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Immunological biomarkers: Catalysts for translational advances in autoimmune diabetes

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Summary

In a recent workshop organized by the JDRF focused on the 'Identification and Utilization of Robust Biomarkers in Type1 Diabetes', leaders in the field of type 1 diabetes (T1D)/autoimmunity and assay technology came together from academia, government and industry to assess the current state of the field, evaluate available resources/technologies and identify gaps that need to be filled for moving the field of T1D research forward. The highlights of this workshop are discussed in this paper, as well as the proposal for a larger, planned consortium effort, incorporating a JDRF Biomarker Core, to foster collaboration and accelerate progress in this critically needed area of T1D research.

Keywords: autoimmunity, diabetes, peptide immunotherapy

Introduction

Immune-mediated diseases present challenges to biomarker development because of their complexity and variety; however, they also provide opportunities for biomarker discovery, because of advances in understanding mechanisms of immune response and dysfunction and their effect on the target organ [1–3]. In type 1 diabetes (T1D), insulin-dependence is preceded by the appearance of autoantibodies against proteins expressed by the pancreas, such as (pre–pro)insulin, glutamic acid decarboxylase-65 (GAD65), islet-associated antigen-2 (IA-2) and the zinc transporter-8 (ZnT8), to name a few, providing a framework for disease prediction superimposed upon an individual's genetic background. However, these autoantibodies are not prognostic biomarkers for monitoring disease progression, nor are they well suited for evaluating therapeutic response. Insulin-secretory capacity measured via the surrogate marker C-peptide, used currently as the outcome measure for T1D intervention clinical trials, lies significantly downstream of important events in the immune pathogenesis of this disease. Thus, there is a major need for the development of biomarkers that focus on the mechanistic elements of islet-specific immunity and β cell loss to characterize each stage of disease, as well as to monitor specific therapeutic interventions associated with these stages.

A broad set of academic and industry leaders representing T1D, immunology and β cell biology, as well as several biomarker technologies, recently held a workshop sponsored by the JDRF to address this gap, focusing on (1) biomarkers of disease pathogenesis and (2) biomarkers as potential surrogate end-points in clinical trials to predict the clinical efficacy response to a treatment intervention. Highlights from these discussions and recommendations are provided below.

Defining the need

There are substantial technical challenges as well as biological challenges that retard progress in T1D biomarker development. One of the current technical obstacles in the T1D field is access to appropriate patient cohorts or stored

biosamples from such cohorts. For the establishment of effective biomarkers, there needs to be confidence in the clinical characterization and phenotyping and storage conditions, as well as sample integrity over time. However, in T1D, a predominantly childhood disease, samples are often limited to small blood volumes collected using a variety of methods. Standardization of sampling, storage and assay performance, as well as sample availability, is recognized as a crucial concern that will require resources and broad participation from the research community as a whole.

The biological challenges are even more profound, as markers of disease pathogenesis should measure loss of immune tolerance, clonal expansion and function of antigen-specific T and B cells and other immune cell subsets. In particular, markers should be indicative of islet-antigen specific immune activity, with a better molecular definition of immune subsets and the identification and characterization of key antigen-presenting cells. At the level of the pancreatic islets, there is need for biomarkers of β and α cell mass, active β cell loss and β cell regeneration, as well as the development of non-invasive imaging technologies [4,5]. Importantly, a metric that could link biomarkers of β cell stress/death with markers of autoimmunity or inflammation would be of immense value to the field. Recent studies of human pancreata obtained *post mortem* from T1D subjects have shown a surprising degree of spatial variability in residual islets and immune activation within a single pancreas [6], raising the perennial issue of whether sampling of peripheral blood provides the required level of insight into the *in-situ* disease process. Animal studies have reported both the positive and negative aspects of this issue and it is clearly an area that requires further attention, addressed potentially by using matching blood samples when tissues are also obtained.

