SUPPLEMENTARY DATA

Supplementary Table S1. T cell phenotypes requiring further replication.

Specificity	T cell phenotype	Observation	Assay	Cell number (PBMC)	Replicated		Proposed next steps	Reference
					Same lab	Independent lab		
Antigen non-specific (agnostic)	CD4 CD25+CD127hi memory T cell frequency	Increased frequency of CD4+CD45RO+CD25+CD127hi T cells in conjunction with IDAA1c, HbA1c, or C-peptide at diagnosis correlates with a longer remission period after diagnosis.	Flow cytometry	1-5 x 10^6	No	No	Confirm in a replication cohort- underway through TrialNet	(1)
	CD4 T cell co-activation transcript signature	Transcript signature of increased CD4 T cell co-stimulation during disease progression in longitudinal samples from subjects during Ab seroconversion and subjects preceding and at diagnosis compared with healthy controls	RNAseq	unknown (<1 x 10^6)	No	No	Confirm in a replication cohort- underway through JDRF	(2)
	CD4 Treg apoptosis	Higher frequency of apoptotic CD4+CD25hi T cells in multiple Ab+ and new onset T1D compared with single Ab+ and longstanding T1D, as well as non-diabetic controls. Apoptosis diminishes after the honeymoon period consistent with loss of beta cell antigen.	Flow cytometry	1-5 x 10^6	Yes	No	Replication in an independent lab and further biomarker validation	(3; 4)
	CD4 and CD8 CD45RA+/RO+ T cell frequency	Increase in CD4 and CD8 CD45RA+CD45RO+ double positive cells in recent onset T1D compared to matched controls. CD4 double positive also increased compared to controls in prediabetic twins.	Flow cytometry	1-5 x 10^6	Yes	No	Replication of result using expanded memory subset markers; biomarker confirmation	(5; 6)
	CD8 short lived effector-like T cell frequency	Increased frequency of CD8+CD28-CD57+CD27-CD127- T cells (associated with CMV viral infection) in at-risk autoantibody positive progressors compared to Ab- subjects and Ab+ non-progressors.	Flow cytometry	1-5 x 10^6	No	No	Confirm in a replication cohort in an independent lab	(7)
T cell receptor	CD4 and CD8 polyclonal TCR diversity	TCR repertoires of both CD4 and CD8 T cells in T1D patients were less diverse compared with those of T2D patients and non-diabetic controls.	TCR sequencing	unknown (10 mL of peripheral blood)	No	No	Confirm in a larger replication cohort in an independent lab	(8)
	Naïve T cell TCR diversity	TCR repertoires in true naïve CD4 T cells in T1D patients were more diverse with reduced clonality compared with those in non-diabetic controls; TCRB CDR3 length was significantly shorter in T1D compared with non-diabetic control subjects in CD4 naive, central memory, Treg, and stem cell memory T cells.	TCR sequencing	True naïve, regulatory, central memory, and stem cell-like memory CD4 T cells isolated from a mean of 9.4 x 10^6 PBMC	No	No	Confirm in a larger replication cohort in an independent lab	(9)
Antigen specific	Islet antigen CD4 T cell activation and clonal expansion	Increased frequency of islet reactive CD4 T cells with a phenotype of recent activation in established T1D compared with healthy controls. Increased clonal expansion of islet reactive CD4 T cells in established T1D vs. healthy controls based on shared TCR clonotypes.	CD154 activation assay and single cell RNA sequencing	4 x 10^7	Yes	No	Confirm in a replication cohort in an independent lab. Extend to disease progression.	(10; 11)
	CD8+CD57+ islet Tem frequency	Change in frequency of islet reactive CD8+CD57+ Tem cells positively correlated with change in c-peptide levels in longitudinal samples from new onset T1D subjects ≤12y	Class I multimers and flow cytometry	1-5 x 10^6	No	No	Confirm in a replication cohort in an independent lab	(12)

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Abbreviations: IDAA1c, insulin dose adjusted glycosylated hemoglobin; HbA1c, glycosylated hemoglobin; Ab, autoantibody; TCR, T cell receptor, T2D, type 2 diabetes; TCRB, TCR beta chain, CDR3, complementarity determining region 3.

