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(54) Title: DIABETES BIOMARKERS

FIG. 1

(57) Abstract: A new markers for insulin production decline in Type 1 diabetes has been found in the ratio the CD4 naïve (CD45RO-CD62L+) to central memory (CD45RO+CD62L+) and in the level of CD4 central memory T-cell subpopulations. A method of diagnosing autoimmunity and its progressiveness, more specifically diabetes, pre-diabetes, a susceptibility to diabetes mellitus, or the level of effectiveness of therapy/intervention modality for one or more of such conditions in a subject can be conducted by determining level of CD4 naive (CD45RO-CD62L+) T-cells by immunofluorescence analysis of a sample extracted from a subject; determining level of CD4 central memory (CD45RO+CD62L+) T-cells by immunofluorescence analysis of a sample extracted from a subject, and quantitatively relating the levels of the CD4 naive and central memory T-cells, wherein a low ratio of CD4 naive T-cells to CD4 central memory T-cells and/or high CD4 central memory T-cell indicates autoimmunity, a susceptibility to autoimmunity, diabetes, pre-diabetes, a susceptibility to diabetes mellitus or ineffectiveness of a treatment for one or more of such conditions.

DIABETES BIOMARKERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001]   The present application claims priority to U.S. provisional application Serial Number 61/651,144 filed May 24, 2012, herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002]   The present invention relates generally to the field of autoimmunity, diabetes and more particularly to Type 1 diabetes and immune markers.

BACKGROUND

[0003]   The most common form of Type 1 diabetes mellitus (T1DM) is an immune-mediated disease where insulin-secreting β-cells are destroyed by an autoimmune response. There are a number of genetic and environmental factors associated with the onset of the disease, which involves the progressive inflammatory infiltration of pancreatic islets by immunocytes targeted specifically to insulin-secreting β-cells. This pathology develops over an indeterminate period of time (months to years) prior the clinical onset (pre-diabetes) and continues after the patient is diagnosed with the disease.

[0004]   There is currently a method for screening and diagnosis of T1DM using antibodies. Where the specific antibodies are present, over time, overt diabetes will often develop. Expression of one or more of: GAD65 autoantibodies (GAAs), ICA512 autoantibodies (ICA512AAs), or anti–insulin autoantibodies (IAAs) is associated with a risk of progression to T1DM. Expression of two or more of: GAD65 autoantibodies (GAAs), ICA512 autoantibodies (ICA512AAs), or anti–insulin autoantibodies (IAAs) is associated with a high risk of progression to T1DM. (Liping Yu et al., *Diabetes August* 2001 vol. 50 no. 8 1735-1740; *Verge CF et al., Diabetes* 45:926–933, 199; *Verge CF. et al, Diabetes* 47:1857–1866, 1998; *and* Bingley PJ, et al., *Diabetes 43:1304–1310, 1994).*

[0005]   However, this screening has limited usefulness for the individual patient. While the antibody screen can detect a heightened level of risk of T1DM, the risk is based on the population in general and cannot inform the individual patient as to whether the disease will, for example, onset within the next several months or if the individual will likely be free of diabetes for the next 5- 10 years. The intensity of the autoimmune destruction varies patient to

patient. Thus, there is a need for a diagnostic method that will inform an individual of personal risk of developing T1DM and can indicate the time frame of disease onset.

[0006]   There is also need for improved primary endpoints for analysis of therapies for T1DM autoimmunity. For a therapy to receive regulatory approval from the FDA, clinical trials must show statistically significant effect at an appropriate primary endpoint. This treatment effect preferably demonstrates some obvious and clinically significant benefit. In case of treatment to prevent T1DM, the delay or absent of development of the clinical disease (this makes these trials 5 or 10 years long). In case of intervention in patients with clinical disease, the appropriate primary endpoints are metabolic control and diabetes complication related. Most commonly applied metabolic primary endpoint - short of the cure- is measurement of self-insulin production (stimulated C-peptide as a surrogate marker for preservation of β-cell function), others are HbA1c and insulin use. Improved β-Cell function in T1DM patient can predict better short and long-term clinical outcome can take several years to assess. For treatment of T1DM and complications, blood sugar levels can be monitored directly and improved glycemic control can be monitored through the levels of glycosylated hemoglobin (e.g. HbA_{1c}), which has been shown to be directly related to the risk of short and long term diabetic complications.

[0007]   For therapies intended to preserve β-cell function in a post clinical phase of the disease, stimulated C-peptide concentration has been used to measure progression of T1DM (Palmer JP, et al., *Diabetes* 2004; 53:250-264). However, the use of C-peptide concentration requires repeated invasive testing (so called Mixed Meal Tolerance Test) over extended period of time, thus the trials last for a long time to allow for assessment of progression of the disease that reduces β-cell function and thus C-peptide concentration.

[0008]   Therefore, there is a need for better markers to serve as primary endpoints for trials (both prevention and intervention trials) as well as better markers that can more rapidly and reliably indicate the progressiveness of T1DM autoimmunity, thus stage the prediabetic and diabetic condition. There is also a need for better assays that can measure the effectiveness of therapies for the treatment of T1DM and/or detect complications.