Type 1 diabetes results from a chronic, progressive autoimmune process that occurs over a time-scale of months, years or even decades, which is potentially tractable to effective interventional therapy. The workshop discussions focused on three categories of biomarkers that could transform translational research in this disease: (i) quantifiable biomarkers that precede the appearance of autoantibodies. These would be early markers of disease susceptibility and genetic penetrance, reflecting changes in the immune system or non-immune tissue that precede autoantibody development and could enable efforts for primary disease prevention in very young children; such markers should of necessity be suitable for testing in large scale studies and populations; (ii) immune biomarkers of disease progression, representing surrogates for the activation and expansion of destructive autoreactivity that could identify individuals in imminent danger of losing glucose-sensitive insulin secretion; such markers would enable a medically actionable early intervention strategy and justify using immunotherapy in subjects who do not yet carry a diagnosis of 'diabetes'. Such immune biomarkers must be coupled with biomarkers of β cell mass/death to confirm the destructive nature of the autoimmune process; and (iii) surrogate biomarkers for response to therapy. These biomarkers should have a significant correlation with the clinical end-point and might differ for distinct therapies, perhaps leading to personalization of treatment options.

Key challenges and opportunities

T cell specificity and number

The central role of effector and regulatory T cells in autoimmunity has focused considerable attention on assay development to characterize such cells in T1D. However, there still remain several challenges for practical biomarker applications. First, significant blood volumes are needed to measure rare lymphocyte populations that are at the centre of this disease. There is as yet no consensus on the precise autoreactive T cell peptide–major histocompatibility complex (MHC) recognition specificities in humans or, indeed, on the likelihood that they are shared between different subjects. The low affinities of autoreactive T cells pose unique challenges for detection, especially with regard to teasing out signal from noise, and it remains incompletely determined whether fresh or frozen samples are best suited for all assays. Several speakers at the workshop discussed T cell assays that reflect new accomplishments in the field, as well as highlighting areas of active assay development and potential roadblocks. Topics included:

- Successful generation of $CD4^+$- and $CD8^+$-specific multimers that allow for higher numbers of low-affinity autoreactive cells to be detected from the peripheral blood [7].
- Application of class II tetramer assays for direct detection of autoreactive $CD4^+$ cells without culture or *in-vitro* expansion [8].
- Functional assays [e.g. cytokine enzyme-linked immunospot assay (ELISPOT)] that use naturally processed and presented epitopes of putative islet autoantigens validated in blinded studies [9].
- Molecular engineering efforts using structure–function studies to improve T cell detection with better MHC binding peptides [10].
- Quantum (Q-) dot assay, for multiplex, sensitive detection of MHC class I-restricted T cell receptors (TCRs), allowing for T cell-based immune signatures of remission and relapse of autoimmunity in the islet transplantation setting; correlative studies of T1D clinical trials; and discovery of new autoreactive T cell epitopes [11,12].
- High-throughput TCR sequence analysis including TCR-β chain deep sequencing within functional populations in T1D subjects [13].

Functional assays of T cell activity

These assays potentially define intermediate immunological phenotypes associated with clinical prognosis. Workshop highlights included the following:

- T cell proliferation assays coupled with phenotypic characterization of surface markers that may be used to align appearance of T cell memory with appearance of autoantibodies in the at-risk populations (unpublished).
- Functional interrogation of disease-specific pathogenic or beneficial T cells as a gauge of T cell 'health', including assays for requisite signalling pathways and other intracellular events downstream of TCR and cytokine receptor engagement [14].

Technologies to accelerate biomarker discovery

At the development stage, both improvements in existing technologies as well as exploration of new technologies are needed. Miniaturizing – most assays still utilize larger than desirable sample volumes – and the limiting factors of procuring, handling and storing of human samples are barriers to rapid evaluation. Other optimization needs include cost reduction, availability of good quality longitudinal samples and distributed validation efforts for a number of newer assays.

Some of the highlighted current immune biomarker technologies at the workshop included the following:

- Epimax: an unbiased technology for the identification of new T1D epitopes and the assessment of antigen-specific T cell repertoires [15].
- Serum-driven transcription profiling to characterize longitudinal changes in inflammatory characteristics of disease over time [16].
- T cell transcriptome profiling as prognostic markers of disease onset/relapse [17].
- Whole blood transcriptome fingerprinting as a measure of disease severity [18].
- Nucleic Acid Programmable Protein Assay (NAPPA) technology platform for profiling autoantibodies in new-onset or prediabetic patient sera [19].
- Detection of β cell-specific methylated DNA in peripheral blood to serve as a predictive or staging marker [20].
- Disappearance of peripheral blood anergic B cells as an early biomarker of T1D risk [21].
- A microengraving technology for the detection of secreted cytokines and antibodies from peripheral blood mononuclear cells [22,23].
- A proposed standardizing method for lymphocyte extractions from blood [24].

