



HAL
open science

Increased frequency of CD14⁺HLA-DR-/low cells in type 2 diabetes patients with poor glyceemic control

M.A. Valtierra-Alvarado, J.E. Castañeda-Delgado, Geanncarlo Lugo-Villarino, F. Dueñas-Arteaga, B. Rivas-Santiago, J.A. Enciso-Moreno, C.J. Serrano

► **To cite this version:**

M.A. Valtierra-Alvarado, J.E. Castañeda-Delgado, Geanncarlo Lugo-Villarino, F. Dueñas-Arteaga, B. Rivas-Santiago, et al.. Increased frequency of CD14⁺HLA-DR-/low cells in type 2 diabetes patients with poor glyceemic control. *Human Immunology*, 2022, 83 (11), pp.789-795. <10.1016/j.humimm.2022.08.011>. <hal-04738359>

HAL Id: hal-04738359

<https://hal.science/hal-04738359v1>

Submitted on 17 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Increased frequency of CD14⁺HLA-DR^{-/low} cells in type 2 diabetes patients with
poor glyceemic control

M.A. Valtierra-Alvarado^{a, b}, J.E. Castañeda-Delgado^c, G. Lugo-Villarino^d, F. Dueñas-Arteaga^e, B. Rivas-Santiago^a, J.A. Enciso-Moreno^a, C.J. Serrano^{a, *}

^a Unidad de Investigación Biomédica Zacatecas, Instituto Mexicano del Seguro Social, México

^b Departamento de Inmunología, Centro de Investigación en Ciencias de la Salud y Biomedicina, Universidad Autónoma de San Luis Potosí (UASLP), México

^c Investigador por México, Consejo Nacional de Ciencia y Tecnología (CONACyT-México), Unidad de Investigación Biomédica Zacatecas, Instituto Mexicano del Seguro Social, Zacatecas, México

^d Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, Toulouse, France

^e Hospital General No. 26, Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE), Zacatecas, México

* Correspondence: Carmen J. Serrano PhD. Unidad de Investigación Biomédica Zacatecas, IMSS. Zacatecas, Zac., México. Interior Alameda No. 45, Zacatecas, Zac., 98000, México. Tel 01 52 (492) 922 6019. carmenyuyu2000@yahoo.com.mx

ABSTRACT

Aims: Type 2 diabetes (T2DM) is associated with alterations of the immune response and T2DM patients have an increased risk for infections and certain sorts of cancers. Although CD14⁺HLA-DR^{-/low} cells have emerged as important mediators of immunosuppression in several pathologies, including cancer and non-malignant diseases, the presence of these cells in T2DM is not fully characterized.

Methods: In this study, we evaluated the frequency of CD14⁺HLA-DR^{-/low} cells in non-obese T2DM patients and their association with glycemic control. Peripheral blood mononuclear cells were isolated from healthy controls (HC, n=24) and non-obese T2DM patients (n=25), the population was evaluated by flow cytometry, and an analysis of correlation between cell frequencies and clinical variables was performed.

Results: CD14⁺HLA-DR^{-/low} monocytes were expanded in patients with T2DM compared to HC regardless of weight. Among the subjects with T2DM, the frequency of CD14⁺HLA-DR^{-/low} was higher in patients with poor glycemic control (HbA1c >9%) compared to those with better glycemic control (HbA1c <9%) and, positively correlated with the years since the diagnosis of T2DM, the age of the patients and the glycemic index.

Conclusions: An increased frequency of CD14⁺HLA-DR^{-/low} cells in the blood of T2DM patients was recorded. The influence of hyperglycemia seems to be independent of obesity, but related to glycemic control and age.

Keywords: monocytes, MDSCs, immunosuppression, diabetes, glycemic control

1. Introduction

Diabetes represents a global epidemic that has reached alarming levels, with 537 million adults with the disease in 2021, 90% of global cases due to type 2 diabetes mellitus (T2DM) [1]. In 2021, it was estimated that 6.7 million adults died because of diabetes complications, being among the leading causes of death worldwide [1].

T2DM is a metabolic disease characterized by high blood glucose levels (hyperglycemia) with altered carbohydrate, fat, and protein metabolism resulting from deficient insulin action (insulin resistance) and/or insulin secretion defects [2, 3]. Chronic hyperglycemia and inflammation increase the risk of macrovascular and microvascular complications [4]. In addition, the immune response is affected in diabetic patients, causing more susceptibility to infections. Actually, poor glycemic control is strongly associated with infection severity [4, 5].

T2DM is associated with impaired immune cells, including alterations in the phenotype and function of human monocytes [4, 6-10]. Monocytes with low or no HLA-DR expression have been called CD14⁺HLA-DR^{-/low} (CD14⁺HLA-DR^{lo/neg}) monocytes. These cells belong to the larger class of suppressive cells termed myeloid-derived suppressor cells (MDSC), specifically to the group of monocytic MDSCs (M-MDSCs) and have been studied as important mediators of tumor-induced immunosuppression [11, 12]. These cells are abundant in different types of cancer, affecting negatively the response to immunotherapy [11, 13-15]. In other inflammatory conditions, such as sepsis, the loss of HLA-DR is considered a hallmark of monocytes functional deactivation, and it has been associated with poor clinical outcomes [11, 16]. Interestingly, changes in the frequency of these cells have

also been reported in Coronavirus disease (COVID)-19 [17]. Therefore, the role of these cells could be relevant in patients with T2DM, who have an increased risk of developing cancers [18] and infections [5].

Concerning CD14⁺HLA-DR^{-/low} cells in the context of diabetes, Friedrich et al. found a higher absolute number in peripheral blood of obese individuals compared to normal and overweight individuals; among obese individuals, those with T2DM showed a higher frequency [19]. Furthermore, we reported low HLA-DR expression (median fluorescence intensity) in monocytes and monocyte-derived macrophages (MDM) of patients with T2DM [7]. However, no studies have been conducted focused on the frequency of the CD14⁺HLA-DR^{-/low} cell phenotype in patients with T2DM discriminating the contribution of obesity. Therefore, the present study aimed to evaluate the frequency of CD14⁺HLA-DR^{-/low} cells in nonobese patients with T2DM and its correlation with clinical parameters that typically assess glycemic and metabolic control.

2. Materials and methods

2.1. Subjects

HC subjects and patients with T2DM (diagnosed using the criteria of the American Diabetes Association) were recruited between April 2018 and December 2019 from the hospitals HGZ1, Dr. Emilio Varela Luján, Instituto Mexicano del Seguro Social (IMSS), and HG26, Instituto de Seguridad Social y Servicios de los Trabajadores del Estado (ISSSTE), in Zacatecas City, Mexico. The IMSS National Research and Ethics Committee approved the study with the number R-2016-785-049. All participants provided their written informed consent.