SUMMARY

[0009]   A new marker for self-insulin production decline in Type 1 diabetes has been found in the ratio the CD4 naïve (CD45RO-CD62L+) to central memory (CD45RO+CD62L+) T-cell

subpopulations and in the central memory (CD45RO+CD62L+) T-cell subpopulation levels. A method of diagnosing diabetes, pre-diabetes, a susceptibility to diabetes mellitus, or the effectiveness of therapy for one or more of such conditions in a subject can be conducted by determining a level of CD4 naïve (CD45RO-CD62L+) T-cells by immunofluorescence analysis of a sample extracted from a patient; determining a level of CD4 central memory (CD45RO+CD62L+) T-cells by immunofluorescence analysis of a sample extracted from a patient, and quantitatively relating the levels of the CD4 naïve and CD4 central memory T-cells, wherein a low or decreasing ratio of CD4 naïve T-cells to CD4 central memory T-cells or a high or increasing CD4 central memory T-cell level indicates autoimmune disease, pre-autoimmune disease, a susceptibility to autoimmune or ineffectiveness of a treatment for one or more of such conditions. In prediabetes setting, the presence of T1DM specific autoantibody indicates the presence of T1DM autoimmunity itself. Thus, in one aspect of the present invention, the method as described herein is combined with determining whether diabetes autoantibodies are present.

[0010]   In one aspect of the present invention, methods for determining the effectiveness of a therapy for diabetic and pre-diabetic condition in a subject are disclosed including the steps of initiating therapy in a subject, extracting a sample from the subject, measuring the ratio the CD4 T-cell naïve (CD45RO-CD62L+) to central memory (CD45RO+CD62L+) subpopulation and /or the level of CD4 central memory T-cells in the sample, and evaluating the effectiveness of the therapy, wherein an increase in the ratio and/or low/decline CD4 central memory T-cells during the therapy indicates effective therapy.

[0011]   In yet other embodiments, the methods can include initiating therapy in the subject, extracting a sample from the subject, measuring the CD4 T-cell central memory (CD45RO+CD62L+) subpopulation in the sample, and evaluating the effectiveness of the therapy, wherein a low or decreasing CD4 central memory T-cell level or a high or increasing ratio of the CD4 T-cell naïve to CD4 central memory T-cell subpopulation during the therapy indicates effective therapy.

[0012]   Some embodiments provide a method of monitoring the effect of an intervention for an autoimmune disease such as diabetes mellitus in a subject comprising: selecting a subject undergoing a therapy for an autoimmune disease or condition, the extracting a sample from the subject, measuring the CD4 central memory (CD45RO+CD62L+) T-cell subpopulation and optionally measuring the CD4 T-cell naïve (CD45RO-CD62L+) subpopulation in the sample,

and evaluating the effectiveness of the therapy, wherein a low or decreasing CD4 central memory T-cell level or a high or increasing ratio of the CD4 T-cell naïve to CD4 central memory T-cell subpopulation during the therapy indicates effective therapy. The samples are extracted from the subject, for example, before the start of therapy (or after the start of therapy but before the onset of changes in cell populations) and at approximately three and/or six months of ongoing therapy.

[0013] In other embodiments, there is provided a method of diagnosing an autoimmune disease, pre-autoimmune disease, a susceptibility to an autoimmune disease, or the effectiveness of therapy for one or more of such conditions in a subject comprising: selecting a subject having or suspected of having an autoimmune disease, pre-autoimmune disease, or a susceptibility to an autoimmune disease, determining a level of CD4 naïve (CD45RO-CD62L+) T-cells by immunofluorescence analysis of a sample extracted from the subject; determining a level of CD4 central memory (CD45RO+CD62L+) T-cells by immunofluorescence analysis of a sample extracted from the subject, and quantitatively relating the levels of the CD4 naïve and CD4 central memory T-cells, wherein a low or decreasing ratio of CD4 naïve T-cells to CD4 central memory T-cells or a high or increasing CD4 central memory T-cell level indicates autoimmune disease, pre-autoimmune disease, a susceptibility to autoimmune or ineffectiveness of a treatment for one or more of such conditions.

[0014] Some embodiments provide a method of determining the time until onset of autoimmune disease comprising: obtaining a subject having an autoantibody specific for the autoimmune condition; extracting one or more sample(s) from the subject; measuring the central memory (CD45RO+CD62L+) T-cell subpopulation level and optionally measuring the CD4 T-cell naïve (CD45RO-CD62L+) subpopulation level in the sample(s), and calculating the change in the CD4 central memory T-cell subpopulation level and/or the change in the ratio of the CD4 T-cell naïve to CD4 central memory T-cell subpopulation between two or more sample or between two or more measurements in one sample made at different time points, wherein a decrease in the ratio and/or higher CD4 central memory T-cell levels correlate with a shorter time to onset of autoimmune disease. As an example, each unit of increase from baseline in Log central memory correlates to a subsequent decrease in C-peptide concentration of approximately -0.178 ng/mL. This correlation can be used to measure and predict the speed

of decline in C-peptide levels as the quantitative change in these T cell populations herald quantitative changes in C-peptide levels to come.