It was noted that technology platforms that remain underutilized in T1D biomarker studies include single-cell assay methods such as flow cytometry or mass spectrometry, and other recent microfluidics technologies, such as single-cell mass cytometry (CyTOF) [25]. These technologies allow scaling of assay platforms to high-throughput levels. To this end, liquid chromatography/mass spectrometry-based proteomics approaches to yield prognostic or early diagnostic biomarkers, including a sophisticated mix of shotgun, differential [26,27] or targeted approaches, were presented [28] at the workshop. These methodologies utilize very low sample volumes and can provide precise, reproducible measurement of either known (targeted) or all (shotgun, differential) peptides or metabolites present, are potentially scalable and are increasingly accessible to less specialized academic and clinical laboratories. However, at present these technologies require considerable expertise, have a comparatively limited dynamic range, can handle a 'medium' sample throughput (~300 per week) and can struggle with labile metabolites, leaving room for improvement. An early-stage assay involving two-dimensional gel electrophoresis/mass spectrometry to screen for inflammatory and metabolic markers with greatest longitudinal changes in T1D was presented at the meeting [29], which awaits further development and validation.

Disease staging: key gaps

While various T1D-specific biorepositories and living biobanks exist, to date no concerted and consolidated effort has emerged to couple new assays and technologies with such sample resources with the goal of establishing and validating a robust set of clinically implementable biomarkers that can be applied to disease staging, prediction, as well as response to therapy. The key gaps highlighted during the workshop are presented below for the prediabetic and diabetic stages of the disease.

In prediabetes, there is a significant dearth of biomarkers all around, and the field will need to actively develop biomarkers that can fill this gap, keeping in mind the heterogeneity of this disease. Active research should be pursued for the following areas:

- Development of cost-effective assays for autoantibody detection and measurement [30].
- Defining differences between progressors and non-progressors to disease (typically after persistent multiple autoantibody positivity). Special emphasis should be placed on the 'elite non- progressors' – autoantibody-positive individuals who do not develop T1D, or develop it slowly – compared with progressors.
- Small-scale, proof-of-concept studies of candidate biomarkers, followed by validation. Much biomarker effort thus far has been in the discovery stage and is ready for this next step.
- Defining early risk biomarkers detectable prior to autoantibody conversion, which could be assessed in cohorts at

genetic risk and subsequently expanded to address 'moderate risk' populations as well; these could include biomarkers of β cell mass/death and β cell stress/function ('omics' approaches appear well-suited for this), and genetic and functional signatures (including epigenetic biomarkers), among others.
- Better understanding of the role of innate immunity and metabolites in the predisease state.

In diabetes, there are gaps in understanding disease progression after onset. The relationship between immune status and insulin resistance, in the period immediately preceding clinical diagnosis of T1D, remains incompletely understood. The following key focus areas in need for attention were identified:

- Short-term adaptive trials with mechanistic biomarkers as end-points. The choice of biomarker should be reflective of the mechanistic pathway targeted by a given intervention. Such trials would allow the determination of the dose, route and timing of therapy and identify responder subgroups. If a desirable effect is achieved, these trials could inform larger trials for longer time-frames that may include individuals with longer-standing disease.
- Studies to define markers of slow *versus* rapid progression of loss of β cell mass *after* disease onset.

The path to translation: necessary considerations

Whether a biomarker/assay is ready for translation will depend on the context and clarity of a study end-point, the extent to which assay validation has been carried out and the stage of the disease to which the biomarker/assay would correspond. In this context, information gained from evaluating longitudinal samples with a comparison of cases *versus* controls would be meaningful towards establishing confidence in the applicability of a given biomarker assay. Following were the identified gaps in knowledge on the path to biomarker translation:

1. *The switch:* when people progress to overt disease, they appear to do so following a 'switch' that is comprised of genetic risk, the presence of autoantibodies and permissive environmental factors [31–37]. This is highlighted in instances where siblings of a similar predisposing genetic make-up do not all become diabetic. In order to understand this phenomenon more clearly, we must study systematically changes in the innate and adaptive immune responses in key cohorts over time. Most studies thus far involving autoreactive $CD4^+$ and $CD8^+$ T cells have focused more extensively on the newly diagnosed population and less on prediabetes. It would be informative to know the immune profile of individuals at the time of, or immediately preceding, autoantibody positivity. Unbiased approaches that interrogate innate immunity would also be gap-filling here [38].
2. *The integrated pipeline:* there is a need for an integrated pipeline of biomarkers with the purpose of utilizing multiple and different approaches to interrogate the same set of samples from a well-defined cohort with genetic risk and follow this cohort immunologically along the course of the disease. It would be desirable to link this approach with high-throughput studies with a large sample size; a cohort would therefore need to include at least 100 individuals. Several existing cohorts that meet this criterion could potentially be accessed for this purpose.
3. *The 'Big Hole':* workshop participants felt that a 'Big Hole' in knowledge is the lack of information about β cell mass/status/function during disease progression. This has contributed to our inability to assess more effectively the cross-talk between the immune system and β cells during loss of tolerance to β cell antigens. The need for molecular or imaging-based markers of β cell loss as well as markers for the loss of β cell function was greatly emphasized, as identifying β cell loss will serve as the ultimate validation of disease progression

Resources: what we have and where we need to go

Existing biobanks

There was general consensus that access to existing repositories needs to be improved. Type 1 diabetes Trial-Net (http://www.diabetestrialnet.org), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; http://www2.niddk.nih.gov), Network of Pancreatic Organ Donors (n-POD; http://www.jdrfnpod.org) and other repositories offer samples suitable for evaluation of biomarkers of different stages of disease. It was noted that the Trial-Net Ancillary Study Committee offers a navigator to help non-diabetes investigators design their studies. It would be meaningful to utilize these resources effectively for biomarker research.

Living biobanks

Living biobanks were felt to be key for moving T1D biomarker efforts forward. A living biobank is a cohort of well-characterized individuals who are followed longitudinally along the course of disease progression, and who have consented to provide 'on-demand' biological samples for research purposes. These biobanks support studies that are novel and preliminary, supply assays that require large sample volume and need to be tested in a large sample size for validation or require fresh cells/samples. It would be reasonable to prioritize optimization or development of T cell-based assays with these cohort samples. Such cohorts would also be ideal for the study of disease progression over long

periods of time and might allow for procuring longitudinal samples at frequent intervals (e.g. every 8 weeks or so), unlike what has been possible in the past. Given the gap-filling roles living biobanks can play in biomarker development, the group discussed whether a large effort could be undertaken by existing independent biobanks both in the United States [TrialNet, Barbara Davis Center for Childhood Diabetes (BDC; http://www.barbaradaviscenter.org), Benaroya Research Institute (BRI; http://www.benaroyaresearch.org), The T1D Exchange (http://www.t1dexchange.org), etc.] and around the world (Germany, Finland, Australia, United Kingdom) to come together with greater co-operation towards a seamless and unified living biobank effort.

Special populations to target in this effort would be:

- Cohorts of genetically at-risk subjects.
- Cohorts of discordant twins, which would offer genetically matched samples suitable for 'omics' approaches. Twins would help to reduce effects of genetic heterogeneity and improve the ability to detect differences that reflect disease.
- Elite non-progressors.
- Affected mothers (to study both mother and infants).
- Patients with T1D.
- Healthy control children (to properly age-match). This is a critical resource and knowledge gap and has been traditionally difficult to achieve.

Gold standard sample repository

There was consensus on a need to begin building a 'Gold Standard' Sample Repository immediately, where samples would be collected prospectively through the living biobank effort. This would allow for later validation of an integrated pipeline of biomarker assays and allow sharing of samples for parallel analysis with multiple approaches. The cohort linked with this effort would thus be a 'validation' cohort. It was noted that the design of this resource should be protocol-driven, with appropriate equipment and procedures to collect the samples. Participants with background in industry settings suggested that the development of less complex assays and protocols to stabilize samples soon after collection should be paramount here. The repository would not need to be in a single physical location, and some assays could be performed by centralized laboratories to reduce variability; however, it would be helpful to export these assays to other laboratories for a comprehensive analysis. An effective strategy would be to create a collection of serum/plasma samples for non-cell-based assays, and a collection of frozen peripheral blood mononuclear cells (PBMC) for non-live-cell-based assays from the same samples following rigorous standardized protocols [39]. Finally, all data generated could link to a centralized database (see next section) to allow for merging of data from different groups.

