CD247, a Novel T Cell–Derived Diagnostic and Prognostic Biomarker for Detecting Disease Progression and Severity in Patients With Type 2 Diabetes

Roy Eldor,[1] Yair Klieger,[2,3] Moshe Sade-Feldman,[2] Ilan Vaknin,[2] Inna Varfolomeev,[2] Camil Fuchs,[4] and Michal Baniyash[2]

Diabetes Care 2015;38:113–118 | DOI: 10.2337/dc14-1544

OBJECTIVE

We have previously shown that chronic inflammation results in immunosuppression associated with CD247 downregulation in T lymphocytes. Type 2 diabetes mellitus (T2DM) is known to be associated with chronic inflammation. We therefore sought to examine CD247 expression levels in patients with T2DM and to assess whether it can serve as a diagnostic and prognostic biomarker for disease complications and outcomes.

RESEARCH DESIGN AND METHODS

Peripheral blood samples from 75 T2DM patients and 40 healthy control subjects were collected and analyzed for the expression level of CD247 in T lymphocytes. Subjects with T2DM underwent a medical interview with physical examination and were followed for an additional average of 19.2 ± 0.9 months to determine the occurrence of major adverse disease end points. The relationship between the level of CD247 expression and disease status at the time of blood draw and the ability of the marker to identify future complications was evaluated.

RESULTS

We observed a significant reduction in CD247 expression levels in T lymphocytes of T2DM patients when compared with healthy volunteers. CD247 downregulation was associated with disease severity, complications, and the occurrence of future cardiovascular events, suggesting its potential use not only as a diagnostic but also as a prognostic biomarker.

CONCLUSIONS

Our results suggest the use of CD247 as a biomarker in diabetic patients for evaluating the state of chronic inflammation that contributes to morbidity and mortality in this disease and for the prediction of future cardiovascular events.

Type 2 diabetes mellitus (T2DM) is associated with low-grade chronic inflammation, which may contribute to the development of diabetes-related complications (1). Several nonspecific biomarkers of inflammation, such as hs-CRP, TNF-α, lipoprotein (a), and sICAM-1, have been implicated in disease progression and activity and found to be significantly elevated in diabetic patients with existing coronary artery

[1]Diabetes Unit, Department of Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel
[2]The Lautenberg Center for Immunology and Cancer Research, Israel-Canada Medical Research Institute, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
[3]Improdia Ltd., Herzliya, Israel
[4]Department of Statistics and Operations Research, Tel-Aviv University, Ramat Aviv, Tel Aviv, Israel

Corresponding author: Michal Baniyash, michalb@ekmd.huji.ac.il.

Received 25 June 2014 and accepted 11 October 2014.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-1544/-/DC1.

R.E. and Y.K. contributed equally to this work.

Table 1—Baseline demographics and characteristics of control subjects and subjects with T2DM

	T2DM	T2DM without complications	T2DM with complications	Control
Number of subjects (*n*)	75	23	52	40
Age (years ± SD)	62.97 ± 11.14	61.5 ± 13.6	63.6 ± 9.92	42.4 ± 15.9
Female (%)	40%	48%	37%	50%
Duration of diabetes (years ± SD)	13.24 ± 9.77	7.9 ± 5.6	15.31 ± 10.29	—
Complications [*n* (%)]				
CVD	26 (34.7%)			—
Retinopathy	24 (32%)			—
Neuropathy	27 (36%)			—
Nephropathy	24 (32%)			—
Cerebrovascular disease	5 (6.7%)			—
PVD	10 (13.3%)			—
Diabetic foot ulcer	4 (5.3%)			—
Medications [*n* (%)]				
Insulin	32 (42.7%)	8 (34.7%)	24 (46.2%)	—
Metformin	53 (70.7%)	17 (73.9%)	36 (69.2%)	—
Sulfonylurea	29 (38.7%)	4 (17.4%)	25 (48.1%)	—
Thiazolidinedione	7 (8.8%)	1 (4.3%)	6 (11.5%)	—
GLP-1 analog/DPP inhibitor	10 (13.4%)	3 (13%)	7 (13.5%)	—
Aspirin	59 (78.7%)	16 (69.5%)	43 (82.7%)	—
Statin	65 (86.7%)	18 (78.3%)	47 (90.4%)	—
A1C (% ± SD)	8.53% ± 1.78	8% ± 1.75	8.82% ± 1.75	—
(mmol/mol)	(70 ± 14.6)	(63.8 ± 13.9)	(72.7 ± 14.4)	—
CRP (mg/dL ± SD)	1.15 ± 2.45	0.99 ± 0.5	2.09 ± 4	<0.5
CD247 expression (% ± SEM)	81.8% ± 2.5	90.9% ± 4	77.7% ± 3	100% ± 2.2