[0015] Some embodiments provide a method of determining the level of effectiveness of different intervention modalities, such as different clinical trials for diabetes mellitus. This method comprises comprising initiating therapy in a subject, extracting a sample from the subject, measuring the CD4 central memory (CD45RO+CD62L+) T-cell subpopulation and optionally measuring the CD4 T-cell naïve (CD45RO-CD62L+) subpopulation in the sample, and evaluating and compare the effectiveness of the different interventions, where increase in the ratio and/or lower CD4 central memory T-cell levels correlate with more effective intervention ---you repeated twice!?. Thus, there is provided a way to reliably and quantitatively compare the effectiveness of intervention modalities used in the active arms of two or more different clinical trials.

[0016] Some embodiments provide a method of targeted drug development for autoimmunity comprising: extracting a sample from one or more subjects, isolating the central memory (CD45RO+CD62L+) T-cell subpopulation and optionally isolating the CD4 T-cell naïve (CD45RO-CD62L+) subpopulation in the sample, and develop drug(s) specifically targeting these or subset of these cells based on their disease specific characteristics.

[0017]   These and other features of the embodiments as will be apparent are set forth and described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018]   The following drawings form part of the present specification and are included to further demonstrate certain aspects of the invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0019]   FIG. 1 is a chart showing the reduction in C-peptide loss per unit change in T cells at a prior visit compared to baseline. Both the decrease in central memory and the increase in naïve/central ratio are shown.

[0020]   FIGS. 2A – 2D show the percent change from baseline of CD4 T cell subsets identified as representing (FIG. 2A) naive and (FIG. 2B) central memory populations as well as

(FIG. 2C) the ratio of naive:central memory and (FIG. 2D) Treg populations, all measured at specified intervals after treatment initiation ("0 months"). Last treatment was at month 24. Closed circles are abatacept treated and open circles placebo; symbols represent mean and the error bars represent 95% confidence intervals. P values and dashed lines indicate that the two groups differ significantly over the timepoints indicated.

DETAILED DESCRIPTION

[0021]   The most common form of Type 1 diabetes mellitus (T1DM) is an immune-mediated disease where insulin-secreting β-cells are destroyed by an autoimmune response. T-cells play a central part in autoimmunity associated with T1DM. To become fully activated, these cells are believed to need at least two crucial signals. (Marelli-Berg FM, Okkenhaug K, Mirenda V. A *Trends Immunol* 2007; **28:** 267–73.)   The first signal is an interaction between an antigen in the groove of the MHC molecule on antigen-presenting cells with the T-cell receptor. The second signal is the interaction between CD80 and CD86 on the antigen presenting cells and CD28 on the T-cells. This co-stimulatory second signal is needed for full activation of cells, and without it they cannot function. Therefore, co-stimulation blockade has been proposed as a therapeutic modality for autoimmunity and transplantation. (Bluestone JA, St Clair EW, Turka LA. *Immunity* 2006; **24:** 233–38.)

[0022]   Naïve T lymphocytes travel to T-cell areas of secondary lymphoid organs in search of antigen presented by antigen presenting cells (APC-s). Once activated, they proliferate vigorously, generating effector cells that can migrate to inflamed tissues to fight infection or in case of autoimmunity destroy tissues. Upon clearance of the antigen a fraction of primed/activated T lymphocytes persists as circulating memory cells that can normally confer protection and give, upon secondary challenge, an enhanced response. Two major types of memory T-cells remain: central memory cells, which patrol lymphoid organs, and effector memory cells that act as sentinels in peripheral tissues such as the skin and the gut.

[0023]   Type 1 diabetes (T1DM) autoimmunity is driven by activated T-lymphocytes. Abatacept is a co-stimulation modulator and blocks full T-lymphocyte activation. The effect of two-year administration of abatacept in a randomized double-masked trial in recently diagnosed T1DM patients has been evaluated. Abatacept slowed decline of beta cell function significantly over two years. Preliminary results from this trial have been published as an article   entitled

"Co-stimulation modulation with abatacept in patients with recent-onset Type 1 diabetes: a randomized, double-blind, placebo-controlled trial" in *The Lancet* (published online June 28, 2011). This paper is included as Appendix A and is part of the presently filed application.

[0024]   Multiple T-cell markers have been analyzed for any correlation to the progression of diabetes (destruction of remaining insulin-secreting β-cells).   For patients treated with a compound that slows the autoimmune destruction in patients having diabetes humoral biomarker(s) (GAA, ICA512AA, IAA), it was found that the CD4 T-cell naïve (CD45RO-CD62L+) to central memory (CD45RO+CD62L+) subpopulation ratios increased significantly from baseline during treatment and then returned to baseline after the therapy concluded. The treatment with abatacept was also found to significantly slow the decline of C-peptide by reducing the levels of CD4 T central memory (CD45RO+CD62L+) cells.

[0025]   For patients not treated to slow the progression of diabetes (destruction of remaining insulin-secreting β-cells), it was found that higher central memory T-cells were significantly associated with subsequent decline in C-peptide. Thus, a decrease in these T-cells in the treated group was significantly associated with slower rate of C-peptide decline and this T immune cell subpopulation (central memory T-cells) can be used as a surrogate immune marker for self-insulin production decline.

