

# Islet Autoreactive CD8 T-cells in Type 1 Diabetes Licensed to Kill?

Bart O. Roep

**W**hile differentiating T-cell autoreactivity against pancreatic islets between type 1 diabetic patients and nondiabetic control subjects has, over time, proven to be a difficult task, the ultimate challenge for cellular markers of this disease will involve the definition of immunological measures associated with disease progression and intervention. With the notion that type 1 diabetes is a T-cell-mediated autoimmune disease, it is therefore logical for investigators to direct research efforts toward studies of T-cells. However, relatively few studies associating T-cell autoreactivity with disease progression have been attempted (i.e., in comparison with efforts monitoring autoantibodies), and those that have been performed are largely limited to CD4 T-cells (1).

Ever since the demonstration of their presence in insulinitic lesions in the pancreas of patients at clinical onset of type 1 diabetes, CD8-positive cytotoxic T-cells have been implicated in the disease process (2). This association and its potential importance for disease was corroborated by the genetic predisposition of HLA class I loci for type 1 diabetes that is independent of linkage disequilibrium with HLA class II (3); a setting that implied the role of HLA class I with type 1 diabetes is more than guilt by genetic association alone (4). Theoretically, HLA class I-restricted CD8 T-cells could be the sole candidate effector pathway for islet autoantigen specific  $\beta$ -cell destruction since autoreactive cytotoxic T-cells recognize peptide epitopes displayed on the  $\beta$ -cell surface in the context of HLA class I molecules. In recent years, a surge of studies has identified and validated such epitopes, derived primarily from  $\beta$ -cell proteins (5–10). The consistencies among these studies have been remarkable as well as encouraging (11).

However, it is still unresolved as to what extent circulating CD8 T-cells may act as correlates of disease progression. One pertinent study addressing this question revealed a strong correlation between increases of autoreactive CD8 T-cells and recurrent autoimmunity, yet this was following islet transplantation and graft failure in type 1 diabetic patients (6). In this issue of *Diabetes*, Martinuzzi et al. (12) addressed this knowledge void and observed a change in CD8 autoreactivity to islet epitopes in the opposite direction; namely, they measured a drop in numbers of islet epitope-specific CD8 T-cells in the first year following disease diagnosis. If confirmed by subse-

quent studies, this could imply that measuring CD8 T-cell autoreactivity in peripheral blood mirrors insulinitis or changes in  $\beta$ -cell mass. This would have important ramifications for studies of T-cells as markers of autoimmunity.

However, it is also important to note a number of items. First, the authors did not compare changes in islet autoreactivity with  $\beta$ -cell function. In addition, the authors used an ELISPOT assay that they previously reported to detect elevated CD8 T-cell autoreactivity to islets (8). Even though their assay suffers from large variations in background cytokine production that may affect their interpretation and impair enumerating changes in response rates, it is clear that the reactivity they detect is associated with disease activity. Yet, cytokine production does not equal cytolytic activity in that the number of cytokine spots may underrepresent the precursor frequency of islet specific CD8 T-cells. Indeed, even with clonal populations of CD8 T-cells, <10% of cells elicit cytokine production in response to their cognate antigen (13). HLA tetramer studies may offer added value in this context. Another issue involved selection of  $\beta$ -cell epitopes (14). While the authors tested a series of validated epitopes, these were selected on their presumed high binding affinity to HLA-A2. Perhaps surprisingly, Ouyang et al. (9) recently observed an inverse correlation between the binding affinity of  $\beta$ -cell peptides to HLA-A2 and CD8 T-cell responses against those peptides in recent-onset type 1 diabetic patients. Ascertainment by high binding affinity, and in particular on the basis of matrix-assisted algorithms such as SYFPEITHI, may be flawed. Indeed, “immunosophically” it would make sense that T-cells recognizing peptides with intermediate or low binding affinity may escape central tolerance (i.e., clonal deletion) during thymic education and therefore turn pathogenic upon peripheral stimulation. With the unambiguous identification of several HLA class I alleles predisposing to type 1 diabetes, the next generation of epitopes may be derived from peptides binding to these HLA variants (3). The new candidate target of  $\beta$ -cells, the cation efflux transporter ZnT8, deserves consideration for this purpose (15). These issues offer great opportunities for future T-cell workshops, preferably in a blinded context, of the Immunology of Diabetes Society, coordinated by a new generation of researchers.