Anthropometric variables and clinical medical history were recorded for each participant, including the following evaluations: body mass index (BMI), blood pressure, thyroid palpation, cardiovascular examination, pharmacotherapy, fasting glucose, HbA1c, hematic biometry, lipid profile, serum creatinine, and estimated glomerular filtration rate. Subjects were not included when they had one or more of the following criteria: hypothyroidism, alcoholism, smoking, advanced retinopathy, renal failure, neuropathy, recent or present infections in any organ, obesity (BMI >29.9 kg/m²), or when the individuals were receiving treatment with insulin or immunosuppressants.

2.2. PBMCs isolation and Flow cytometry

Blood samples were drawn from participants using tubes with EDTA. Then, human peripheral blood mononuclear cells (PBMCs) were purified using Lymphoprep (AXIS-SHIELD, Scotland) density gradient centrifugation and washing with phosphate-buffered saline 1X (PBS) according to the manufacturer's instructions.

PBMCs were incubated at 4°C for 15 min with heat-inactivated human AB serum (Sigma- Aldrich, USA) to avoid nonspecific binding of antibodies. Then, cells were stained with FITC anti-CD14 mAb (clone M5E2; BD, USA) and APC anti-HLA-DR mAb (clone G46-6; BD, USA) for 20 min at 4°C in darkness, washed with PBS and fixed with 4% paraformaldehyde. The samples were acquired in a FACS Canto II flow cytometer (BD Biosciences, USA), and the data were analyzed using FlowJo VX software (BD, USA).

2.3. Statistical analysis

Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software Inc. CA, USA). First, the normality of variables was calculated with the Kolmogorov-

Smirnov test, and the comparison between groups was performed by *t* student test or the Mann-Whitney U test. The Spearman's correlation coefficient for data with a non-normal distribution was used to assess the correlations. $p < 0.05$ was considered statistically significant.

3. Results

3.1. *Clinical, anthropometric, and laboratory characteristics of participants*

The clinical characteristics of HC (n=24) and T2DM (n=25) subjects are shown in **Table 1**. As expected, participants with diabetes compared to HC had higher values for fasting plasma glucose levels ($p < 0.0001$) and HbA1c ($p < 0.0001$). Also anthropometric data and metabolic variables such as BMI ($p = 0.041$), triglycerides ($p < 0.0001$), and VLDL-cholesterol ($p < 0.0001$) showed statistically significant differences. Additionally, in diabetic patients compared to HC a lower concentration of HDL-cholesterol ($p = 0.0003$) was found. The time since T2DM diagnosis for patients showed a median of 3 years (range 0-17 years) and the patients analyzed were receiving pharmacological treatment with metformin (88.00%) or metformin plus glibenclamide (56.00%).

3.2. *A higher frequency of CD14⁺HLA-DR^{-/low} is observed in T2DM patients*

As mentioned before, CD14⁺HLA-DR^{-/low} cells have been associated with an MDSC immunosuppressive phenotype, therefore an increased frequency of these cells could be associated with impairment of immune protective functions. The frequency of CD14⁺HLA-DR^{-/low} (negative or low expression of HLA-DR) cells in HC and T2DM patients was evaluated by flow cytometry. To this end, the CD14⁺HLA-DR^{-/low}

population was defined by high CD14 expression and HLA-DR downregulation was delimited by fluorescence minus one (FMO) control [12, 17, 19]. The gating strategy used to define CD14⁺HLA-DR^{-/low} cells is shown in **Fig. 1A**. A higher frequency of CD14⁺HLA-DR^{-/low} cells was observed in patients with T2DM compared to subjects HC ($p < 0.0001$) as depicted in **Fig. 1B**. Although subjects with obesity (BMI > 30 kg/m²) were not included in our study, some participants (with or without T2DM) were overweight (Ow). Therefore, all participants were stratified into normal weight (BMI 18.5-24.9 kg/m²) or Ow (BMI 25.0-29.9 kg/m²), and their percentage of CD14⁺HLA-DR^{-/low} cells were compared. These results are shown in **Fig. 1C**, therefore, among the entire population studied no significant differences ($p = 0.727$) attributable to weight were found.

3.3. CD14⁺HLA-DR^{-/low} frequency exhibits an association with glycemic control

MDSC cells and other populations of monocytes have been associated with suppressive immune functions and metabolic alterations. First, we looked for differences in CD14⁺HLA-DR^{-/low} frequency due to weight in patients with T2DM, finding no differences as illustrated in **Fig. 2A**. To determine the influence of hyperglycemia on CD14⁺HLA-DR^{-/low} frequency, we stratified the T2DM patients into good glycemic control (GGC, HbA1c < 9%) and poor glycemic control (PGC, HbA1c > 9%). As shown in **Fig. 2B**, the percentages of CD14⁺HLA-DR^{-/low} cells were higher in patients with PGC compared to patients with GGC ($p = 0.019$).

To evaluate whether the increase in CD14⁺HLA-DR^{-/low} frequency in patients with T2DM was associated with metabolic/glycemic control parameters, we performed a correlation analysis (**Table 2**). The correlation analyses with significant values are

shown in **Fig. 2C-F**. For HbA1c, a value close to the significance ($r = 0.390$, $p = 0.053$) was found, while a significant positive correlation was found between the phenotype frequency and years since T2DM diagnosis ($r = 0.397$, $p = 0.048$), glycemic index (%HbA1c x self-reported years with diabetes; [20]) ($r = 0.453$, $p = 0.022$) and, age ($r = 0.601$, $p = 0.001$). No correlation was found with BMI, FGP, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, serum creatinine, and monocytes total counts in T2DM patients. None of the parameters analyzed for correlation analysis were statistically significant for HC subjects (**Table 2**).

4. Discussion

The impaired immune system in T2DM patients increases their susceptibility to several infections and cancers, it becomes important to understand how immune cell populations are affected, including those that suppress the immune response. CD14⁺HLA-DR^{-/low} cells have been studied as important mediators of immunosuppression, they are expanded in cancer and under chronic inflammation [11]. In human obesity, considered a low-level chronic inflammation state frequently associated with T2DM, a high frequency of CD14⁺HLA-DR^{-/low} cells has been reported [19, 21]. In addition, in subjects with morbid obesity a higher absolute number of this phenotype was found in the patients with T2DM compared to non-diabetics; while a positive correlation between the frequency of CD14⁺HLA-DR^{-/low} cells and BMI, fat mass, waist circumference, triglycerides, and C-reactive protein was also reported [19]. Given the reported impact of obesity in the CD14⁺HLA-DR^{-/low} population, it is important to discriminate the impact of hyperglycemia *versus*

obesity in T2DM patients. For this reason, in the present study we focus on patients with T2DM without obesity or advanced vascular complications, which covers a wide range of glycemic control levels (HbA1c from 6 to 14%). Our findings show that CD14⁺HLA-DR^{-/low} monocytes expand in patients with T2DM regardless of weight and their frequency is even higher during poor glycemic control. In diabetic patients the frequency of CD14⁺HLA-DR^{-/low} cells positively correlated with years since T2DM diagnosis, patients age, and glycemic index (HbA1c x self-reported years with diabetes), suggesting their expansion is induced under high glucose microenvironment and is enhanced by age.