[0026]   Thus, it is hypothesized that abatacept blocks naïve cells from becoming activated and the presence of a higher concentration of CD4 naïve T-cells as compared to the CD4 memory T-cells indicates that the compound is effective at delaying the onset of T1DM in pre-diabetic subjects and is effective delaying the decline of insulin production in T1DM patients. Abatacept exert its effect on autoimmunity by reducing CD4 central memory T-cells levels as it blocks CD4 naïve to CD4 central memory T-cell activation process   This biomarker can also be used in the absence of a compound such as abatacept for the diagnosis of progressivness of diabetes or pre-diabetes (in conjunction with diabetes antibodies) as well as to determine the susceptibility to fast progressing diabetes mellitus. This marker can monitor the intensity and aggressiveness of autoimmune destruction, the speed of loss of the insulin-secreting β-cells.

[0027]   The biomarker analysis as described herein may be provided in conjunction with known antibody testing. Such a combination provides both a determination of susceptibility to diabetes as well as a time frame for onset. Post-clinical onset, it can predict the time to the total loss of self-insulin production- time to "total diabetes".

[0028]   C-peptide is a 31 amino acid peptide that acts as a structural connection in proinsulin.   It is released into circulation along with insulin when the proinsulin is enzymatically cleaved. Thus, low to undetectable levels of C-peptide are found in T1DM while T2DM patients earlier in their disease often have higher than normal insulin/C-peptide level. However, there can be several reference ranges for C-peptide levels dependent upon factors such as the type of assay used, patient age, and whether or not a patient has fasted prior to the test.   Any known assay method may be used to quantify C-peptide such as the radioimmuno assay (RIA) and immunochemiluminometric assay (ICMA). In the RIA method, C-peptide can be measured using goat anti-C-peptide. The antibody, which also recognizes proinsulin, has no cross-reactivity with insulin. The analytic sensitivity of the test is generally 0.125 ng/ml and an overnight fast is required. The RIA method provides a reference range for normal adults of 0.5 - 2 ng/mL. In the ICMA method, a competitive immunoassay having two incubation cycles is used to provide an analytic sensitivity of approximately 0.3 ng/mL. The ICMA method provides a reference range for normal adults of 0.9 - 4 ng/mL and the patient must be fasting. For children less than 12 years old, the reference range is 0.0 to 0.3 ng/mL. For children 10 – 12 years, the reference range is 0.4 to 3.3 ng/mL and for individuals 17 years and up. (LABCORP). In order to assess the capacity of the insulin-secreting β-cells to produce insulin food challenge tests are used, most commonly used test is called Mixed Meal Tolerance Test (MMTT) where several blood samples are collected over 2 or 4 hours time for C-peptide measurement.

[0029]   As used herein, the term "subject" is a human or other animal, having or expected to have an autoimmune disorder. Thus, in some embodiments the subject will be in need of the therapeutic treatment as provided herein.   Preferred subjects are mammals.   Examples of subjects include but are not limited to, humans, horses, monkeys, dogs, cats, mice, rates, cows, pigs, goats and sheep. In some embodiments, "subjects" are generally human patients having or expected of having diabetes. In some embodiments, "subjects" are human patients who have been diagnosed with diabetes within the last 200, 100, or 50 days. In some embodiments, "subjects" are human patients who have Type 1 diabetes mellitus. In some embodiments, "subjects" are human patients who are pre-diabetic. In some embodiments, "subjects" are human patients who have been recently diagnosed with diabetes mellitus but still have residual beta-cell function. In some embodiments, "subjects" are human patients who have autoimmunity other then Type 1 diabetes. Such autoimmunity includes, but is not limited to rheumatoid arthritis and multiple sclerosis.

[0030]   A subject having or expected of having an autoimmune disease or condition or one having or suspected of having an autoimmune disease, pre-autoimmune disease, or a susceptibility to an autoimmune disease, can be selected by evaluating subjects based on the diagnosis criteria for the, i.e., autoimmune disease.   Alternatively, or in addition, this patient population can be selected by evaluating any genetic marketers or autoantibodies or other biomarkers known to be correlated with the autoimmune disease, pre-autoimmune disease, or a susceptibility to an autoimmune disease.

[0031]   The term "treatment" or "treating" as used herein is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease.   Treatment is intended to encompass preventing the onset, slowing the progression, reversing or otherwise ameliorating, improve, or affect the disease, the symptoms or of disease or the predisposition toward disease. For example, treatment of a subject, e.g., a human subject, with a composition described herein, can slow, improve, or stop the ongoing autoimmunity, e.g., a reaction against pancreatic β-cells, in a subject before, during, or after the clinical onset of Type 1 diabetes.

[0032]   The term a "diabetic condition" as used herein is intended to encompass diabetes, pre-diabetes, or a susceptibility to diabetes.

[0033]   The treatment may be treatment using an approved pharmaceutical ingredient for clinical testing or may be the treatment occurring during a clinical trial or a pre-clinical trial.

[0034]   The phrase "delaying the progression" as used herein in the context of delaying the progression of diabetes mellitus means that the loss of functional residual β-cell mass, before or after the clinical onset of Type 1 diabetes is delayed. The delay, for example, may be a delay of 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24 or more months, or it may be a delay of 2, 3, 4, or more years.

[0035]   As used herein, the terms "administering" or "administration" are intended to encompass all means for directly and indirectly delivering a pharmaceutical composition to its intended site of action.