disease (2–4), diabetic nephropathy (3,5–7), and retinopathy. However, neither of these parameters is indicative of chronic inflammation or the patients' immune functionality. For example, conflicting results exist as to the correlation between CRP and diabetes-related micro- and macrovascular complications and its usefulness as a biomarker of disease progression (8). Additionally, the lack of CRP specificity regarding its ability to distinguish between acute and chronic inflammation and susceptibility to the effects of certain anti-inflammatory medications such as statins and aspirin (4), frequently used in diabetic patients, may diminish its usefulness in clinical practice.

We have previously shown that chronic inflammation leads to T-cell immunosuppression associated with CD247 downregulation (9). CD247 (also referred to as the ζ-chain) is part of the T-cell antigen receptor (TCR) complex, which plays a key role in receptor expression and signaling leading to optimal effector T-cell functions (10,11). Interestingly, in T cells, the observed immunosuppression is associated with a unique CD247 downregulation while the remaining TCR subunits are unaffected. This phenomenon characterizes chronic inflammation and does not occur during an acute inflammatory response (9,12). Moreover, neutralization of the chronic inflammatory environment leads to CD247 expression recovery and immune function recuperation to normal levels, indicating a reversible phenomenon. CD247 downregulation was previously described in chronic inflammatory diseases, including celiac disease (13), chronic obstructive pulmonary disease (14), and systemic lupus erythematosus (15) as well as different types of cancer (9). Thus, CD247 may serve as a potential biomarker for evaluating the immune status in pathologies characterized by chronic inflammation.

Currently, the widely used biomarkers for disease severity and progression in diabetes rely on metabolic parameters and are limited in their ability to improve risk stratification with regards to disease-related outcomes (16). Identifying a more mechanistically relevant biomarker that is linked not only to the metabolic processes but also to the underlying inflammatory process may provide an invaluable clinical tool to predict disease progression and effectiveness of therapeutic interventions.

In this study we hypothesize that the expression levels of T-cell CD247, which is a unique biomarker that senses chronic inflammation and associated immunosuppression, in patients with T2DM are associated with parameters of disease severity and progression. Herein, we present evidence of such an association and suggest a possible clinical use for measurement of CD247 expression levels as a novel biomarker for diabetes-related outcomes.

RESEARCH DESIGN AND METHODS

Subjects

A total of 115 subjects participated in the study (40 healthy subjects and 75 subjects with T2DM). Healthy control subjects were recruited among blood donors in the Hadassah University Hospital blood bank facility. Only healthy donors without comorbidities such as diabetes, heart disease, etc., and who did not receive in a period of 3 weeks before the blood donation any anti-inflammatory drugs, were selected to participate in the experiment. The information was obtained by a detailed medical questionnaire obtained at the time of blood donation. Subjects with T2DM were recruited after signing a written informed consent to participate in the study. The patients underwent a full medical interview with physical examination. The diagnosis of diabetes and diabetes-related complications was based on medical history obtained during the interview

and medical records available in the Hadassah Hospital database. Seventy-five subjects with diabetes were followed through office visits and medical records for an average of 19.2 ± 0.9 months to determine the occurrence of major adverse disease end points (a composite of death from any cause, cerebrovascular event, ST segment elevation myocardial infarction, non–ST segment elevation myocardial infarction, cardiac catheterization, cardiac artery bypass graft surgery, pulmonary edema with hospitalization, or angina pectoris with hospitalization).

Determination of CD247 Expression

Peripheral Blood Collection

Peripheral blood samples were collected into heparin-vacutainer tubes and were gradually frozen within 6 h in freezing buffer that contained 20% DMSO and 80% FCS. Blood samples were kept frozen until thawed for analysis.