In contrast to the findings from Friedrich et al., we did not find a correlation with BMI, triglycerides, and HDL cholesterol; however, it should be noted that their correlation analysis included together subjects with normal weight, OW and with morbid obesity (BMI 46.9 ± 1.1 kg/m²) [19]. On the other hand, our results are consistent with Restrepo and colleagues, who reported a low HLA-DR median fluorescence intensity (MFI) in monocytes subsets from T2DM patients, regardless of their dyslipidemic state [8]. Our participants did not include subjects with obesity, only with normal weight, and some overweight (BMI range 22-29 kg/m²) within the two studied groups. Therefore, our results suggest an independent influence of weight and dyslipidemia over CD14⁺HLA-DR^{-/low} frequency.

MDSCs include the population CD14⁺HLA-DR^{-/low} cells, but there is no consensus in the markers to define all MDSCs. Data suggest that MDSCs' ability to regulate inflammation and immunosuppression slow down the progress of diabetes, however, MDSCs play different functions in particular complications of diabetes ranging from participating in specific organ damage to possible therapeutic effects [22]. MDSCs

with particular phenotypes have been reported in the diabetic microenvironment, consistent with our results. There are few studies in human blood, reporting a higher proportion of these cells in patients with T2DM compared to healthy subjects [23-25]. Fernández-Ruiz and colleagues found that the frequency of mononuclear MDSCs CD33⁺HLA-DR^{-/low} producers of TGF- β and IL-10 was higher in T2DM patients compared to non-T2DM individuals [23]. Wang and colleagues reported an increased frequency of CD11b⁺CD33⁺ MDSCs in the peripheral blood of T2DM patients. MDSCs inhibited proliferation and modulated cytokine secretion of CD4⁺ T cells *in vitro* and delayed the development of diabetes in NOD/SCID mice [24]. Additionally, polymorphonuclear MDSCs (CD14⁻CD33⁺CD11b⁺CD15⁺) have been reported to expand in patients with T2DM with diabetic nephropathy (T2DN) and that high levels of these cells correlate with the risk of renal progression [25]. Therefore, our results add to the knowledge about MDSCs populations in T2DM patients, suggesting an impact of hyperglycemia independent of complications associated with diabetes over the frequency for CD14⁺HLA-DR^{-/low} cells.

The present results complement previous reports of low MFI HLA-DR expression in human monocytes [7, 8] and monocyte-derived macrophages [7, 26], from patients with T2DM. However, to our knowledge, the present study is the first to report the CD14⁺HLA-DR^{-/low} population as a frequency in non-obese patients with T2DM without advanced vascular complications and therefore to start exploring the impact of glycemic control in the expansion of these cells, especially during poor glycemic control and the association with the length of hyperglycemia (years since T2DM diagnosis and glycemic index). The loss of HLA-DR on monocytes has been reported in different conditions with a common inflammatory component (patients

with severe burns, acute and chronic liver inflammation, pancreatitis, after surgical procedures), but the precise mechanism of HLA-DR loss have yet to be elucidated in each disease, including T2DM [11]. Down-regulated HLA-DR has been reported induced by many factors such as IL-1 β [27], TGF- β [28], IL-10 [29], and cortisol [30]. Many of these factors are critical mediators of immunosuppressive state [11]. However, the specific mechanism of hyperglycemia as a possible contributor to immunosuppression by down-regulation of HLA-DR is unknown.

MDSCs including CD14⁺HLA-DR^{-/low} cells expand in different types of cancer, affecting the response to immunotherapy. Interestingly, T2DM is associated with a high risk of developing various cancers; some studies link elevated glucose or HbA1c levels to an increased risk of certain cancers, and hyperglycemia can promote its progression [18, 31-33]. Our study suggests a strong expansion of CD14⁺HLA-DR^{-/low} monocytes associated with poor glycemic control and long-term hyperglycemia, which calls for further research on the mechanisms involved.

Furthermore, an increased frequency of CD14⁺HLA-DR^{-/low} cells has been reported in infections. High frequencies were found in patients with sepsis, with the decrease in HLA-DR expression in monocytes that induces the immunosuppressive state called “immunoparalysis” associated with fatal outcomes [11, 16, 34]. In patients with COVID-19, an elevated frequency of CD14⁺HLA-DR^{-/low} cells was positively correlated with oropharyngeal viral loads and length of hospital stay [17]. Huang and colleagues found a higher percentage of CD14⁺HLA-DR^{-/low} cells in patients with chronic Hepatitis B virus infection compared with HC, the frequency was positively correlated with serum viral load, and these cells could suppress hepatitis B virus-specific CD8⁺T cells [35]. Significantly, patients with T2DM have an increased risk

of severe infections, hospitalization, and mortality [5, 36, 37], highly associated with poor glycemic control (where we found the higher frequency of CD14⁺HLA-DR^{-/low} cells among T2DM patients). Together with the critical role of HLA-DR on antigen presentation to T cells, immunosuppression mediated by CD14⁺HLA-DR^{-/low} cells may be involved in the development and unfavorable outcomes for infections in patients with T2DM.

A limitation of our study is the lack of functional studies. While we did not evaluate CD14⁺HLA-DR^{-/low} cells function, the literature reports the immunosuppressive function of these cells in cancer and non-malignant conditions with an inflammatory component [11, 16, 17, 34, 35]. Therefore, we hypothesize the implication of these cells in the context of T2DM. Furthermore, the down-regulation of HLA-DR in monocytes has been reported in inflammatory conditions [11, 16, 27]; however, we did not evaluate whether inflammatory mediators (such as IL-1 β , IL-6, C-reactive protein, etc.) in patients with diabetes are also correlated with the increase in CD14⁺HLA-DR^{-/low} cells we report here. Our results could be the sum of hyperglycemia and inflammatory conditions during T2DM. Consequently, our results warrant future studies with larger human cohorts, including the study of the functional and clinical implications of CD14⁺HLA-DR^{-/low} blood cells in patients with T2DM. More research is needed on the origin of such molecular and metabolic explanations regarding the link between metabolite alterations such as glucose and myeloid cell differentiation.