[0036]   The term "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A

therapeutically effective amount of a pharmaceutical composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the pharmaceutical composition elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the pharmacological agent are outweighed by the therapeutically beneficial effects.

[0037]   While the above description provides examples and specific details of various embodiments, it will be appreciated that some features and/or functions of the described embodiments admit to modification without departing from the scope of the described embodiments. The above description is intended to be illustrative of the invention, the scope of which is limited only by the language of the claims appended hereto.

EXAMPLES

[0038]   Aspects of the applicant's teachings may be further understood in light of the following examples, which should not be construed as limiting the scope of the applicant's teachings in any way.

Example 1 – Trial

[0039]   As described in the above-referenced Lancet paper, herein incorporated by reference in its entirety, a phase 2 clinical trial was conducted of the use of abatacept for patients diagnosed with Type 1 diabetes. Eligible patients had been diagnosed with Type 1 diabetes within the past 100 days and had at least one diabetes-related autoantibody (microassayed insulin antibodies; glutamic acid decarboxylase-65 [GAD-65] antibodies; islet-cell antigen-512 [ICA-512] antibodies; or islet-cell autoantibodies) and had stimulated C-peptide concentrations of 0·2 nmol/L or higher.

[0040]   Patients were randomly assigned in a 2:1 ratio, stratified by participating site, to receive experimental treatment with abatacept or placebo using a double blind protocol. Abatacept (Orencia, Bristol-Myers Squibb, Princeton, NJ, USA) was given on days 1, 14, and 28, and then every 28 days with the last dose on day 700 (total 27 doses) as a 30-min intravenous infusion at a dose of 10 mg/kg (maximum 1000 mg per dose) in a 100 mL 0·9% sodium chloride infusion. Normal saline infusion was used as placebo. Patients did not receive any premedication.

[0041]   Blood samples were analyzed centrally. C-peptide concentrations were measured from frozen plasma with a two-site immunoenzymometric assay (Tosoh Bioscience, South San Francisco, CA, USA). Blood samples were obtained at 3, 6, 12, 18, and 24 months as well as at 30 months, six months after the end of the dosing.

[0042]   Of the 112 patients enrolled in the study, 77 were randomly assigned to receive experimental treatment with abatacept and 35 were assigned to receive placebo. Results showed that over 2 years co-stimulation modulation with abatacept slows the reduction in β-cell function in recent-onset Type 1 diabetes by 9·6 months. At two year, the abatacept treated group had a 59% higher self-insulin production compare to placebo group. The abatacept treated group also had a significantly better HbA1c (measurement of level of blood sugar control) throughout the trial (with same insulin usage). The early intervention beneficial effect suggests that T-cell activation still occurs around the time of clinical diagnosis of Type 1 diabetes, even though the disease course has presumably been in progress for several years.

Example 2 – Flow Cytometry

[0043]   Flow cytometry analysis was performed on blood samples from subjects of the clinical trial in Example 1 for both abatacept and placebo arms at 0, 3, 6, 12, and 24 months with an additional analysis done six months after the end of the trial (30 months). Flow cytometry is a routine technique for counting and examining microscopic particles, such as cells, by suspending them in a stream of fluid and passing them one cell at the time by laser and an electronic detection apparatus. Modern instruments usually have multiple lasers and fluorescence detectors. Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers.

[0044]   Fluorescence-activated cell sorting (FACS), a specialized type of flow cytometry, was used in the analysis. FACS provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell and characterizing them. It is a useful scientific instrument, as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. Fluorescent signal comes from the fluorescent labeled antibodies the cells have been incubated with prior the FACS. With multiple labeling, each antibody is coupled with a different fluorophore. Antibodies used are specific for the cell marker of interest. To detect CD4+

cells, antiCD4 antibody was labeled with a fluorophore. For the simultaneously detection of CD45RO, a specific antiCD45RO antibody with another fluorophore was also used. (Fluorescence-labeled antiCD4 and antiCD45RO antibodies are commercially available from various sources, such as BD Biosciences of San Jose, CA.) Each fluorophore has a characteristic peak excitation and emission wavelength, thus make it possible to distinguish between them, e.g., by using a fluorescence-activated cell sorting instruments, such as the Becton-Dickinson FACSCalibur or FACSAria system.

[0045]   In three 5-color assays seven T-cell markers were studied. There were no changes from baseline found in the placebo group for any of these markers. In the treated group we saw no change in CD4 and CD8 T-cells or in naïve and memory subsets of CD8 T-cells.

[0046]   However, the CD4 T-cell naïve (CD45RO-CD62L+) to central memory (CD45RO+CD62L+) subpopulation ratio increased significantly from baseline in the abatacept group during treatment and then returned to baseline off therapy. During the trial for the placebo group, higher CD4 central memory T-cells were significantly associated with subsequent decline in C-peptide. A decrease in these T-cells in abatacept group was significantly associated with slower rate of C-peptide decline.

[0047]   The study also found that the regulatory T-cell population (CD4+CD25high) decreased from baseline in the abatacept group then returned to baseline off therapy. However, the reduction in these regulatory T-cells showed no correlation with the changes in naïve/memory populations or with changes in C-peptide levels.