Flow Cytometry Analysis

Human whole blood cells were stained using anti-CD247, anti-CD3, and anti-CD56 antibodies, all purchased from Biolegend and used according to the manufacturer's protocol. For cell surface labeling of CD3 and CD56, cells were incubated 30 min at 4°C with the antibodies and washed with FACS buffer containing 2% BSA and 0.05% NaN3 in PBS. The cells then underwent intracellular labeling of CD247; cells were fixed for 20 min with 1% paraformaldehyde at 4°C, permeabilized for 10 min with 0.1% saponin/PBS at room temperature, and then washed with FACS buffer and labeled by a similar procedure as used for cell surface labeling. Samples were collected in a FACS Calibrator using Cell Quest software (BD) and analyzed using the FCS express software (De Novo Software). Expression of a given molecule per cell was detected by FACS analysis.

Data Analysis

The data are presented as the mean ± SEM unless specified otherwise. Statistical analysis of CD247 expression level in different subpopulations and with different diabetes-related complications was performed using Student *t* test. Event occurrence after measurement of CD247 expression levels was plotted as a Kaplan-Meier cumulative event curve and assessed by log-rank test using SSPS software. In all tests, $P < 0.05$ was considered statistically significant.

RESULTS

Patient Population

Seventy-five subjects with T2DM and 40 healthy control subjects were recruited. Mean duration of diabetes was 14.04 ± 9.93 and mean A1C was 8.53% ± 1.78 (70 ± 14.6 mmol/mol). Of the diabetic subjects, 69% had diabetes-related complications, with 34.7% suffering from cardiovascular disease (CVD), 32% from retinopathy, 36% neuropathy, and 32% nephropathy. A majority of the diabetic subjects were treated with metformin (70.7%), a statin (86.7%), and aspirin (78.7%). When compared with healthy volunteers, subjects with T2DM were generally older and had relatively higher CRP levels (<0.5 mg/dL in healthy subjects vs. 0.86 ± 2.55 in subjects with diabetes). When compared with T2DM subjects without complications, T2DM subjects with complications had a longer duration of disease (15.31 ± 10.29 vs. 7.9 ± 5.6 years, $P < 0.001$). Numeric differences in A1C and CRP did not reach statistical significance (Table 1).

Significant Reduction in CD247 Expression Levels in Subjects With T2DM

CD247 expression levels were determined in T cells from blood samples of control subjects and subjects with T2DM by gating on $CD3\varepsilon^{+}CD56^{-}$ T cells, as depicted in Fig. 1*A*. A significant reduction in CD247 expression levels was noted in subjects with T2DM (% CD247 expression 81.8% ± 2.5 in diabetic vs. 100% ± 2.2 in healthy subjects, $P < 0.001$) (Fig. 1*B*). To verify that the observed reduction in CD247 expression was not the result of TCR internalization due to acute T-cell activation, a process in which all TCR subunits are downregulated, we measured in all samples CD3ε expression levels as a representative of remaining TCR complex. No apparent differences in CD3ε expression (99 ± 2 in diabetic vs. 98.8 ± 0.8 in healthy subjects, $P = 0.462$) (Fig. 1*C*) were observed between healthy subjects and subjects with T2DM. These results indicate a chronic inflammatory environment in subjects with T2DM and associated

