A divergent population of autoantigen-responsive CD4+ T cells in infants prior to β cell autoimmunity. Sci Transl Med 2017;9.pii: eaaf8848.

Submitted Nov 19, 2017. Accepted for publication Nov 29, 2017.
doi: 10.21037/atm.2017.12.14
View this article at: http://dx.doi.org/10.21037/atm.2017.12.14

Although it can arise at any age, type 1 diabetes (T1D) represents one of the most prevalent and severe chronic diseases in childhood and adolescence that results from the autoimmune-mediated destruction of insulin-producing β cells in the pancreatic islets of Langerhans causing insulin deficiency and hyperglycemia (1). There is currently no cure or prevention for T1D, leading to lifelong dependence on exogenous insulin therapy, which is essential in managing metabolic symptoms, but does not interfere with the underlying autoimmune process and is frequently associated with serious secondary complications such as retinal, renal, and neurological disease (2).

T1D represents a multifactorial disease caused by a combination of events, including inherited genetic susceptibility, as well as environmental and immunological factors (3). The strong genetic component of T1D is reflected by a major contribution of specific alleles and haplotypes of human leukocyte antigen (HLA) class II genes. Polymorphisms in multiple non-HLA genes (such as insulin, *PTPN22*, *CTLA4*, and *IL2RA*) some of which exert important functions in immune regulation, also contribute to T1D risk. In genetically susceptible individuals, manifestation of clinical T1D is a multi-step process that is precipitated by ill-defined environmental factors (viruses, allergens, toxins, etc.) in conjunction with conditions of immune dysregulation [e.g., impaired function of Foxp3$^+$ regulatory T (Treg) cells], leading to the generation of pathogenic CD4$^+$ and CD8$^+$ T effector cells that mediate progressive β cell destruction. Notably, loss of self-tolerance and initiation of an autoimmune response to β cell antigen occurs well before clinical onset of T1D: the T cell-mediated selective loss of pancreatic β cell mass is preceded by an asymptomatic pre-clinical period of variable duration, which is characterized by the gradual emergence of a number of autoantibodies (AAbs) with high affinity to β cell-associated self-antigens, such as insulin and 65 kDa glutamic acid decarboxylase (GAD65). AAbs with β cell reactivity are thought to be dispensable for T1D pathogenesis, but represent valuable serum biomarkers for β cell autoimmunity prior to manifestation of clinical T1D and evaluation of disease risk in individuals with an inherited HLA predisposition (4,5). Importantly, naïve CD4$^+$CD45RA$^+$ T cells with β cell reactivity (insulin, GAD65) can already be detected in cord blood of healthy neonates at frequencies that positively correlate with HLA-dependent genetic T1D susceptibility (6). However, the mechanisms underlying their inappropriate activation and differentiation into diabetogenic CD4$^+$ T effector cells, as

well as their relationship to β cell-specific B cells secreting high affinity AAbs has remained largely elusive. As the human pancreas (and thus pancreas-infiltrating immune cells) is not readily accessible, the study of immune events that precede the generation of $CD4^+$ T effector cells and AAbs reactive to β cell antigen is inevitably dependent on peripheral blood mononuclear cells (PBMC). Major challenges include the limited availability of PBMC samples from cohorts of individuals with high genetic T1D risk and exceedingly low frequencies of β cell-specific T cells among PBMCs.

Now, a recent study by Bonifacio and colleagues (7) has shed new light on early immunological events in T1D, suggesting that a unique subset of naïve $CD4^+CD45RA^+$ T cells with β cell reactivity and an unusual proinflammatory T helper (T_H)-like phenotype marks a latent phase of autoimmune susceptibility, which might be of diagnostic value well before the first appearance of memory $CD4^+CD45RO^+$ T cells and seroconversion to multiple AAbs.