[0048]   Table 1 provides the least squares mean change from baseline, given as a log, of the ratio of CD4 naïve T-cells to CD4 central memory T-cells and the standard deviation and p values for 3, 6, 12, and 24 months from baseline. The 30-months from baseline, where the subjects are off therapy is given as the 30-month data. The p values between drug and placebo groups are between groups at the same visit.

TABLE 1

LOG (NAÏVE T-CELLS/ CENTRAL MEMORY T-CELLS)

	Month from baseline	Mean Change	Standard Error	p value
Abatacept	3	2.137	0.1318	p=NS
Placebo	3	1.753	0.1927	
Abatacept	6	2.517	0.1305	p=0.0002
Placebo	6	1.636	0.1949	
Abatacept	12	2.698	0.1305	p=0.0002
Placebo	12	1.793	0.1951	
Abatacept	24	2.656	0.1328	p=0.0001
Placebo	24	1.698	0.1954	
Abatacept	30	1.731	0.1323	p=NS
Placebo	30	1.623	0.2075	

[0049]   The C-peptide concentration for these samples and time points were also measured. Analyzing both group data, prior changes both in CD4 T cell naïve to central memory ratios and in CD4 central memory T cell levels predicted subsequent C-peptide loss in T1DM (FIG. 1) whereas contemporaneous T-cell measurements were not significantly associated with C-peptide change from baseline. It has been found that an increase in central memory T-cells is significantly associated with subsequent declines in C-peptide (self insulin production). Specifically, each unit of increase from baseline in Log central memory ratio is estimated to decrease C-peptide on average by -0.178 ng/ml. FIG. 1 provides the decrease of CD4 central memory T cell and increase in CD4 naïve/central memory T cell ratio resulting in reduction in C-peptide loss. The number of the unit change can be compared and quantitatively express providing a measure indicating the aggressiveness of the autoimmune processes and also the level of effectiveness of different intervention modalities. The level of effectiveness of different intervention modalities can then be ranked based on the number of units.

[0050]   The time lags we looked at were 3 or 6 months. So for our purposes we need two different measurements of this cell population to assess the subsequent C-peptide loss or lack of it. Changes in these T-cell populations – both CD4 naïve/central memory cell ratios and CD4 central memory cell levels- predict subsequent C-peptide loss. FIGS. 2A – 2D shows the change in naive, memory and naive/memory T cell over time relative to baseline values. These show the changes as they occur over 30 months. While we looked at 3 and 6 months, as can be seen from FIG 2, other times and time intervals would work as well, and may include a

baseline measurement taken prior to treatment, and/or measurements at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24, 27, 30, 33, 36, or more months.

[0051]   The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described in any way. While the applicant's teachings are described in conjunction with various embodiments, it is not intended that the applicant's teachings be limited to such embodiments. On the contrary, the applicant's teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

CLAIMS

1.   A method of determining the effectiveness of a therapy for an autoimmune disease or
        condition in a subject comprising:
           selecting a subject undergoing a therapy for an autoimmune disease or condition,
           extracting a sample from said subject,
           measuring the CD4 central memory (CD45RO+CD62L+) T-cell subpopulation and
               optionally measuring the CD4 T-cell naïve (CD45RO-CD62L+) subpopulation
               in said sample, and
           evaluating the effectiveness of the therapy, wherein
               a low or decreasing CD4 central memory T-cell level or
               a high or increasing ratio of the CD4 T-cell naïve to CD4 central memory T-cell
               subpopulation during said therapy indicates effective therapy.

2.   The method of claim 1, wherein said sample is incubated with a labeled antiCD45RO
        antibody and a labeled antiCD62L antibody prior to the measuring step.

3.   The method of claim 1 or of claim 2, wherein measuring comprises subjecting said
        sample to flow cytometry.

4.   The method of any one of the preceding claims, wherein said sample is a blood sample.

5.   The method of any one of the preceding claims, wherein the decreasing CD4 central
        memory T-cell level or the increasing ratio is relative to the level or ratio from said
        extracted sample at different time points.

6.   The method of any one of claims 1 - 4, wherein the decreasing CD4 central memory T-
        cell level or the increasing ratio is relative to a standardized level or ratio, or to level or
        ratio obtained from a sample extracted before therapy starts.

7.   The method of any one of the preceding claims, wherein a low or decreasing CD4
        central memory T-cell level during said therapy indicates effective therapy.

8.   The method of any one of the preceding claims, wherein measuring comprises
        measuring both the CD4 central memory T-cell subpopulation and the CD4 T-cell naïve

subpopulation and wherein a high or increasing ratio of the CD4 T-cell naïve to CD4 central memory T-cell subpopulation during said therapy indicates effective therapy.