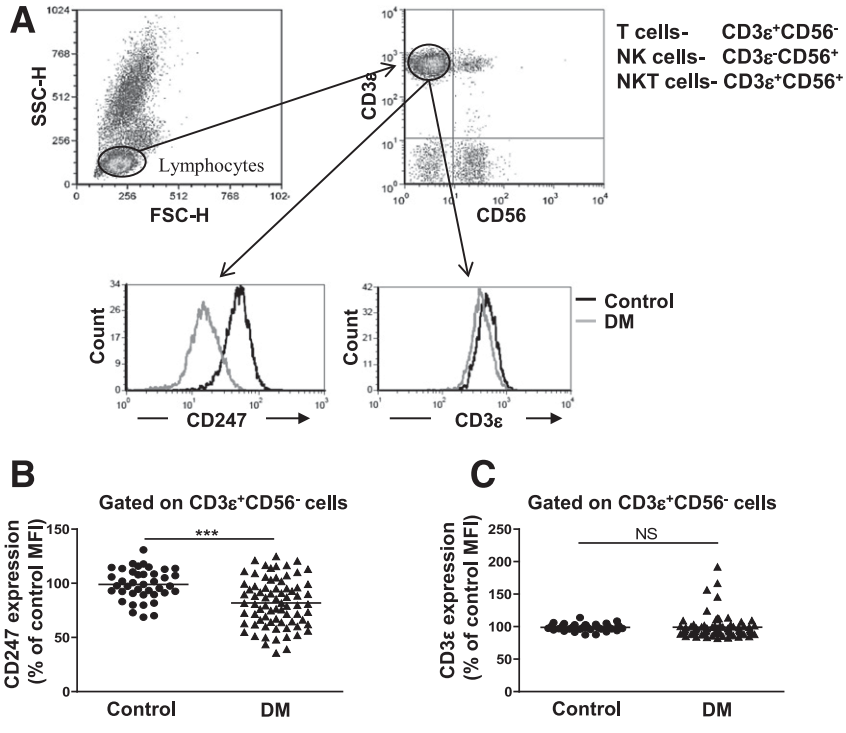

Figure 1—Decreased CD247 expression in T cells isolated from whole blood of diabetic vs. healthy control subjects. CD247 and CD3ε expression levels in T cells were analyzed by flow cytometry gating on the $CD3\varepsilon^{+}CD56^{-}$ cells within the lymphocyte fraction (*A*). Only the expression level of CD247 (*B*) but not of CD3ε (*C*) was significantly reduced in diabetic (DM) vs. healthy control subjects (control). $P < 0.0001$ and $P = 0.462$, respectively. NS, not significant; MFI, mean fluorescence intensity.

immunosuppression, as reflected by the unique CD247 downregulation.

CD247 Expression Is Related to Disease Severity and Complications

To further characterize whether changes in CD247 expression levels in subjects with T2DM are associated with disease-related complications, we compared its expression levels in subjects with and without diabetes-related complications. Whereas there was only a slight but significant reduction in CD247 expression between diabetic subjects without complications and healthy subjects (% CD247 expression 100 ± 2.2 in healthy subjects [control] vs. 90.9 ± 4 diabetic subjects without complications [DM], $P = 0.03$) (Fig. 2*A*), a more prominent and significant reduction in CD247 expression was noted in diabetic subjects with complications (% CD247 expression 77.7 ± 3 in diabetic subjects with diabetes-related complications [DM-c] vs. diabetic subjects without complications [$P = 0.006$] and vs. healthy subjects [$P < 0.0001$]) (Fig. 2*A*). When assessing the relation between A1C and complications, we found that the mean A1C value was higher in subjects with diabetes-related complications versus those without (8.82 ± 0.27 [73 ± 1 mmol/mol] for DM-c vs. 8 ± 0.35 [64 ± 1.3 mmol/mol] for DM, $P = 0.04$) (Fig. 2*B*). It is important to note that other disease parameters such as age and CRP did not differ between diabetic subjects with or without complications (age 61.38 ± 2.72 years for DM vs. 63.62 ± 1.37 years for DM-c, $P = 0.2$; CRP 0.8 ± 0.12 for DM vs. 0.92 ± 0.1 for DM-c, $P = 0.26$) (Fig. 2*C* and *D*). Interestingly, whereas a significant correlation was observed between CD247 expression levels and disease duration ($P = 0.017$), no clear correlation was observed between CD247 expression levels and A1C, CRP, sex, or age (Supplementary Fig. 1). Moreover, no relation between CD247 expression levels and various treatments such as aspirin, statins, and metformin was observed (Supplementary Fig. 2)

To better understand the dynamics of change in CD247 expression in relation to diabetes-related complications, we compared CD247 expression levels to the actual number of diagnosed complications/subject and specific complications. As shown in Fig. 3*A*, CD247 expression levels significantly decreased as the number of diabetes-related complications increased. Among previously diagnosed individual complications, only in subjects with retinopathy and nephropathy was a significant reduction in CD247 expression noted when compared with all subjects in the cohort without these complications (retinopathy: CD247 expression 86 ± 3.3% without retinopathy vs. 74.6 ± 3.9% with retinopathy, $P = 0.015$; nephropathy: CD247 expression 87.8 ± 2.9% without nephropathy vs. 68.4 ± 4.3% with nephropathy, $P < 0.001$). Nevertheless, a trend toward reduced CD247 expression was also observed in subjects previously diagnosed with ischemic heart disease (IHD) (CD247 expression 85.1 ± 3.2 without IHD vs. 76.3 ± 4.7 with IHD, $P = 0.063$), cerebrovascular accident (CVA) (CD247 expression without prior CVA 82.8 ± 2.7 vs. 65.9 ± 9.7 with CVA, $P = 0.063$), and diabetic foot ulcer (DFU) (CD247 expression 82 ± 2.7 without DFU vs. 69 ± 5.3 with DFU, $P = 0.056$) (Fig. 3*B*).