Although an increasing number of studies, including that of Martinuzzi et al., have demonstrated the presence of circulating islet autoreactive CD8 T-cells around the clinical onset of disease, clinical evidence for a role for these T-cells in  $\beta$ -cell destruction is still lacking. The new report corroborates that CD8 T-cell populations may decrease in periphery with  $\beta$ -cell mass waning, and this observation supports a direct correlation with  $\beta$ -cell destruction active insulinitis. This study also supports the use of peripheral blood as source of biomarkers of disease activity (Fig. 1). Would CD8 T-cells be suitable targets for

From the Department of Immunohaematology & Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands.

Corresponding author: Bart O. Roep, LUMC, Dept. IHB, E3-Q, P.O. Box 9600, NL-2300 RC Leiden, Netherlands. E-mail: boroep@lumc.nl.

DOI: 10.2337/db08-0264

© 2008 by the American Diabetes Association.

See accompanying original article, p. 1312.

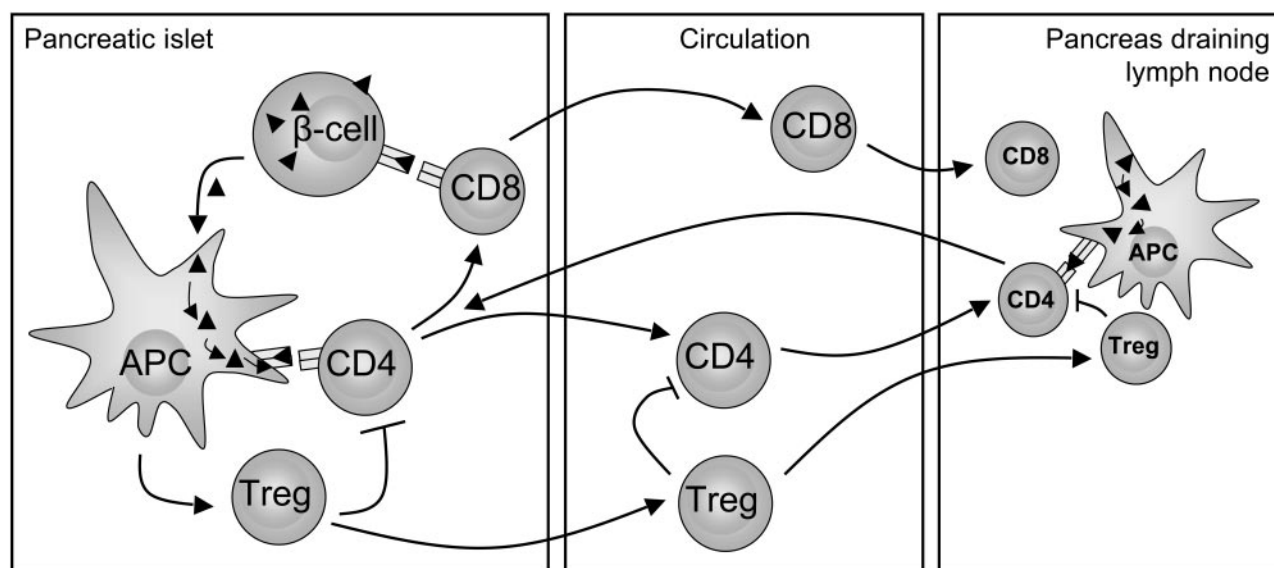


FIG. 1. Recycling of insulinitic effector T-cells and disease-modifying regulatory T-cells offers opportunities for their detection in peripheral blood.

immune intervention therapy in type 1 diabetes? Immunotherapy with modified monoclonal antibodies against the common T-cell marker CD3 resulted in the induction of CD8 lymphocytosis and inversion of the CD4-to-CD8 ratio that was perhaps partly reflecting reactivation of Epstein-Barr virus-specific CD8 T-cells (16). Interestingly, the anti-CD3 mAb hOKT3 $\gamma$ 1(Ala-Ala) caused activation of CD8+ T-cells and induced regulatory CD8+CD25+ T-cells expressing CTLA4 and Foxp3 (17,18). These cells inhibited the responses of CD4+ cells to the mAb itself and to the antigen. Foxp3 was also induced on CD8+ T-cells in patients during mAb treatment, which suggests a potential mechanism of the anti-CD3 mAb immune modulatory effects involving induction of a subset of regulatory CD8+ T-cells. It would therefore be important to consider and appraise pathogenic as well as regulatory features of CD8 T-cells in type 1 diabetes.

With the identification of circulating immune correlates of disease activity, the opportunity arises to use such validated markers for monitoring progression of type 1 diabetes in the context of immune intervention trials in order to assess the immunological, as well as clinical efficacy, of candidate therapies in type 1 diabetes.