5. Conclusions

In conclusion, our results show an increased frequency of CD14⁺HLA-DR^{-/low} cells in the blood during T2DM disease. A higher frequency of these cells was observed in patients with poor glycemic control and was independent of weight in the entire population studied and in the non-obese patients with T2DM analyzed. CD14⁺HLA-DR^{-/low} frequency directly correlated with the years since T2DM diagnosis, the age of patients, and glycemic index. Together our data suggest that hyperglycemia and the length of T2DM influence CD14⁺HLA-DR^{-/low} frequency independent of weight.

Funding

This work was supported by the Instituto Mexicano del Seguro Social (grant FIS/IMSS/PROT/G17-2/1717) and CONACYT (grant A1-S-48232). CONACYT also provided a scholarship to MAVA for her Ph.D. studies (584982).

Declaration of Competing Interest

The authors report that they have no conflicts of interest relevant to this article. The authors alone are responsible for the intellectual property of this article.

Acknowledgments

The authors thank the participants who kindly donated blood samples for our study. The authors are grateful to M.D. José Antonio Luna-Roldán and M.D. René Padilla Rodríguez, for providing all facilities needed to carry out the laboratory evaluation of

samples of diabetic patients and healthy controls at H.G.26, ISSSTE, in Zacatecas City.

Authors' Contributions

MAVA: Investigation; Methodology; Analysis; Writing-original draft. JECD: Methodology; Review & editing. GLV: Writing-review & editing. FDA: Medical evaluation and recruitment of participants. BRS: Review & editing. JAEM: Review & editing. CJS: Conceptualization; Resources; Project administration; Supervision; Writing-original draft.

REFERENCES

<http://www.pasteur.fr/fr/recherche/ressources-scientifiques/publications-scientifiques/research-microbiology/instructions-authors>

- [1] Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen Let al. : IDF Diabetes Atlas: Estimation of Global and Regional Gestational Diabetes Mellitus Prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. *Diabetes Res Clin Pract* 2022;183:109050.
- [2] Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539.
- [3] American Diabetes A: Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;32 Suppl 1:S62.
- [4] Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R: Type 2 Diabetes and its Impact on the Immune System. *Curr Diabetes Rev* 2020;16:442.
- [5] Critchley JA, Carey IM, Harris T, DeWilde S, Hosking FJ, Cook DG: Glycemic Control and Risk of Infections Among People With Type 1 or Type 2 Diabetes in a Large Primary Care Cohort Study. *Diabetes Care* 2018;41:2127.
- [6] Fadini GP, de Kreutzenberg SV, Boscaro E, Albiero M, Cappellari R, Krankel Net al. : An unbalanced monocyte polarisation in peripheral blood and bone marrow of patients with type 2 diabetes has an impact on microangiopathy. *Diabetologia* 2013;56:1856.
- [7] Valtierra-Alvarado MA, Castaneda Delgado JE, Ramirez-Talavera SI, Lugo-Villarino G, Duenas-Arteaga F, Lugo-Sanchez Aet al. : Type 2 diabetes mellitus metabolic control correlates with the phenotype of human monocytes and monocyte-derived macrophages. *J Diabetes Complications* 2020;34:107708.

- [8] Restrepo BI, Twahirwa M, Jagannath C: Hyperglycemia and dyslipidemia: Reduced HLA-DR expression in monocyte subpopulations from diabetes patients. *Hum Immunol* 2021;82:124.
- [9] Restrepo BI, Twahirwa M, Rahbar MH, Schlesinger LS: Phagocytosis via complement or Fc-gamma receptors is compromised in monocytes from type 2 diabetes patients with chronic hyperglycemia. *PLoS One* 2014;9:e92977.
- [10] Gomez DI, Twahirwa M, Schlesinger LS, Restrepo BI: Reduced Mycobacterium tuberculosis association with monocytes from diabetes patients that have poor glucose control. *Tuberculosis (Edinb)* 2013;93:192.
- [11] Mengos AE, Gastineau DA, Gustafson MP: The CD14(+)/HLA-DR(lo/neg) Monocyte: An Immunosuppressive Phenotype That Restrains Responses to Cancer Immunotherapy. *Front Immunol* 2019;10:1147.
- [12] Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten T et al. : Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* 2016;7:12150.
- [13] Meyer C, Cagnon L, Costa-Nunes CM, Baumgaertner P, Montandon N, Leyvraz L et al. : Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. *Cancer Immunol Immunother* 2014;63:247.
- [14] Kitano S, Postow MA, Ziegler CG, Kuk D, Panageas KS, Cortez C et al. : Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes. *Cancer Immunol Res* 2014;2:812.
- [15] Giallongo C, Parrinello NL, La Cava P, Camiolo G, Romano A, Scalia M et al. : Monocytic myeloid-derived suppressor cells as prognostic factor in chronic myeloid leukaemia patients treated with dasatinib. *J Cell Mol Med* 2018;22:1070.
- [16] Volk HD, Thieme M, Heym S, Docke WD, Ruppe U, Tausch W et al. : Alterations in function and phenotype of monocytes from patients with septic disease--predictive value and new therapeutic strategies. *Behring Inst Mitt* 1991:208.
- [17] Xue G, Jiang M, Zhao R, Le A, Li J: Elevated frequencies of CD14(+)/HLA-DR(lo/neg) MDSCs in COVID-19 patients. *Aging (Albany NY)* 2021;13:6236.
- [18] Collins KK: The diabetes-cancer link. *Diabetes Spectr* 2014;27:276.
- [19] Friedrich K, Sommer M, Strobel S, Thrum S, Bluher M, Wagner U et al. : Perturbation of the Monocyte Compartment in Human Obesity. *Front Immunol* 2019;10:1874.
- [20] Restrepo BI, Khan A, Singh VK, Erica d-L, Aguilon-Duran GP, Ledezma-Campos E et al. : Human monocyte-derived macrophage responses to M. tuberculosis differ by the host's tuberculosis, diabetes or obesity status, and are enhanced by rapamycin. *Tuberculosis (Edinb)* 2021;126:102047.
- [21] Bao Y, Mo J, Ruan L, Li G: Increased monocytic CD14(+)/HLA-DR(lo/neg) myeloid-derived suppressor cells in obesity. *Mol Med Rep* 2015;11:2322.
- [22] Wang S, Tan Q, Hou Y, Dou H: Emerging Roles of Myeloid-Derived Suppressor Cells in Diabetes. *Front Pharmacol* 2021;12:798320.
- [23] Fernandez-Ruiz JC, Galindo-De Avila JC, Martinez-Fierro ML, Garza-Veloz I, Cervantes-Villagrana AR, Valtierra-Alvarado M et al. : Myeloid-Derived Suppressor Cells Show Different Frequencies in Diabetics and Subjects with Arterial Hypertension. *J Diabetes Res* 2019;2019:1568457.
- [24] Wang T, Wen Y, Fan X: Myeloid-derived suppressor cells suppress CD4+ T cell activity and prevent the development of type 2 diabetes. *Acta Biochim Biophys Sin (Shanghai)* 2018;50:362.