SUPPLEMENTARY DATA

Supplemental References

1. Moya R, Robertson HK, Payne D, Narsale A, Koziol J, Type 1 Diabetes TrialNet Study G, Davies JD: A pilot study showing associations between frequency of CD4(+) memory cell subsets at diagnosis and duration of partial remission in type 1 diabetes. Clin Immunol 2016;166-167:72-80
2. McKinney E, Lee J, Jayne D, Lyons P, Smith K: T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. Nature 2015;523:612-616
3. Glisic-Milosavljevic S, Waukau J, Jailwala P, Jana S, Khoo HJ, Albertz H, Woodliff J, Koppen M, Alemzadeh R, Hagopian W, Ghosh S: At-risk and recent-onset type 1 diabetic subjects have increased apoptosis in the CD4+CD25+ T-cell fraction. PloS one 2007;2:e146
4. Glisic-Milosavljevic S, Wang T, Koppen M, Kramer J, Ehlenbach S, Waukau J, Jailwala P, Jana S, Alemzadeh R, Ghosh S: Dynamic changes in CD4+ CD25+(high) T cell apoptosis after the diagnosis of type 1 diabetes. Clin Exp Immunol 2007;150:75-82
5. Smerdon RA, Peakman M, Hussain MJ, Alviggi L, Watkins PJ, Leslie RD, Vergani D: Increase in simultaneous coexpression of naive and memory lymphocyte markers at diagnosis of IDDM. Diabetes 1993;42:127-133
6. Peakman M, Alviggi L, Hussain MJ, Lo SS, Hawa M, Leslie RD, Vergani D: Increased expression of T-cell markers of immunological memory associated with protection from type I diabetes. A study of identical twins. Diabetes 1994;43:712-717
7. Harms RZ, Lorenzo-Arteaga KM, Ostlund KR, Smith VB, Smith LM, Gottlieb P, Sarvetnick N: Abnormal T Cell Frequencies, Including Cytomegalovirus-Associated Expansions, Distinguish Seroconverted Subjects at Risk for Type 1 Diabetes. Front Immunol 2018;9:2332
8. Tong Y, Li Z, Zhang H, Xia L, Zhang M, Xu Y, Wang Z, Deem MW, Sun X, He J: T Cell Repertoire Diversity Is Decreased in Type 1 Diabetes Patients. Genomics, proteomics & bioinformatics 2016;14:338-348
9. Gomez-Tourino I, Kamra Y, Baptista R, Lorenc A, Peakman M: T cell receptor beta-chains display abnormal shortening and repertoire sharing in type 1 diabetes. Nature communications 2017;8:1792
10. Yang J, Wen X, Xu H, Torres-Chinn N, Speake C, Greenbaum CJ, Nepom GT, Kwok WW: Antigen-Specific T Cell Analysis Reveals That Active Immune Responses to beta Cell Antigens Are Focused on a Unique Set of Epitopes. Journal of immunology (Baltimore, Md : 1950) 2017;199:91-96
11. Cerosaletti K, Barahmand-Pour-Whitman F, Yang J, DeBerg HA, Dufort MJ, Murray SA, Israelsson E, Speake C, Gersuk VH, Eddy JA, Reijonen H, Greenbaum CJ, Kwok WW, Wambre E, Prlic M, Gottardo R, Nepom GT, Linsley PS: Single-Cell RNA Sequencing Reveals Expanded Clones of Islet Antigen-Reactive CD4(+) T Cells in Peripheral Blood of Subjects with Type 1 Diabetes. Journal of immunology (Baltimore, Md : 1950) 2017;199:323-335
12. Yeo L, Woodwyk A, Sood S, Lorenc A, Eichmann M, Pujol-Autonell I, Melchiotti R, Skowera A, Fidanis E, Dolton GM, Tungatt K, Sewell AK, Heck S, Saxena A, Beam CA, Peakman M: Autoreactive T effector memory differentiation mirrors beta cell function in type 1 diabetes. J Clin Invest 2018;128:3460-3474