Longitudinal tracking of β cell-responsive $CD4^+$ T cells in at-risk children

Taking advantage of a collection of PBMC samples, which were regularly obtained from infancy through early childhood of at-risk individuals (family history and HLA susceptibility) and made available through the BABYDIET study (8), the authors had the rare opportunity to correlate quantitative and qualitative differences in β cell-specific $CD4^+$ T cell responses with the progression to β cell autoimmunity and overt T1D (i.e., before, at and after appearance of AAbs). Overall, the study encompassed longitudinal samples from 16 at-risk children who developed β cell-reactive AAbs during the observation period. For comparison, samples of 12 genetically susceptible children who remained free of any signs for β cell autoimmunity were also included.

Low frequencies of β cell-reactive $CD4^+$ T cells were identified among total PBMCs based on their proliferative capacity (dilution of a fluorescent dye) and acquisition of the early T cell activation marker CD25, upon *in vitro* re-stimulation with whole protein antigen (proinsulin, GAD65). This highly specific approach further allowed discriminating β cell-specific $CD4^+$ T cells with a naïve or memory phenotype based on differential surface expression of CD45RA and CD45RO, respectively. At 6 months of age, none of the 28 at-risk children initially included in the study exhibited detectable memory $CD4^+CD45RO^+$ T cells or AAbs to β cell antigen. However, in the cohort of children who developed β cell autoimmunity at later time points, the first appearance of β cell-specific AAbs closely correlated with the detection of β cell-reactive $CD4^+$ T cells that exhibited a $CD45RO^+$ memory phenotype. On the basis of their frequency and proliferation profile after *in vitro* re-stimulation with cognate self-antigen, the *in vivo* abundance of β cell-reactive $CD4^+RO^+$ T cells was estimated to represent less than 0.1% of PBMC-derived $CD4^+$ T cells, underscoring the high sensitivity of this assay. In striking contrast, $CD4^+CD45RO^+$ memory T cells and AAbs to β cell antigen remained below the detection limit in the control cohort. Hence, reminiscent of adoptive immune responses to foreign (i.e., non-self) antigens, β cell antigens appear to synchronously drive activation and differentiation of both $CD4^+$ T cells and AAb-producing B cells.

Consistent with the previous observation of β cell-specific, naïve $CD4^+CD45RA^+$ T cells in healthy neonates (6), both AAb-positive and -negative cohorts of at-risk children were found to be largely comparable with regard to frequencies of β cell-specific $CD4^+$ T cells with a naïve $CD45RA^+$ phenotype, as judged by the up-regulation of CD25 expression and low, but clearly detectable proliferative responsiveness *in vitro* to proinsulin or GAD65 protein. Surprisingly, subsequent gene expression profiling revealed that many children who later on developed β cell autoimmunity exhibit a strong bias towards a rather unusual naïve β cell-reactive $CD4^+$ T cell phenotype with proinflammatory features well before the appearance of memory $CD4^+$ T cells and AAbs reactive to β cell antigen.

Transcriptional signatures of diabetogenic $CD4^+$ T cell subphenotypes

Gene expression profiling at the single-cell level offers the opportunity to unravel inter-cellular transcriptomic variations within a defined cell population and to identify cell subsets involved in disease pathogenesis, which are otherwise obscured by the bulk analysis of those populations. Yet, despite recent technological advances, single cell transcriptomic has remained challenging, in particular, with small cell populations and rare clinical samples. To address these issues, Heninger *et al.* took advantage of a robust quantitative RT-PCR-based approach, employing fluorescence activated cell sorting-based purification of single β cell-responsive $CD4^+$ T cells in conjunction with multiplex primers, interrogating