- [25] Islam J, Lee HJ, Yang SH, Kim DK, Joo KW, Kim YSet al. : Expansion of Myeloid-Derived Suppressor Cells Correlates with Renal Progression in Type 2 Diabetic Nephropathy. *Immune Netw* 2020;20:e18.
- [26] Valtierra-Alvarado MA, Lugo-Villarino G, Duenas-Arteaga F, Gonzalez-Contreras BE, Lugo-Sanchez A, Castaneda-Delgado JEet al. : Impact of Type 2 Diabetes on the capacity of human macrophages infected with Mycobacterium tuberculosis to modulate monocyte differentiation through a bystander effect. *Immunol Cell Biol* 2021.
- [27] Rohn W, Tang LP, Dong Y, Benveniste EN: IL-1 beta inhibits IFN-gamma-induced class II MHC expression by suppressing transcription of the class II transactivator gene. *J Immunol* 1999;162:886.
- [28] Gonzalez-Junca A, Driscoll KE, Pellicciotta I, Du S, Lo CH, Roy Ret al. : Autocrine TGFbeta Is a Survival Factor for Monocytes and Drives Immunosuppressive Lineage Commitment. *Cancer Immunol Res* 2019;7:306.
- [29] Koppelman B, Neefjes JJ, de Vries JE, de Waal Malefyt R: Interleukin-10 down-regulates MHC class II alphabeta peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. *Immunity* 1997;7:861.
- [30] Le Tulzo Y, Pangault C, Amiot L, Guilloux V, Tribut O, Arvieux Cet al. : Monocyte human leukocyte antigen-DR transcriptional downregulation by cortisol during septic shock. *Am J Respir Crit Care Med* 2004;169:1144.
- [31] Li W, Zhang X, Sang H, Zhou Y, Shang C, Wang Yet al. : Effects of hyperglycemia on the progression of tumor diseases. *J Exp Clin Cancer Res* 2019;38:327.
- [32] Stattin P, Bjor O, Ferrari P, Lukanova A, Lenner P, Lindahl Bet al. : Prospective study of hyperglycemia and cancer risk. *Diabetes Care* 2007;30:561.
- [33] Joshu CE, Prizment AE, Dluzniewski PJ, Menke A, Folsom AR, Coresh Jet al. : Glycated hemoglobin and cancer incidence and mortality in the Atherosclerosis in Communities (ARIC) Study, 1990-2006. *Int J Cancer* 2012;131:1667.
- [34] Janols H, Bergenfelz C, Allaoui R, Larsson AM, Ryden L, Bjornsson Set al. : A high frequency of MDSCs in sepsis patients, with the granulocytic subtype dominating in gram-positive cases. *J Leukoc Biol* 2014;96:685.
- [35] Huang A, Zhang B, Yan W, Wang B, Wei H, Zhang Fet al. : Myeloid-derived suppressor cells regulate immune response in patients with chronic hepatitis B virus infection through PD-1-induced IL-10. *J Immunol* 2014;193:5461.
- [36] Kumar A, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla Vet al. : Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. *Diabetes Metab Syndr* 2020;14:535.
- [37] Reilly ML, Schillie SF, Smith E, Poissant T, Vonderwahl CW, Gerard Ket al. : Increased risk of acute hepatitis B among adults with diagnosed diabetes mellitus. *J Diabetes Sci Technol* 2012;6:858.

FIGURE LEGENDS

Fig. 1. Gating strategy and comparison of the frequency of CD14⁺HLA-DR^{-/low} cells between groups. PBMCs from healthy controls (HC, n=24) and diabetic patients

(T2DM, n=25) were stained and evaluated by flow cytometry. A) Representative gating strategy used to identify CD14⁺HLA-DR^{-/low} cells, with exemplary dot plot for an HC (above) and a T2DM subject (below). B) Frequency of CD14⁺HLA-DR^{-/low} cells in HC (black circles) and T2DM (white circles) subjects. C) Percentage of CD14⁺HLA-DR^{-/low} cells in normal weight (n=17, black circles) and overweight (Ow, n=32, white circles) individuals. Each symbol represents an individual. Statistical analysis was performed with the Mann-Whitney U test, ***p<0.0001. Scatter plots show median with interquartile range.

Fig. 2. Effect of glycemic control on CD14⁺HLA-DR^{-/low} cells frequency in T2DM patients. PBMCs from diabetic patients (T2DM, n=25) were stained and analyzed by flow cytometry. Data are shown for subjects with T2DM. A) Frequency of CD14⁺HLA-DR^{-/low} cells in individuals with normal weight (n=6) or overweight (Ow, n=19). B) Percentage of CD14⁺HLA-DR^{-/low} cells in patients with T2DM stratified as with good glycemic control (GGC, n=10) and poor glycemic control (PGC, n=15). Analysis of correlation of the percentage of CD14⁺HLA-DR^{-/low} cells with C) HbA1c, D) years with the diagnosis of T2DM, E) glycemic index, and F) age of diabetic subjects. Statistical analysis was performed with (A) Mann-Whitney U and (B) Unpaired *t*-test. Scatter plots show (A) median with interquartile range and (B) mean with SD. Each circle represents an individual. (C-F) Correlation analysis provided Spearman's correlation coefficient (r) and the corresponding p values. *p<0.05.

Table 1. Clinical characteristics of participants.

Characteristics	Healthy Controls (HC)	T2DM patients	<i>p</i> value
Numbers of cases (n)	24	25	NA
Years with T2DM diagnosis ^ζ	NA	3.00 ± 8.50	NA
Glycemic index ^ζ	NA	27.96 ± 99.49	NA
Age, years	42.58 ± 7.30	46.64 ± 7.01	0.053
BMI (kg/m ²)	24.79 ± 2.28	26.15 ± 2.24	0.041*
HbA1c (%)	5.27 ± 0.32	9.68 ± 2.71	<0.0001***
FPG (mg/dl)	84.65 ± 6.86	208.10 ± 96.81	<0.0001***
Total cholesterol (mg/dl)	170.00 ± 31.08	182.10 ± 58.18	0.36
HDL- cholesterol (mg/dl)	53.36 ± 10.44	42.15 ± 8.60	0.0003***
LDL- cholesterol (mg/dl)	104.80 ± 31.49	105.00 ± 43.85	0.98
VLDL- cholesterol (mg/dl) ^ζ	19.50 ± 11.50	38.40 ± 48.75	<0.0001***
Triglycerides (mg/dl) ^ζ	95.40 ± 51.67	189.00 ± 227.90	<0.0001***
Serum creatinine (mg/dl) ^ζ	0.70 ± 0.30	0.70 ± 0.20	0.33
Monocytes (10 ³ /μl) ^ζ	0.46 ± 0.25	0.49 ± 0.12	0.36
Hypertension, n (%)	0 (0)	7 (28.00)	NA
Metformin, n (%)	0 (0)	22 (88.00)	NA
Glibenclamide, n (%)	0 (0)	14 (56.00)	NA
Metformin + Glibenclamide, n (%)	0 (0)	14 (56.00)	NA

t student tests were performed to identify differences between groups unless otherwise specified; ζ : Mann-Whitney *U* tests were performed for these variables; mean (\pm standard deviation) or ζ : median (\pm interquartile range) are shown for quantitative variables. Glycemic index = HbA1c x self-reported years with diabetes. BMI: body mass index; HbA1c: glycated hemoglobin %; FPG: fasting plasma glucose; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low-density lipoprotein; NA: non-applicable.