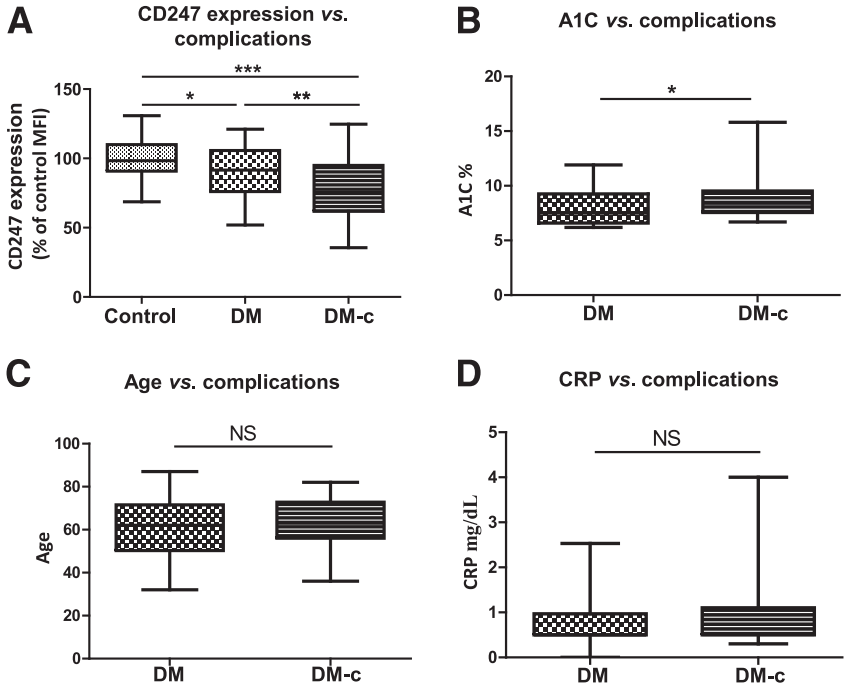

Figure 2—CD247 expression is reduced in subjects with diabetes-related complications. Subjects with T2DM that had a documented history of diabetes-related complications (DM-c) (including CVD, retinopathy, neuropathy, nephropathy, cerebrovascular disease, PVD, and DFU) had significantly lower levels of CD247 expression when compared with both healthy (control) (***$P <$ 0.0001) and diabetic subjects without complications (DM) (**$P = 0.006$). A significant reduced CD247 expression was observed in DM subjects when compared with healthy control subjects ($P = 0.03$) (*A*). (*B*) A1C was significantly lower in DM as compared with subjects with DM-c (*$P =$ 0.04). No difference between DM and DM-c was observed with regards to age ($P = 0.2$) (*C*) and CRP levels ($P = 0.26$) (*D*). NS, not significant.

CD247 Expression Levels as a Potential Biomarker for Impending Cardiovascular Events

Based on the results described above, we further analyzed the potential use of CD247 expression levels as a biomarker for predicting the occurrence of a composite end point of inflammatory and atherosclerotic cardiovascular and cerebrovascular events (including death from any cause, cerebrovascular event, ST segment elevation myocardial infarction, non–ST segment elevation myocardial infarction, cardiac catheterization, cardiac artery bypass graft surgery, pulmonary edema with hospitalization, or angina pectoris with hospitalization). Figure 3*C* presents a Kaplan-Meier plot of disease end point occurrence in the subset of subjects with CD247 expression levels <70 or ≥70% at baseline. Cutoffs of 60 and 80% were also tested, but the 70% cutoff was found to better differentiate the two outcome populations. Using the log-rank test, a significant difference ($P = 0.048$) was noted between these two populations, suggesting that a baseline CD247 expression level <70% may be predictive