## REFERENCES

- Roep BO, Kallan AA, Duinkerken G, Arden SD, Hutton JC, Bruining GJ, de Vries RR: T-cell reactivity to beta-cell membrane antigens associated with beta-cell destruction in IDDM. *Diabetes* 44:278–283, 1995
- In't Veld P, Lievens D, De GJ, Ling Z, Van der Auwera B, Pipeleers-Marichal M, Gorus F, Pipeleers D: Screening for insulinitis in adult autoantibody-positive organ donors. *Diabetes* 56:2400–2404, 2007
- Nejentsev S, Howson JM, Walker NM, Szeszeko J, Field SF, Stevens HE, Reynolds P, Hardy M, King E, Masters J, Hulme J, Maier LM, Smyth D, Bailey R, Cooper JD, Ribas G, Campbell RD, Clayton DG, Todd JA: Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature* 450:887–892, 2007
- Roep BO: Diabetes: missing links. *Nature* 450:799–800, 2007
- Toma A, Haddouk S, Briand JP, Camoin L, Gahery H, Connan F, Dubois-Laforgue D, Caillat-Zucman S, Guillet JG, Carel JC, Muller S, Choppin J, Boitard C: Recognition of a subregion of human proinsulin by class I-restricted T cells in type 1 diabetic patients. *Proc Natl Acad Sci U S A* 102:10581–10586, 2005
- Pinkse GG, Tysma OH, Bergen CA, Kester MG, Ossendorp F, van Veelen PA, Keymeulen B, Pipeleers D, Drijfhout JW, Roep BO: Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. *Proc Natl Acad Sci U S A* 102:18425–18430, 2005
- Hassainya Y, Garcia-Pons F, Kratzer R, Lindo V, Greer F, Lemonnier FA, Niedermann G, van Endert PM: Identification of naturally processed HLA-A2-restricted proinsulin epitopes by reverse immunology. *Diabetes* 54:2053–2059, 2005
- Mallone R, Martinuzzi E, Blancou P, Novelli G, Afonso G, Dolz M, Bruno G, Chaillous L, Chatenoud L, Bach JM, van EP: CD8+ T-cell responses identify  $\beta$ -cell autoimmunity in human type 1 diabetes. *Diabetes* 56:613–621, 2007
- Ouyang Q, Standifer NE, Qin H, Gottlieb P, Verchere CB, Nepom GT, Tan R, Panagiotopoulos C: Recognition of HLA class I-restricted  $\beta$ -cell epitopes in type 1 diabetes. *Diabetes* 55:3068–3074, 2006
- Standifer NE, Ouyang Q, Panagiotopoulos C, Verchere CB, Tan R, Greenbaum CJ, Pihoker C, Nepom GT: Identification of novel HLA-A\*0201-restricted epitopes in recent-onset type 1 diabetic subjects and antibody-positive relatives. *Diabetes* 55:3061–3067, 2006
- Pinkse GG, Boitard C, Tree TI, Peakman M, Roep BO: HLA class I epitope discovery in type 1 diabetes: independent and reproducible identification of proinsulin epitopes of CD8 T cells: report of the IDS T Cell Workshop Committee. *Ann N Y Acad Sci* 1079:19–23, 2006
- Martinuzzi E, Novelli G, Scotto M, Blancou P, Bach GM, Chaillous L, Bruno G, Chatenoud L, van Endert P, Mallone R: The frequency and immunodominance of islet-specific CD8+ T-cell responses change after type 1 diabetes diagnosis and treatment. *Diabetes* 57:1312–1320, 2008
- Unger WW, Pinkse GG, Mulder-Van der Kracht S, van der Slik AR, Kester MG, Ossendorp F, Drijfhout JW, Serreze DV, Roep BO: Human clonal CD8 autoreactivity to an IGRP islet epitope shared between mice and men. *Ann N Y Acad Sci* 1103:192–195, 2007
- Di Lorenzo TP, Peakman M, Roep BO: Translational mini-review series on type 1 diabetes: systematic analysis of T cell epitopes in autoimmune diabetes. *Clin Exp Immunol* 148:1–16, 2007
- Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, Hutton JC: The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci U S A* 104:17040–17045, 2007
- Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Cremer L, De BC, Seigneurin JM, De PP, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L: Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med* 352:2598–2608, 2005
- Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA: Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med* 346:1692–1698, 2002
- Bisikirska B, Colgan J, Luban J, Bluestone JA, Herold KC: TCR stimulation with modified anti-CD3 mAb expands CD8+ T cell population and induces CD8+CD25+ Tregs. *J Clin Invest* 115:2904–2913, 2005