Highly relevant to the Gold Standard Repository discussion are efforts in place with the n-POD. There is already a system in place in this network for tissue/sample processing, archiving and efficient distribution to investigators. It was noted that n-POD has begun instituting working groups that study, collaboratively, samples from the same patients with a multitude of approaches. Importantly, the design of the approach is discussed collectively, whereby critical details are worked out that allow for maximizing co-ordination and the potential for discovery; for example, co-ordinating tissue sections allows for examinations of multiple parameters by different investigators on the same islets (using serial sections). Results are shared within the groups in real time to guide study progression further and incorporate changes or developments. Finally, n-POD offers the opportunity to correlate emerging biomarkers with pathology in the pancreas (for example, markers of β cell stress, mass, etc.) and a number of ongoing n-POD projects are generating data on these aspects at this time [40].

Database

A central, shared database for the Gold Standard-type biomarker samples was deemed critical to make real progress in the field of T1D. Access to the repositories would imply that data are shared to facilitate discovery, standardization and integration across platforms. Given the importance of standardization of data, the community could benefit strongly from a centralized database that would merge all data provided by investigators/groups, and which would also include pilot study and/or basic discovery data. The strategy would be to barcode all samples from all repositories through a single system and have them linked with the data maintained in the database: a system that could potentially be modelled after that of the Immune Tolerance Network (ITN), which already has such methodologies in place. Policies could be put into place that would allow a 6–18-month embargo or until publication (whichever is earlier), for public release of all data deposited into the database. It was noted that independent studies such as The Environmental Determinants of Diabetes in the Young (TEDDY; http://www.teddy.epi.usf.edu) have instituted such guidelines.

Clinical studies with mechanistic end-points

There was interest in considering the design of small and short trials with focus on biomarkers as end-points, to identify dose and responses that would appropriately inform larger, longer and more expensive trials. It was noted that such strategies are currently under consideration by organizations such as Trial-Net and the ITN. Representatives from industry commented that robust responses and proof-of-concept data could be achieved with as few as 10 patients and controls, and therefore small cohort sizes should not be

a deterrent factor in these pilot trials. Biomarkers utilized here must have first passed validation quality control testing in longitudinal cohorts with frequent samplings to establish their range of variability. Ultimately, the factors impacting a given trial design will vary, depending upon the type of drug and the type of biomarker assayed. Overall, this approach would help to define disease heterogeneity and address the issues of individualized therapy in the long term.

Conclusions

In summary, this was a highly dynamic workshop that stimulated the exchange of knowledge and ideas among scientists from various sectors of the community in a common desire to move forward the biomarkers field in T1D. It was clear at the end of this workshop that the T1D scientific community sensed an imminent need for biomarkers associated with all aspects of T1D and realistic opportunities for major advances were identified. It also became apparent that this endeavour may need to be a multi-step process, perhaps starting with very distinct and well-defined populations of T1D subjects for discovery and small-scale clinical confirmation efforts, before expanding into larger cohorts. An effective and gap-filling path to accelerating progress would be to create collaborative consortia comprised of co-operative groups led by physicians/scientists working hand-in-hand with groups of relevant technology experts. In this way, assay optimization efforts can occur concurrently with clinical confirmation efforts that together feed a validation pipeline with a superior biomarker product or biomarker panel. Such a strategic approach should ameliorate many of the hurdles currently in existence with regulatory approvals or the engagement of industry in this space and hopefully provide the necessary toolkit for accelerating T1D research.

JDRF next steps

In recognition of the critical gap in biomarker tools for T1D research, JDRF released a Request For Applications (RFA) entitled 'Biomarker Discovery/Validation for Staging and Assessment of T1D' in early 2012 and subsequently funded a number of applications that ranged from discovery efforts to assay optimization and clinical validation efforts. If successful, these could be applied to disease staging, patient stratification for therapy or clinical response to therapy. JDRF plans to bring together its funded biomarker investigators to establish a Collaborative Biomarkers Consortium that will foster collaboration and data-sharing among its members. An integral component of this consortium will be a recently funded JDRF Biomarker Core and Validation Center (CAV), which should play a key role in undertaking gap-filling projects when applicable, co-ordinating data and sample-sharing and conducting validation assays as projects mature. Ultimately, as part of its larger strategic goal, JDRF hopes to expand both the Core's and Consortium's bandwidth to include other promising T1D biomarker efforts/technologies from academia or other sectors of the scientific community. Importantly, a key goal will be to engage regulatory agencies such as the Food and Drug Administration (FDA) at key points along the way for the qualification of validated biomarkers and their ultimate implementation in the clinic.