the expression of a selected set of 19 mRNA transcripts encoding proteins (transcription factors, cytokines, surface markers, chemokine receptors) with well-defined roles in $CD4^+$ T cell-mediated effector function (e.g., IFN-γ, TNF, IL-21) and immune regulation (e.g., IL-10, FOXP3). Despite the observed quantitative similarities in their overall responsiveness to proinsulin and GAD65, children at 6 months of age exhibited a remarkable transcriptional heterogeneity among the β cell-reactive naïve $CD4^+$ T cell population, which could be attributed to the presence of distinct subphenotypes with predominantly immune regulatory (e.g., FOXP3, TGF-β, and CTLA-4, in the absence of proinflammatory cytokines) or proinflammatory features. The latter included a naïve $CD4^+CD45RA^+$ T cell subset, which could be defined by consistently increased co-expression of a unique set of genes (*CCR6*, *IL21*, *TBX21*, *TNF*, *RORC*, *EGR2*, *TGFB1*, and *ICOS*) that play essential roles in different aspects of $CD4^+$ T_H responses, such as naïve T-cell activation/proliferation, differentiation, and acquisition of T_H effector functions. This proinflammatory phenotype appears rather unusual, as it combines genes whose protein products are involved in the overall control of T_H differentiation (e.g., EGR2, TGF-β1) with individual facets of functionally distinct $CD4^+$ T_H lineages (T_H1: T-bet, TNF; T_H17: ROR-γT, CCR6; follicular T_H (T_{FH}): IL-21, ICOS), whereas the expression of the Treg cell lineage specification factor Foxp3 and the T_H17 signature cytokine IL-17 were found to be underrepresented.

Strikingly, in 3 of the 6 children included in the gene expression survey, the T_H-like phenotype was shared among the vast majority of naïve $CD4^+CD45RA^+$ T cells with both proinsulin- and GAD65-responsiveness, preceding subsequent development of memory $CD4^+$ T cells and AAbs reactive to β cell antigen. Importantly, this particular proinflammatory subphenotype was essentially absent in all tested at-risk children who remained free of any signs for β cell autoimmunity, and was clearly discernable from the transcriptional profile of naïve $CD4^+CD45RA^+$ T cells responsive to the non-β cell antigen tetanus toxoid, formally excluding the possibility that genetically susceptible children with β cell autoimmunity exhibit an overall bias in their antigen-specific $CD4^+$ T cell responses. A predictive algorithm for progression to β cell autoimmunity based on the proinflammatory gene signature of naïve $CD4^+CD45RA^+$ T cells with β cell responsiveness further supported the relevance of these findings.

Transition to memory $CD4^+$ T cell responses and overt β cell autoimmunity

Their independent identification in several at-risk children as early as 6 months of age and selective predominance in those who progressed to β cell autoimmunity already indicated that the mixed '$T_H1/T_H17/TF_H$'-like phenotype of naïve β cell-responsive $CD4^+$ T cells may represent a transitional but rather long-lasting stage of partial differentiation with the potential to give rise to fully differentiated, diabetogenic $CD4^+$ T effector cells. Consistently, comparative gene expression profiling of single β cell-responsive $CD4^+$ T cells before and after seroconversion provided direct evidence that the progression towards β cell autoimmunity is indeed accompanied by the transition to a more homogeneous T_H1-type response characterized by the expression of *IFNG*, *TNF* and *CCR7*, with low frequencies of cells expressing *IL17*, *IL4*, *IL10* or *FOXP3* mRNA. However, the replacement of semi-committed '$T_H1/T_H17/TF_H$'-like T cells was not dominantly driven by proliferative clonal expansion, as the paired analysis of TCR/α/β chains at single-cell resolution revealed a largely diverse TCR repertoire expressed by β cell-responsive memory $CD4^+$ T cells and provided no evidence for the selection of dominant or public clones.

Naïve T_H-like $CD4^+$ T cells with β cell reactivity: marker for early T1D diagnosis and target for autoimmune intervention?

In addition to providing novel insight into the immune pathogenesis of T1D, the present study is likely to have important implications for disease diagnosis and prevention of β cell autoimmunity. So far, β cell-specific AAbs have been most commonly used as immune biomarker with predictive value for progression of genetically susceptible individuals to clinical T1D. A T-cell-readout based on the naïve $CD4^+$ T_H-like phenotype reported by Heninger *et al.* may improve early detection of β cell autoimmunity prior to the establishment of β cell-specific memory $CD4^+$ T cell responses and seroconversion, thereby further strengthening diagnosis and prevention of metabolic complications often associated with clinical T1D onset. The mixed proinflammatory transcriptional signature was detected in naïve β cell-responsive $CD4^+$ T cells of some (50%) but not all at-risk children who progressed to β