Table 2. Correlation analysis of clinical variables with the frequency of CD14⁺HLA-DR^{-/low} cells.

Variable	Spearman correlation coefficient	p value
<i>Healthy Controls (HC)</i>		
Age, years	0.235	0.267
BMI (kg/m ²)	0.245	0.248
HbA1c (%)	-0.204	0.336
FPG (mg/dl)	0.200	0.348
Total cholesterol (mg/dl)	-0.072	0.737
HDL- cholesterol (mg/dl)	0.229	0.316
LDL- cholesterol (mg/dl)	-0.183	0.438
VLDL- cholesterol (mg/dl)	-0.220	0.351
Triglycerides (mg/dl)	-0.326	0.119
Serum creatinine (mg/dl)	0.400	0.052
Monocytes (10 ³ /μl)	0.365	0.079
<i>Type 2 diabetes mellitus patients (T2DM)</i>		
Years since T2DM diagnosis	0.397	0.048*
Glycemic index	0.453	0.022*
Age, years	0.601	0.001**
BMI (kg/m ²)	-0.373	0.066
HbA1c (%)	0.390	0.053
FPG (mg/dl)	0.259	0.210
Total cholesterol (mg/dl)	0.085	0.684
HDL- cholesterol (mg/dl)	0.329	0.115
LDL- cholesterol (mg/dl)	0.086	0.693
VLDL- cholesterol (mg/dl)	-0.180	0.400
Triglycerides (mg/dl)	-0.203	0.328
Serum creatinine (mg/dl)	0.174	0.403
Monocytes (10 ³ /μl)	0.346	0.089

Correlations were calculated with Spearman correlation coefficient. Glycemic index = HbA1c x self-reported years with diabetes. BMI: body mass index; HbA1c: glycated hemoglobin %; FPG: fasting plasma glucose; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low-density lipoprotein.

Table 1. Clinical characteristics of participants.

Characteristics	Healthy Controls (HC)	T2DM patients	<i>p</i> value
Numbers of cases (n)	24	25	NA
Years with T2DM diagnosis ^ζ	NA	3.00 ± 8.50	NA
Glycemic index ^ζ	NA	27.96 ± 99.49	NA
Age, years	42.58 ± 7.30	46.64 ± 7.01	0.053
BMI (kg/m ²)	24.79 ± 2.28	26.15 ± 2.24	0.041*
HbA1c (%)	5.27 ± 0.32	9.68 ± 2.71	<0.0001***
FPG (mg/dl)	84.65 ± 6.86	208.10 ± 96.81	<0.0001***
Total cholesterol (mg/dl)	170.00 ± 31.08	182.10 ± 58.18	0.36
HDL- cholesterol (mg/dl)	53.36 ± 10.44	42.15 ± 8.60	0.0003***
LDL- cholesterol (mg/dl)	104.80 ± 31.49	105.00 ± 43.85	0.98
VLDL- cholesterol (mg/dl) ^ζ	19.50 ± 11.50	38.40 ± 48.75	<0.0001***
Triglycerides (mg/dl) ^ζ	95.40 ± 51.67	189.00 ± 227.90	<0.0001***
Serum creatinine (mg/dl) ^ζ	0.70 ± 0.30	0.70 ± 0.20	0.33
Monocytes (10 ³ /μl) ^ζ	0.46 ± 0.25	0.49 ± 0.12	0.36
Hypertension, n (%)	0 (0)	7 (28.00)	NA
Metformin, n (%)	0 (0)	22 (88.00)	NA
Glibenclamide, n (%)	0 (0)	14 (56.00)	NA
Metformin + Glibenclamide, n (%)	0 (0)	14 (56.00)	NA

t student tests were performed to identify differences between groups unless otherwise specified; ζ : Mann-Whitney *U* tests were performed for these variables; mean (\pm standard deviation) or ζ : median (\pm interquartile range) are shown for quantitative variables. Glycemic index = HbA1c x self-reported years with diabetes. BMI: body mass index; HbA1c: glycated hemoglobin %; FPG: fasting plasma glucose; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low-density lipoprotein; NA: non-applicable.