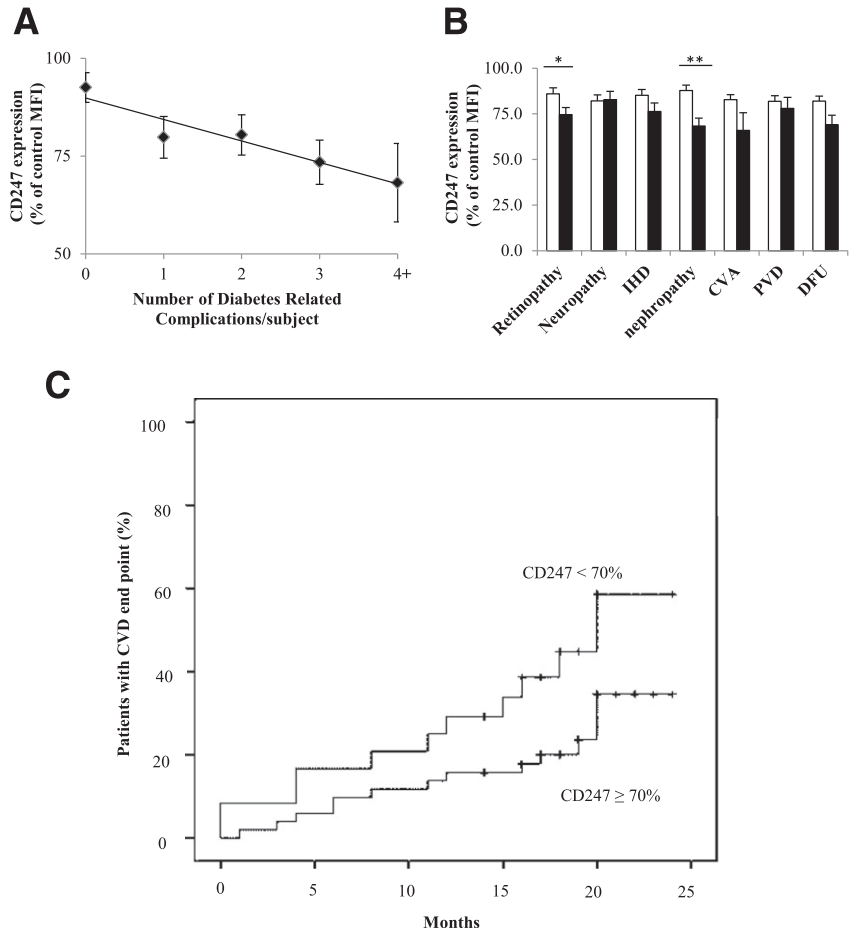

Figure 3—Reduced CD247 expression is associated with disease progression. *A*: Reduced CD247 expression levels significantly correlate with cumulative diabetes-related complications ($P = 0.005$ between the mean CD247 expression and number of diabetes-related complications/subject). *B*: CD247 expression is significantly reduced in subjects with retinopathy and nephropathy (*, retinopathy [$P = 0.015$]; **, nephropathy [$P < 0.001$]). *C*: Kaplan-Meier plot of disease end points in subjects with CD247 expression level <70 and ≥70%. Disease end points were a composite of the following events: death from any cause, cerebrovascular event, ST segment elevation myocardial infarction, non–ST segment elevation myocardial infarction, cardiac catheterization, cardiac artery bypass graft surgery, pulmonary edema with hospitalization, or angina pectoris with hospitalization. CD247 expression levels were measured at baseline and subjects were followed for an average of 19.2 ± 0.9 months for the occurrence of CVD end points. ($n = 75$ [$n = 24$ CD247 <70% and $n = 51$ CD247 ≥70%], $P = 0.048$ using the log-rank test). NS, not significant.

of future CV events in diabetic patients. This predictive significance of low CD247 expression was also observed in the subset of subjects without a previous diagnosis of macrovascular disease (IHD, CVA, and/or peripheral vascular disease [PVD], $n = 10$ subjects with CD247 expression <70% vs. $n = 31$ subjects with CD247 expression ≥70%, $P = 0.016$) (Supplementary Fig. 3).

CONCLUSIONS

Preventing the development of the ominous micro- and macrovascular complications associated with diabetes necessitates a comprehensive understanding of the interaction between glycemia and vascular inflammatory processes as well as identifying predictive biomarkers linking these seemingly distinct events. We present here, evidence that T cell–specific CD247 expression levels may pose as a sensitive and predictive biomarker for chronic inflammation in diabetes, possibly linking glycemic disease processes with inflammatory outcomes such as CVD and nephropathy.