cell autoimmunity, perhaps due to the modest cohort size or technical peculiarities inherent to the RT-PCR-based analysis of single cells. However, it appears important to emphasize that the environmental factor(s) driving the initial activation of β cell-responsive $CD4^+$ T cells have yet to be determined. Indeed, it might well be that quantitatively and qualitatively different environmental cues, which impinge early on non-committed, truly naïve $CD4^+$ T cells during initial priming with β cell antigen, result in imprinting of distinct proinflammatory $CD4^+$ T cell subphenotypes. Provided that the entire spectrum of pancreas-infiltrating T cell subsets is reflected in the blood, genome-wide single-cell transcriptomic of PBMC-derived β cell-responsive T cells may provide first hints towards the identity of the involved environmental stimuli. Clearly, further studies are warranted to further corroborate the predictive value and diabetogenic potential of the newly identified naïve $CD4^+$ T cell subset.

With regard to autoimmune intervention, the development of immune-modifying approaches to halt the underlying autoimmune response targeting β cell antigen has been a long-standing goal in T1D, but encouraging observations in animal models of T1D have not yet demonstrated comparable efficacy in humans. Such efforts have now gained considerable momentum through the identification of antigen-specific $CD4^+Foxp3^+$ Treg cells as key mediators of dominant tolerance and immune homeostasis. The central role of $Foxp3^+$ Treg cells in autoimmune protection of pancreatic β cells is probably best exemplified by the observation that T1D represents a hallmark of the IPEX (immune dysfunction, polyendocrinopathy, enteropathy, X-linked) syndrome in male patients lacking functional $Foxp3^+$ Treg cells due to mutations in the *FOXP3* gene (9). Conversely, $Foxp3^+$ Treg cell-based therapy for prevention and even interference with established β cell autoimmunity has shown promise in preclinical animal models of T1D and other autoimmune diseases (10,11). This includes the adoptive transfer of β cell-specific $Foxp3^+$ Treg cells and tolerogenic administration of β cell antigen (such as proinsulin) to promote both recessive (deletion and anergy of diabetogenic T effector cells) and dominant (enhancement of Treg cell activity) mechanisms of immunological tolerance. While additional treatment options without pharmacological immunosuppression such as immunoprotective artificial pancreas systems (12) are also emerging, the transplantation of pancreatic islets to restore endogenous insulin secretion is still restricted to a small subset of T1D patients with high metabolic lability, due to the long-term need for systemic immunosuppression and shortage of donor organs. Thus, it appears highly desirable to preserve endogenous β cell mass and insulin production by interference with autoimmune responses prior to the destruction of the majority of insulin-producing β cells. However, the successful development of efficacious T1D prevention strategies will require detection of the earliest events in the autoimmune process. In this context, it appears tempting to speculate that β cell-responsive naïve $CD4^+$ T cells with a semi-committed T_H-like phenotype may represent a suitable target for the assessment of early T cell responses to immune-modulating T1D prevention strategies, in particular tolerogenic vaccination with β cell antigen.

Several clinical $Foxp3^+$ Treg cell-based trials that have been recently conducted in patients at different time points after T1D onset. This includes cell-based immunotherapy by intravenous infusion of children (13,14) and adults (15) with *ex vivo*-expanded autologous polyclonal $FOXP3^+$ Treg cells, as well as antigen-based immunotherapy of adult patients using repeated intradermal injections of an immunodominant proinsulin peptide antigen to promote $Foxp3^+$ Treg cell function (16). Both treatment strategies were well tolerated and no severe adverse effects were observed, strongly encouraging further clinical trials to provide more detailed insight into the potential of Treg cell-based therapy to halt cell autoimmunity in patients with established T1D. Notably, results of the Pre-POINT clinical trial provided first evidence that self-antigen-based therapy might indeed be suitable to prevent progression to overt β cell autoimmunity: oral administration of insulin protein to genetically susceptible children prior to seroconversion (i.e., β cell AAb-negative children) elicited an antigen-specific immune response, which, among other things, was characterized by insulin-responsive $CD4^+$ T cells with Treg cell-like properties, while hyperglycemia or other severe side effects were not observed (17). In light of the present study by Heninger *et al.*, it will be of considerable interest to assess in future clinical studies whether early tolerogenic vaccination of at-risk children can interfere with the establishment of the unique proinflammatory phenotype of the naïve β cell-responsive $CD4^+$ T cells, preventing their developmental progression to memory $CD4^+$ T cells, production of β cell-reactive AAbs and clinical T1D.