9. The method of any one of the preceding claims, wherein said therapy is for a diabetic condition.

10. The method of claim 9, further comprising:
    determining the presence of a diabetes-related autoantibody.

11. The method of claim 9, wherein the diabetic condition is Type 1 diabetes mellitus.

12. A method of diagnosing an autoimmune disease, pre-autoimmune disease, a susceptibility to an autoimmune disease, or the effectiveness of therapy for one or more of such conditions in a subject comprising:
    selecting a subject having or suspected of having an autoimmune disease, pre-autoimmune disease, or a susceptibility to an autoimmune disease,
    determining a level of CD4 naïve (CD45RO-CD62L+) T-cells by immunofluorescence analysis of a sample extracted from the subject;
    determining a level of CD4 central memory (CD45RO+CD62L+) T-cells by immunofluorescence analysis of a sample extracted from the subject, and
    quantitatively relating the levels of the CD4 naïve and CD4 central memory T-cells, wherein a low or decreasing ratio of CD4 naïve T-cells to CD4 central memory T-cells or a high or increasing CD4 central memory T-cell level indicates autoimmune disease, pre-autoimmune disease, a susceptibility to autoimmune or ineffectiveness of a treatment for one or more of such conditions.

13. The method of claim 12, wherein said autoimmune disease is diabetes mellitus or pre-diabetes, wherein a low or decreasing ratio of CD4 naïve T-cells to CD4 central memory T-cells or a high or increasing CD4 central memory T-cell level indicates diabetes, pre-diabetes, or a susceptibility to diabetes mellitus.

14. The method of claim 13, further comprising: determining the presence of a diabetes-related antibody selected from glutamic acid decarboxylase-65 antibodies, islet-cell antigen-512 antibodies, insulin antibodies or other islet-cell autoantibodies.

15.   The method of any one of claims 12-14, wherein the diabetic condition is Type 1 diabetes mellitus.

16.   The method of any one of claims 12-15, wherein the sample is incubated with a labeled antiCD45RO antibody prior to analysis.

17.   The method of any one of claims 12-16, wherein measuring comprises subjecting said sample to flow cytometry.

18.   The method of any one of claims 12-17, wherein said sample is a blood sample.

19.   The method of any one of claims 12-18, wherein said decreasing and/or increasing level or ratio is relative to the level or ratio from said extracted sample at different time points or is relative to a standardized level or ratio.

20.   A method of determining the time until onset of autoimmune disease or the level of effectiveness of different intervention modalities comprising:

          obtaining a subject;

          extracting one or more sample(s) from said subject;

          measuring the central memory (CD45RO+CD62L+) T-cell subpopulation level and
               optionally measuring the CD4 T-cell naïve (CD45RO-CD62L+) subpopulation
               level in said sample(s), and

          calculating the change in said CD4 central memory T-cell subpopulation level
               and/or the change in the ratio of the CD4 T-cell naïve to CD4 central memory
               T-cell subpopulation between two or more sample or between two or more
               measurements in one sample made at different time points,

     wherein a decrease in said ratio and/or higher CD4 central memory T-cell levels
     correlate with either a shorter time to onset of autoimmune disease or less effective
     intervention.

21.   The method of claim 20, wherein said subject has autoantibodies or other biomarkers specific for the autoimmune condition.

22.   The method of claim 20 or claim 21, wherein said subject has at least one diabetes-related antibody selected from glutamic acid decarboxylase-65 antibodies, islet-cell antigen-512 antibodies, insulin antibodies or other islet-cell autoantibodies, and wherein

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a decrease in said ratio and/or higher CD4 central memory T-cell levels correlate with shorter time to onset of diabetes mellitus.

23.   The method of any one of claims 20-22, wherein each unit of increase from baseline in Log central memory T cells correlates to a decrease in C-peptide concentration of approximately -0.178 ng/mL.

24.   The method of any one of claims 20-23, wherein at least two samples are extracted from said subject at between 2 and 12 months apart.

25.   The method of claim 24, wherein at least two samples are extracted from said subject at between 3 and 6 months apart.

26.   The method of any one of claims 20-23, wherein the sample extracted from said subject is measured at at least two different time points that are between 1 and 6 months apart.

27.   The method of any one of claims 20-26, wherein said sample is incubated with a labeled antiCD45RO antibody prior to the measuring step.

28.   The method of any one of claims 20-27, wherein measuring comprises subjecting said sample to flow cytometry.

29.   The method of any one of claims 20-28, wherein said sample is a blood sample.

30.   The method of any one of claims 20-29, wherein an increase in said level and/or a decrease in said ratio is relative to the level or ratio before therapy is started or relative to a standardized level or ratio.

31.   The method of any one of claims 20-29, wherein an increase in said level and/or a decrease in said ratio is relative to the level or ratio at an earlier time point from the same sample.

32.   A method of targeted drug development for autoimmunity comprising:
      extracting a sample from one or more subjects,
      isolating the central memory (CD45RO+CD62L+) T-cell subpopulation and optionally
         isolating the CD4 T-cell naïve (CD45RO-CD62L+) subpopulation in said sample,
         and

develop drug(s) specifically targeting these or subset of these cells based on their disease specific characteristics.