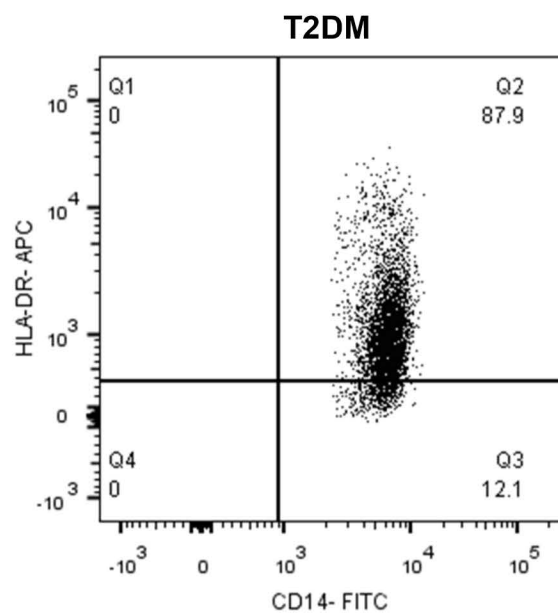
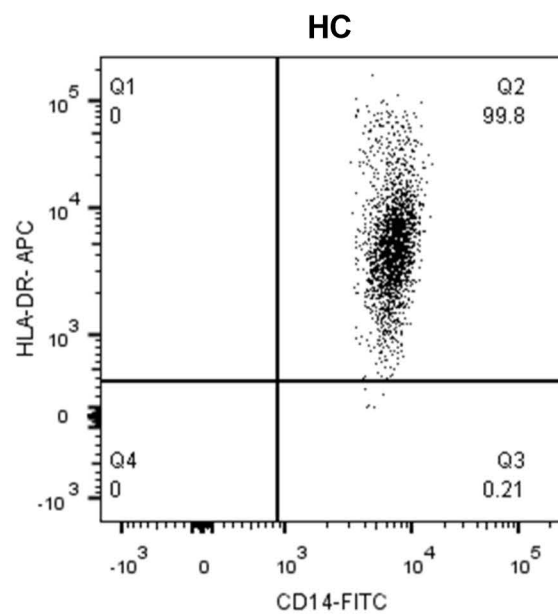
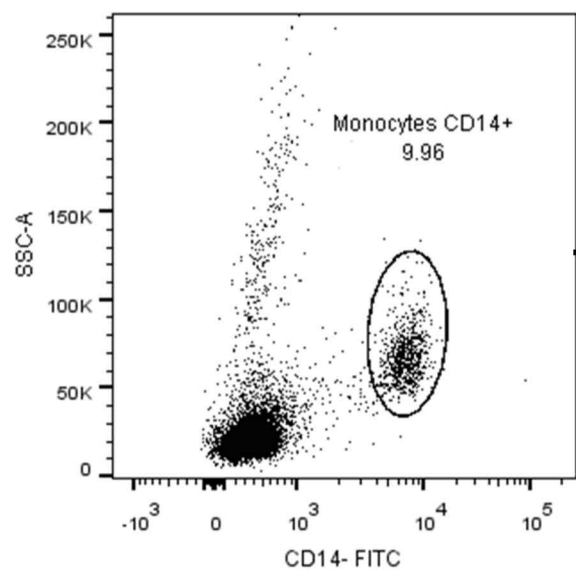
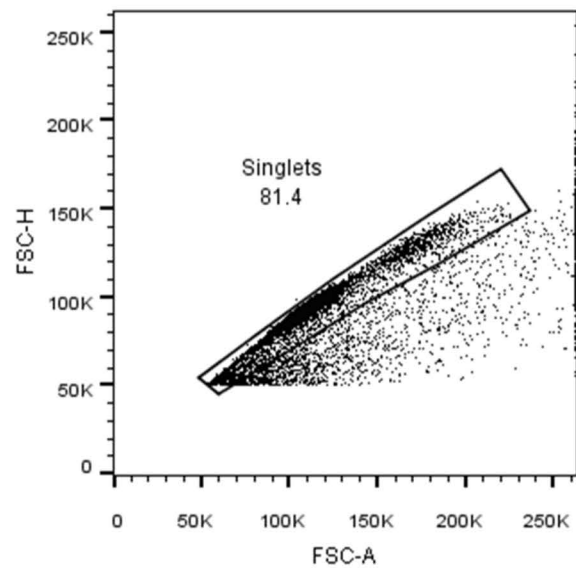
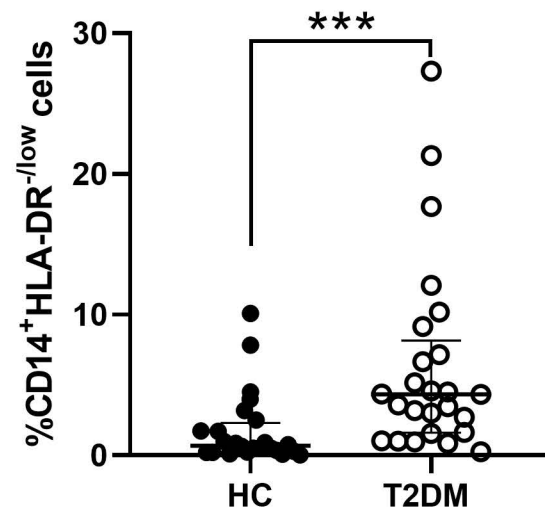
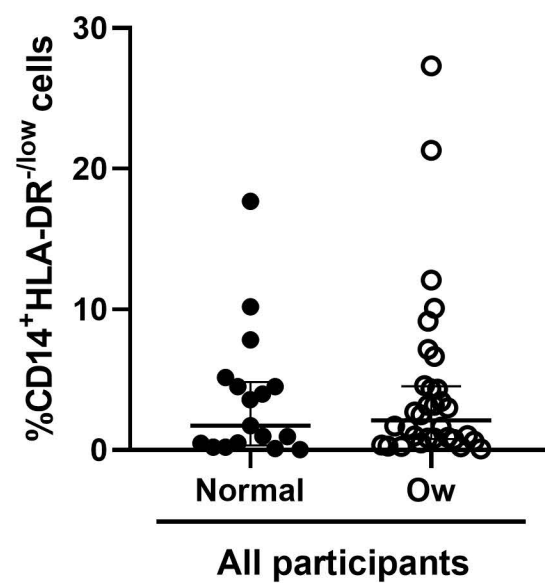
Table 2. Correlation analysis of clinical variables with the frequency of CD14⁺HLA-DR^{-/low} cells.