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List of attendees

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Disclosure

None.

References

1 Roep BO, Peakman M. Surrogate end points in the design of immunotherapy trials: emerging lessons from type 1 diabetes. Nat Rev Immunol 2010; **10**:145–52.
2 Carey C, Purohit S, She JX. Advances and challenges in biomarker development for type 1 diabetes prediction and prevention using omic technologies. Expert Opin Med Diagn 2010; **4**:397–410.
3 Roep BO, Buckner J, Sawcer S, Toes R, Zipp F. The problems and promises of research into human immunology and autoimmune disease. Nat Med 2012; **18**:48–53.
4 van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol Rev 2011; **91**:79–118.
5 Gaglia JL, Guimaraes AR, Harisinghani M *et al.* Noninvasive imaging of pancreatic islet inflammation in type 1A diabetes patients. J Clin Invest 2011; **121**:442–5.
6 Rowe PA, Campbell-Thompson ML, Schatz DA, Atkinson MA. The pancreas in human type 1 diabetes. Semin Immunopathol 2011; **33**:29–43.
7 Bulek AM, Cole DK, Skowera A *et al.* Structural basis for the killing of human beta cells by CD8(+) T cells in type 1 diabetes. Nat Immunol 2012; **13**:283–9.
8 Nepom GT. MHC class II tetramers. J Immunol 2012; **188**:2477–82.
9 Herold KC, Brooks-Worrell B, Palmer J *et al.*, Type 1 Diabetes TrialNet Research Group. Validity and reproducibility of measurement of islet autoreactivity by T-cell assays in subjects with early type 1 diabetes. Diabetes 2009; **58**:2588–95.
10 Crawford F, Stadinski B, Jin N *et al.* Specificity and detection of insulin-reactive CD4+ T cells in type 1 diabetes in the nonobese diabetic (NOD) mouse. Proc Natl Acad Sci USA 2011; **108**:16729–34.
11 Velthuis JH, Unger WW, Abreu JR *et al.* Simultaneous detection of circulating autoreactive CD8+ T-cells specific for different islet cell-associated epitopes using combinatorial MHC multimers. Diabetes 2010; **59**:1721–30.
12 Abreu JR, Martina S, Verrijn Stuart AA *et al.* CD8 T cell autoreactivity to preproinsulin epitopes with very low human leucocyte antigen class I binding affinity. Clin Exp Immunol 2012; **170**:57–65.
13 Robins H, Desmarais C, Matthis J *et al.* Ultra-sensitive detection of rare T cell clones. J Immunol Methods 2012; **375**:14–9.
14 Long SA, Cerosaletti K, Bollyky PL *et al.* Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. Diabetes 2010; **59**:407–15.
15 Cao T, Ueno H, Glaser C, Fay JW, Palucka AK, Banchereau J. Both Langerhans cells and interstitial DC cross-present melanoma antigens and efficiently activate antigen-specific CTL. Eur J Immunol 2007; **37**:2657–67.
16 Levy H, Wang X, Kaldunski M *et al.* Transcriptional signatures as a disease-specific and predictive inflammatory biomarker for type 1 diabetes. Genes Immun 2012; **13**:593–604.
17 McKinney EF, Lyons PA, Carr EJ *et al.* A CD8+ T cell transcription signature predicts prognosis in autoimmune disease. Nat Med 2010; **16**:586–91, 1 p following 91.
18 Kaizer EC, Glaser CL, Chaussabel D, Banchereau J, Pascual V, White PC. Gene expression in peripheral blood mononuclear cells from children with diabetes. J Clin Endocrinol Metab 2007; **92**:3705–11.
19 Qiu J, LaBaer J. Nucleic acid programmable protein array a just-in-time multiplexed protein expression and purification platform. Methods Enzymol 2011; **500**:151–63.
20 Akirav EM, Lebastchi J, Galvan EM *et al.* Detection of beta cell death in diabetes using differentially methylated circulating DNA. Proc Natl Acad Sci USA 2011; **108**:19018–23.
21 O'Neill SK, Liu E, Cambier JC. Change you can B(cell)eive in: recent progress confirms a critical role for B cells in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes 2009; **16**:293–8.
22 Bradshaw EM, Kent SC, Tripuraneni V *et al.* Concurrent detection of secreted products from human lymphocytes by microengraving: cytokines and antigen-reactive antibodies. Clin Immunol 2008; **129**:10–8.
23 Bradshaw EM, Raddassi K, Elyaman W *et al.* Monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines inducing Th17 cells. J Immunol 2009; **183**:4432–9.
24 Burger DE, Wang L, Ban L *et al.* Novel automated blood separations validate whole cell biomarkers. PLoS One 2011; **6**:e22430.
25 Newell EW, Sigal N, Bendall SC, Nolan GP, Davis MM. Cytometry by time-of-flight shows combinatorial cytokine expression and