Acknowledgements

We would like to thank Annette Ingeborg Garbe for

critically reading the manuscript and helpful discussion. *Funding:* Karsten Kretschmer was supported by the Competence Network for Diabetes mellitus funded by the Federal Ministry of Education and Research (BMBF; FKZ 01GI0805-07), by a BMBF grant to the German Center for Diabetes Research (DZD e.V., FKZ01GI0924), and by the FZT 111 (DFG, Center for Regenerative Therapies Dresden, Cluster of Excellence).

Footnote

Conflicts of Interest: The authors have no conflicts of interests to declare.

References

1. Wållberg M, Cooke A. Immune mechanisms in type 1 diabetes. Trends Immunol 2013;34:583-91.
2. McKnight JA, Wild SH, Lamb MJ, et al. Glycaemic control of Type 1 diabetes in clinical practice early in the 21st century: an international comparison. Diabet Med 2015;32:1036-50.
3. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 2010;464:1293-300.
4. LaGasse JM, Brantley MS, Leech NJ, et al. Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. Diabetes Care 2002;25:505-11.
5. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013;309:2473-9.
6. Heninger AK, Monti P, Wilhelm C, et al. Activation of islet autoreactive naive T cells in infants is influenced by homeostatic mechanisms and antigen-presenting capacity. Diabetes 2013;62:2059-66.
7. Heninger AK, Eugster A, Kuehn D, et al. A divergent population of autoantigen-responsive CD4+ T cells in infants prior to beta cell autoimmunity. Sci Transl Med 2017;9. pii: eaaf8848.
8. Hummel S, Pfluger M, Hummel M, et al. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care 2011;34:1301-5.
9. Barzaghi F, Passerini L, Bacchetta R. Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome: a paradigm of immunodeficiency with autoimmunity. Front Immunol 2012;3:211.
10. Petzold C, Riewaldt J, Watts D, et al. Foxp3+ regulatory t cells in mouse models of type 1 diabetes. J Diabetes Res 2013;2013:940710.
11. Petzold C, Schallenberg S, Stern JN, et al. Targeted antigen delivery to DEC-205(+) dendritic cells for tolerogenic vaccination. Rev Diabet Stud 2012;9:305-18.
12. Ludwig B, Reichel A, Steffen A, et al. Transplantation of human islets without immunosuppression. Proc Natl Acad Sci U S A 2013;110:19054-8.
13. Marek-Trzonkowska N, My liwiec M, Dobyszuk A, et al. Therapy of type 1 diabetes with CD4(+)CD25(high) CD127-regulatory T cells prolongs survival of pancreatic islets - results of one year follow-up. Clin Immunol 2014;153:23-30.
14. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, et al. Administration of CD4+CD25highCD127- regulatory T cells preserves beta-cell function in type 1 diabetes in children. Diabetes Care 2012;35:1817-20.
15. Bluestone JA, Buckner JH, Fitch M, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. Sci Transl Med 2015;7:315ra189.
16. Alhadj Ali M, Liu YF, Arif S, et al. Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes. Sci Transl Med 2017;9. pii: eaaf7779.
17. Bonifacio E, Ziegler AG, Klingensmith G, et al. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 2015;313:1541-9.

Cite this article as: Schallenberg S, Kretschmer K. New insight into type 1 diabetes development: resolving early diabetogenic CD4$^+$ T cell responses that precede seroconversion. Ann Transl Med 2018;6(3):58. doi: 10.21037/atm.2017.12.14