Currently, commonly used biomarkers for disease progression and outcome, such as A1C, assess the glycemic exposure but reveal little as to the actual pathologic inflammatory process underlying the progression of micro- and macrovascular complications (16). This has been recently highlighted by evidence from megatrials such as ACCORD (Action to Control Cardiovascular Risk in Diabetes) and ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation), suggesting a dissociation between changes in A1C upon implementation of intensive antihyperglycemic therapy and progression of macrovascular outcomes (17,18). Likewise, a recent study showed that assessment of A1C values in the context of CVD risk assessment provided little incremental benefit for prediction of CVD risk (16).

In light of these studies, a more process-specific biomarker of the pathologic inflammation in diabetes is needed. A biomarker that may shed light on the level of chronic inflammation is expected to both improve our understanding of the factors contributing to the progression of complications in diabetes and offer a mode for assessing therapeutic efficacy and novel therapeutic targets. Commonly used biomarkers for inflammation, such as CRP, have shown a loose correlation to actual disease outcome and may not be sufficiently specific to the diabetic disease process (8). Moreover, CRP levels fluctuate in successive measurements and respond to both chronic and acute inflammatory process, impeding its validity as a biomarker for chronic processes (19).

It is well established that downregulation of the TCR component CD247 is associated with chronic inflammation-induced immunosuppression, as previously described for various types of cancer, chronic infections (HIV, hepatitis C, and leprosy), and autoimmune diseases (arthritis, contact eczema, and lupus) (9). Thus, it is suggested as a biomarker for evaluating the immune status of patients with pathologies characterized by chronic inflammation. Along these observations, we present here evidence of a specific decline in the expression levels of CD247, in patients with T2DM and in patients with a more progressive disease (manifested by the occurrence of diabetes-related complications). Of the specific diabetes complications examined, subjects with nephropathy and/or retinopathy had significantly reduced CD247 expression levels, and a tendency

toward a reduced expression was noted in subjects with diagnosed IHD, prior CVA, and DFU when compared with subjects without these specific complications. The lack of significant correlation with other known inflammatory diabetes complications may be due to an inherent characteristic of the process leading to a reduced expression of CD247 or may be the result of the relatively low number of events/subjects tested. For example, only 5 of 75 subjects tested had a previous diagnosis of cerebrovascular disease. Moreover, this analysis is confounded by the fact that 70% of subjects with complications had more than one complication diagnosed.

Assessment of baseline CD247 expression levels in diabetic patients as a biomarker for a composite end point of vascular events revealed a worse outcome in the subgroup of patients with reduced CD247 expression levels. These results underscore a potential predictive value of CD247 measurements to stratify the CVD risk in diabetic patients. Moreover, the significant predictive value of low CD247 expression was maintained in the subset of subjects without a previous diagnosis of macrovascular disease, suggesting a potential use of this biomarker in primary prevention of CVD.

CD247 expression levels did not correlate with other parameters of disease severity such as age, A1C, and CRP. This may suggest that CD247 is less amenable to relatively acute fluctuations in glycemic control (which rapidly affect A1C) or acute inflammation (which rapidly affects CRP) and may be better linked to the hypothesized underlying chronic low-grade inflammatory processes that are thought to contribute to the emergence of diabetes-related complications. Interestingly, the ability of both A1C and low CD247 expression to distinguish between patients with and without diabetes-related complications, and the fact that these two markers did not correlate, raise the possibility that they are indeed affected by different disease pathways. Therefore, a combined approach utilizing these two markers may improve our ability to stratify a patient's risk of developing diabetes-related complications and enhance the efficacy of primary therapeutic interventions.

The study we present is confounded by its relatively small sample size and short follow-up time. However, our promising results suggest a novel approach to risk stratification in diabetes. Rather than focusing on the typical markers of the diabetic metabolic disorder such as glycemia and lipids, CD247 may shed light on the actual pathologic process leading to end organ damage. Understanding these processes may open a new venue for treatment in diabetes, not only addressing the metabolic disturbances but also optimizing and personalizing treatment to prevent the resultant chronic inflammatory processes that are at its core.

Acknowledgments. The authors thank the support of the Society of Research Associates of the Lautenberg Center, the Concern Foundation of Los Angeles, and the Harold B. Abramson Chair in Immunology.