33. The method of claim 32, wherein said method is a method of targeted drug development for diabetes.
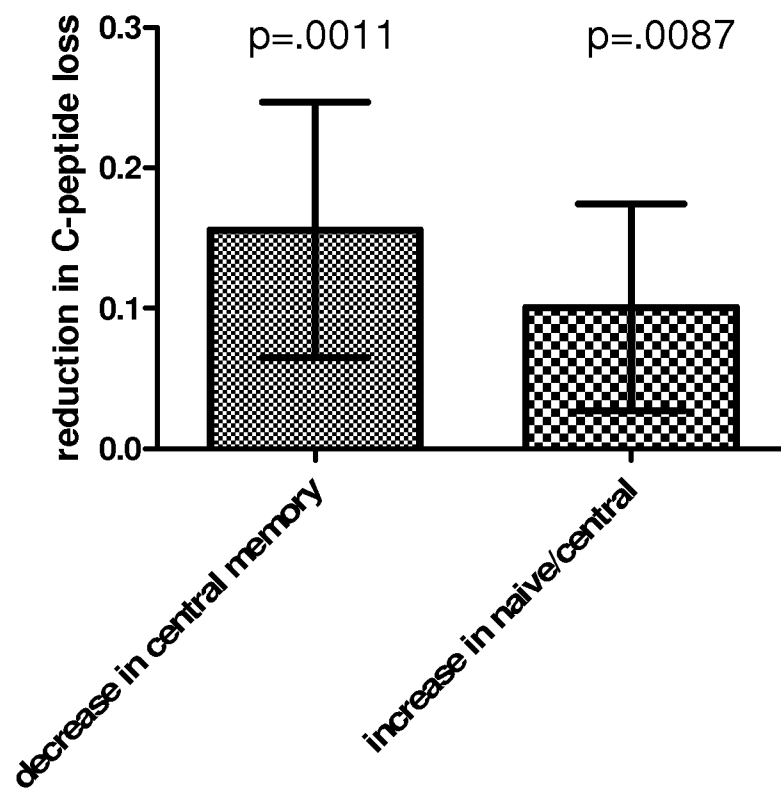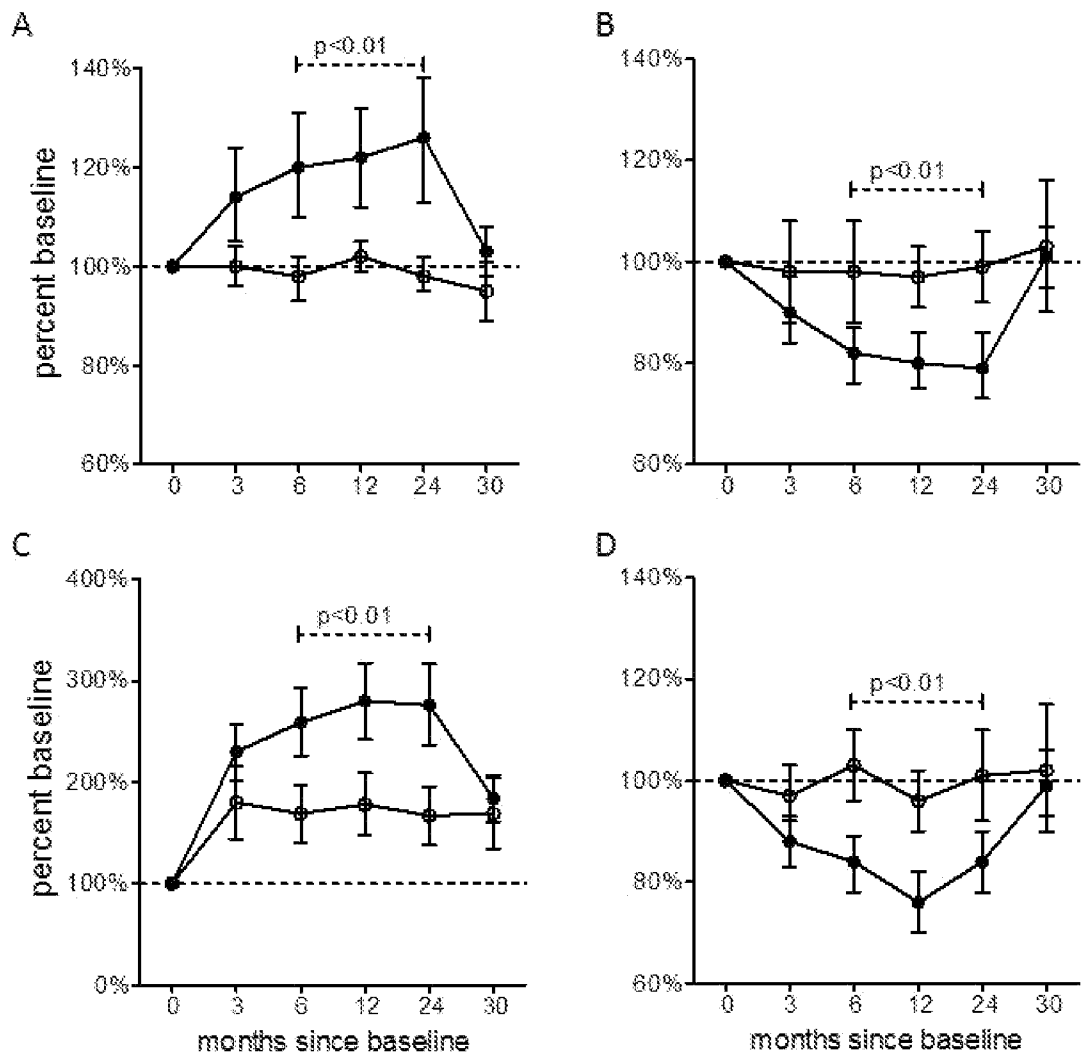
FIG. 1

FIGS. 2A – 2D