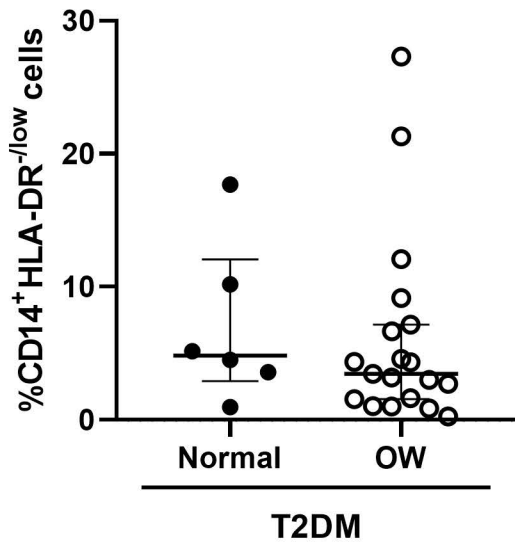
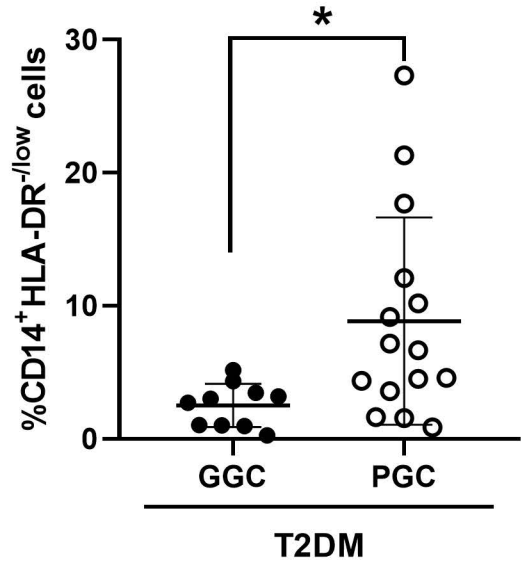
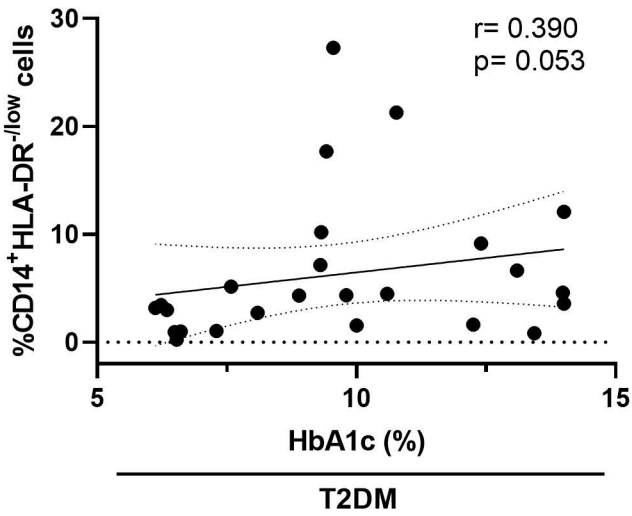
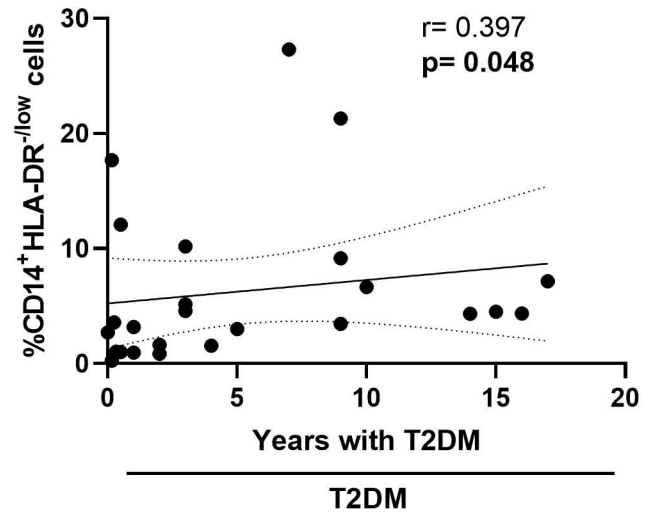
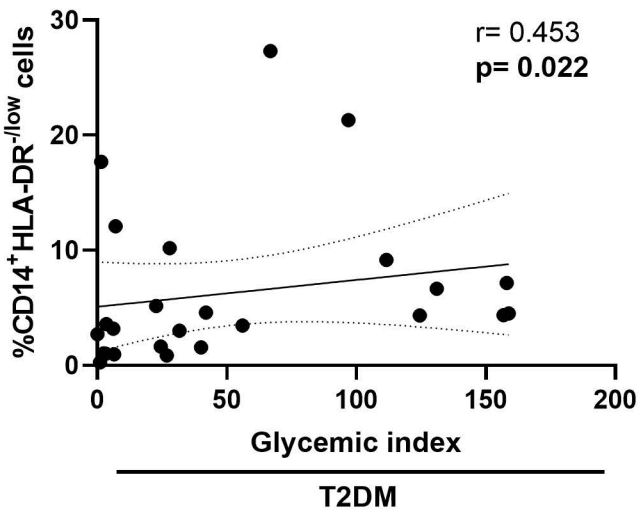
Variable	Spearman correlation coefficient	p value
<i>Healthy Controls (HC)</i>		
Age, years	0.235	0.267
BMI (kg/m ²)	0.245	0.248
HbA1c (%)	-0.204	0.336
FPG (mg/dl)	0.200	0.348
Total cholesterol (mg/dl)	-0.072	0.737
HDL- cholesterol (mg/dl)	0.229	0.316
LDL- cholesterol (mg/dl)	-0.183	0.438
VLDL- cholesterol (mg/dl)	-0.220	0.351
Triglycerides (mg/dl)	-0.326	0.119
Serum creatinine (mg/dl)	0.400	0.052
Monocytes (10 ³ /μl)	0.365	0.079
<i>Type 2 diabetes mellitus patients (T2DM)</i>		
Years since T2DM diagnosis	0.397	0.048*
Glycemic index	0.453	0.022*
Age, years	0.601	0.001**
BMI (kg/m ²)	-0.373	0.066
HbA1c (%)	0.390	0.053
FPG (mg/dl)	0.259	0.210
Total cholesterol (mg/dl)	0.085	0.684
HDL- cholesterol (mg/dl)	0.329	0.115
LDL- cholesterol (mg/dl)	0.086	0.693
VLDL- cholesterol (mg/dl)	-0.180	0.400
Triglycerides (mg/dl)	-0.203	0.328
Serum creatinine (mg/dl)	0.174	0.403
Monocytes (10 ³ /μl)	0.346	0.089

Correlations were calculated with Spearman correlation coefficient. Glycemic index = HbA1c x self-reported years with diabetes. BMI: body mass index; HbA1c: glycated hemoglobin %; FPG: fasting plasma glucose; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low-density lipoprotein.

Figure 1. Gating strategy and comparison of the frequency of CD14⁺HLA-DR^{-/low} cells between groups. PBMCs from healthy controls (HC, n=24) and diabetic patients (T2DM, n=25) were stained and evaluated by flow cytometry. A) Representative gating strategy used to identify CD14⁺HLA-DR^{-/low} cells, with exemplary dot plot for a HC (above) and a T2DM subject (below). B) Frequency of CD14⁺HLA-DR^{-/low} cells in HC (black circles) and T2DM (white circles) subjects. C) Percentage of CD14⁺HLA-DR^{-/low} cells in normal weight (n=17, black circles) and overweight (Ow, n=32, white circles) individuals. Each symbol represents an individual. Statistical analysis was performed with the Mann-Whitney U test, ***p<0.0001. Scatter plot shows median with interquartile range.

Figure 2. Effect of glycemic control on CD14⁺HLA-DR^{-/low} cells frequency. PBMCs from healthy controls (HC, n=24) and diabetic patients (T2DM, n=25) were stained and analyzed by flow cytometry. The data are shown only for subjects with T2DM. A) Frequency of CD14⁺HLA-DR^{-/low} cells in individuals with normal weight (n=6) or overweight (Ow, n=19) in diabetic patients. B) Percentage of CD14⁺HLA-DR^{-/low} cells in T2DM patients stratified as good glycemic control (GGC, n=10) and poor glycemic control (PGC, n=15). Correlation of the percentage of CD14⁺HLA-DR^{-/low} cells with C) HbA1c, D) years with T2DM diagnosis, E) glycemic index, and F) age of diabetic subjects. Statistical analysis was performed with (A) Mann-Whitney U and (B) Unpaired t-test. (C-F) Correlation analysis providing Spearman's correlation coefficient (r) and corresponding p values. *p<0.05. Scatter plots show (A) median with interquartile range and (B) mean with SD. Each circle represents an individual.

A)**B)****C)**

A)**B)****C)****D)****E)****F)**