Funding. This study was supported by the Israel Science Foundation, the Israeli Ministry of Health, the Joint German-Israeli Research Program, the Israel Cancer Research Fund, the MAGNET program (Nofar track, funded by the Israel Office of the Chief Scientist of the Ministry of Industry, Trade, and Labor), and the Joseph and Matilda Melnick Funds.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. R.E. was involved in the study design, collected the research data, analyzed the data, and wrote the manuscript. Y.K. performed the data analysis, researched data, and wrote the manuscript. M.S.-F. performed some of the analyses and collected the research data. I.Vak. was responsible for the blood sample collection and was involved in the study design and research data collection. I.Var. performed some of the analyses. C.F. performed the statistical analyses. M.B. initiated the conception, was involved in the study design, and edited the manuscript. M.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Garcia C, Feve B, Ferré P, et al. Diabetes and inflammation: fundamental aspects and clinical implications. Diabetes Metab 2010;36:327–338
2. Jin C, Lu L, Zhang RY, et al. Association of serum glycated albumin, C-reactive protein and ICAM-1 levels with diffuse coronary artery disease in patients with type 2 diabetes mellitus. Clin Chim Acta 2009;408:45–49
3. Kornum JB, Thomsen RW, Riis A, Lervang HH, Schønheyder HC, Sørensen HT. Type 2 diabetes and pneumonia outcomes: a population-based cohort study. Diabetes Care 2007;30:2251–2257
4. Prasad K. C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev 2006;24:33–50
5. Abrahamian H, Endler G, Exner M, et al. Association of low-grade inflammation with nephropathy in type 2 diabetic patients: role of elevated CRP-levels and 2 different gene-polymorphisms of proinflammatory cytokines. Exp Clin Endocrinol Diabetes 2007;115:38–41
6. Del Cañizo Gómez FJ, Fernández Pérez C, Moreno Ruiz I, et al. Microvascular complications and risk factors in patients with type 2 diabetes. Endocrinol Nutr 2011;58:163–168
7. Navarro JF, Mora C, Maca M, Garca J. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. Am J Kidney Dis 2003;42:53–61
8. Fronczyk A, Molęda P, Safranow K, Piechota W, Majkowska L. Increased concentration of C-reactive protein in obese patients with type 2 diabetes is associated with obesity and presence of diabetes but not with macrovascular and microvascular complications or glycemic control. Inflammation 2014;37:349–357
9. Baniyash M. TCR zeta-chain downregulation: curtailing an excessive inflammatory immune response. Nat Rev Immunol 2004;4:675–687
10. Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell 1991;64:891–901
11. Weiss A, Irving BA, Tan LK, Koretzky GA. Signal transduction by the T cell antigen receptor. Semin Immunol 1991;3:313–324
12. Bronstein-Sitton N, Cohen-Daniel L, Vaknin I, et al. Sustained exposure to bacterial antigen induces interferon-gamma-dependent T cell receptor zeta down-regulation and impaired T cell function. Nat Immunol 2003;4:957–964
13. Lahdenperä AI, Fälth-Magnusson K, Högberg L, Ludvigsson J, Vaarala O. Expression pattern of T-helper 17 cell signaling pathway and mucosal inflammation in celiac disease. Scand J Gastroenterol 2014;49:145–156
14. Grundy S, Plumb J, Lea S, Kaur M, Ray D, Singh D. Down regulation of T cell receptor expression in COPD pulmonary CD8 cells. PLoS ONE 2013;8:e71629
15. Gorman CL, Russell AI, Zhang Z, Cunninghame Graham D, Cope AP, Vyse TJ. Polymorphisms in the CD3Z gene influence TCRzeta expression in systemic lupus erythematosus patients and healthy controls. J Immunol 2008;180:1060–1070
16. Di Angelantonio E, Gao P, Khan H, et al.; Emerging Risk Factors Collaboration. Glycated hemoglobin measurement and prediction of cardiovascular disease. JAMA 2014;311:1225–1233
17. Gerstein HC, Miller ME, Byington RP, et al.; Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545–2559
18. Patel A, MacMahon S, Chalmers J, et al.; ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560–2572
19. Bogaty P, Brophy JM, Boyer L, et al. Fluctuating inflammatory markers in patients with stable ischemic heart disease. Arch Intern Med 2005;165:221–226