virus-specific cell niches within a continuum of CD8+ T cell phenotypes. Immunity 2012; **36**:142–52.
26 Sevinsky JR, Cargile BJ, Bunger MK *et al*. Whole genome searching with shotgun proteomic data: applications for genome annotation. J Proteome Res 2008; **7**:80–8.
27 Paweletz CP, Wiener MC, Bondarenko AY *et al*. Application of an end-to-end biomarker discovery platform to identify target engagement markers in cerebrospinal fluid by high resolution differential mass spectrometry. J Proteome Res 2010; **9**:1392–401.
28 Wang TJ, Larson MG, Vasan RS *et al*. Metabolite profiles and the risk of developing diabetes. Nat Med 2011; **17**:448–53.
29 Zhi W, Sharma A, Purohit S *et al*. Discovery and validation of serum protein changes in type 1 diabetes patients using high throughput two dimensional liquid chromatography-mass spectrometry and immunoassays. Mol Cell Proteomics 2011; **10**:M111 012203.
30 Tiberti C, Yu L, Lucantoni F *et al*. Detection of four diabetes specific autoantibodies in a single radioimmunoassay: an innovative high-throughput approach for autoimmune diabetes screening. Clin Exp Immunol 2011; **166**:317–24.
31 Hober D, Sane F, Jaidane H, Riedweg K, Goffard A, Desailloud R. Immunology in the clinic review series; focus on type 1 diabetes and viruses: role of antibodies enhancing the infection with Coxsackievirus-B in the pathogenesis of type 1 diabetes. Clin Exp Immunol 2012; **168**:47–51.
32 Jaidane H, Sane F, Hiar R *et al*. Immunology in the clinic review series; focus on type 1 diabetes and viruses: enterovirus, thymus and type 1 diabetes pathogenesis. Clin Exp Immunol 2012; **168**:39–46.
33 Lind K, Huhn MH, Flodstrom-Tullberg M. Immunology in the clinic review series; focus on type 1 diabetes and viruses: the innate immune response to enteroviruses and its possible role in regulating type 1 diabetes. Clin Exp Immunol 2012; **168**:30–8.
34 Grieco FA, Sebastiani G, Spagnuolo I, Patti A, Dotta F. Immunology in the clinic review series; focus on type 1 diabetes and viruses: how viral infections modulate beta cell function. Clin Exp Immunol 2012; **168**:24–9.
35 Stene LC, Rewers M. Immunology in the clinic review series; focus on type 1 diabetes and viruses: the enterovirus link to type 1 diabetes: critical review of human studies. Clin Exp Immunol 2012; **168**:12–23.
36 Coppieters KT, Wiberg A, Tracy SM, von Herrath MG. Immunology in the clinic review series: focus on type 1 diabetes and viruses: the role of viruses in type 1 diabetes: a difficult dilemma. Clin Exp Immunol 2012; **168**:5–11.
37 Gale EA. Viruses and type 1 diabetes: ignorance acquires a better vocabulary. Clin Exp Immunol 2012; **168**:1–4.
38 Han D, Cai X, Wen J *et al*. Innate and adaptive immune gene expression profiles as biomarkers in human type 1 diabetes. Clin Exp Immunol 2012; **170**:131–8.
39 Mallone R, Mannering SI, Brooks-Worrell BM *et al*. Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive T cell responses: position statement of the T-Cell Workshop Committee of the Immunology of Diabetes Society. Clin Exp Immunol 2011; **163**:33–49.
40 Campbell-Thompson M, Wasserfall C, Kaddis J *et al*. Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. Diabetes Metab Res Rev 2012; **28**:608–17.