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An exploratory human study investigating the influence of type 2 diabetes on macrophage phenotype after myocardial infarction

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ABSTRACT

Background: Myocardial infarction (MI) is the primary cause of death in subjects with type 2 diabetes (T2D) and their in-hospital mortality after MI is still elevated compared with those without T2D. Therefore, it is of crucial importance to identify possible mechanisms of worse clinical outcomes and mortality in T2D subjects. Monocyte/macrophage-mediated immune response plays an important role in heart remodelling to limit functional deterioration after MI. Indeed, first pro-inflammatory macrophages digest damaged tissue, then anti-inflammatory macrophages become prevalent and promote tissue repair. Here, we hypothesize that the worse clinical outcomes in patients with T2D could be the consequence of a defective or a delayed polarization of macrophages toward an anti-inflammatory phenotype.

Methods and results: In an exploratory human study, circulating monocytes from male patients with or without T2D at different time-points after MI were *in vitro* differentiated toward pro- or anti-inflammatory macrophages. The results of this pilot study suggest that the phenotype of circulating monocytes, as well as the pro- and anti-inflammatory macrophage polarization, or the kinetics of the pro- and anti-inflammatory polarization, is not influenced by T2D.

Conclusion: Further studies will be necessary to understand the real contribution of macrophages after MI in humans.

1. Introduction

Myocardial infarction (MI) is the primary cause of death in subjects with type 2 diabetes (T2D) [1]. While patients with T2D without previous history of coronary artery disease (CAD) have the same risk of major cardiac events than those with CAD, the risk of MI recurrence is approximatively 40 % higher in patients with T2D with a history of MI [2]. Among patients undergoing percutaneous coronary intervention for ST-segment elevation MI, 25 % had a history of T2D, approximately 10 % had previously undiagnosed T2D, and 38.7 % had pre-diabetes [3]. Moreover, the in-hospital mortality of MI patients with T2D is still elevated compared with those without T2D [4]. Therefore, it is of crucial importance to understand the possible mechanisms leading to worse clinical outcomes and increased mortality after MI in T2D subjects.

Innate immune response, mediated by monocytes/macrophages, has recently been described as a crucial mechanism in the scar deposition and heart remodelling to prevent myocardial rupture and limit functional deterioration after MI (for review see [5]). Generally, human circulating monocytes can be divided into three subgroups, based on the expression levels of CD14 (lipopolysaccharide (LPS) coreceptor) and CD16 (FcγRIII): classical (CD14++CD16−), intermediate (CD14++CD16+), and non-classical (CD14+CD16+) monocytes [6], displaying functional differences, such as inflammatory, migratory, and phagocytic responses [7].

After MI occurrence, circulating pro-inflammatory CD14+CD16− and anti-inflammatory CD14+CD16+ monocytes increased, peaking on days 3 and 5 after the event, respectively [8]. Post-MI myocardial salvage was decreased in patients with high peak levels of circulating CD14+CD16− [8]. Analysis of post-mortem cardiac tissue showed an influx of CD14+CD16− cells in the border zone in the

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inflammatory phase after MI, surrounding the necrotic infarct core, and the presence of CD14+CD16+ cells in the infarct core in the subsequent post-MI proliferative phase [9]. Macrophages, derived from monocyte differentiation, are heterogeneous cells adapting their phenotype to micro-environmental signals [10]. Th1 cytokines, such as interferon γ (IFNγ) or interleukin 1β (IL-1β), induce a pro-inflammatory activation M1 profile with macrophages primarily producing TNFα, IL-6 and IL-12, while Th2 cytokines, such as IL-4 and IL-13, induce anti-inflammatory M2 macrophages that produce anti-inflammatory factors (IL-10, transforming growth factor β (TGFβ) and IL-1 receptor antagonist (IL-1Ra)), scavenge debris and promote angiogenesis, tissue remodelling and repair [11,12]. Moreover, macrophages are plastic cells since they can switch from one activated state to another, depending on specific signals [13]. The infiltration of monocytes/macrophages in infarcted tissue occurs in two coordinated sequential phases. Indeed, in the first days following a MI event in mice, from day 3 to 5, macrophages with pro-inflammatory phenotype predominated in the infarcted heart to digest damaged tissue and clean up dead cells. At later stages (from 4 to 7 days post-MI), macrophages with anti-inflammatory properties become more prevalent, promoting extra-cellular matrix remodelling, scar formation, and angiogenesis [14]. Moreover, infiltrated macrophages in a murine model of MI, after 5 days, undergo a metabolic reprogramming toward an increased oxidative phosphorylation, a typical characteristic of the anti-inflammatory phenotype [15].

Effective infarct healing requires timely and coordinated resolution of inflammation: a prolonged pro-inflammatory phase or a compromised anti-inflammatory phase predisposes to heart failure [16]. Indeed, the maintenance of a good balance between pro- and anti-inflammatory macrophages is crucial to ensure a correct heart remodelling after MI, by increasing tissue repair and preventing post-MI heart failure.

Moreover, T2D alters specific monocyte and macrophage markers associated with their function, which can lead to immune dysfunctions associated to increased susceptibility to pathogenic infection observed in these patients [17]. Indeed, macrophage phagocytic and chemotactic functions are impaired by T2D [18].

T2D patients presented an imbalanced M1/M2 ratio, due to a reduction in M2 (with unchanged M1), compared to controls. This M1/M2 ratio was directly correlated with waist circumference and HbA1c [19]. Among T2D patients, M2 reduction and M1/M2 increase was associated with microangiopathy, particularly nephropathy [17,19]. Interestingly, M1 macrophages from atherosclerotic T2D patients displayed more pro-angiogenic and proteolytic activities than non-diabetic subjects [20]. Here, we hypothesize that the worse clinical outcomes after MI observed in patients with T2D could be the consequence of a defective or a delayed polarization of macrophages toward an anti-inflammatory phenotype. To test that, blood monocytes were isolated after MI from patients with or without T2D and *in vitro* differentiated in the presence of IL-1 β to obtain pro-inflammatory M1 macrophages (M(IL-1β)) or with IL-4 to induce the anti-inflammatory M2 macrophage phenotype (M(IL-4)).

2. Materials and methods

2.1. Research subjects

The study, registered on the "clinicaltrials.gov" website under the number NCT02768935, was conducted according to the guidelines of the 1975 Declaration of Helsinki, and approved by the "Comité de Protection des Personnes Sud-Méditerranée", Ethics Committee (protocol 2016-A00793-48, approved on the 16th September 2016). Informed consent was obtained from all subjects enrolled. Male subjects were hospitalized for MI occurrence in the Department of cardiology, University Hospital of Nice, France, from October 2017 to December 2019. MI was defined by clinical symptoms of ischemia associated with ECG changes indicative of new ischemia (new ST-T changes or new left bundle branch block or development of pathological Q waves) and significant troponin rise (High Sensitivity Troponin I, Siemens-Healthineers). None of the patients received reperfusion therapy but all of them were subjected to coronary angioplasty.

T2D was defined based on the glycated hemoglobin (HbA1c) values and the medical records. 16 male subjects without T2D (ND) and 11 with T2D (D) were included (Fig. 1). None of the patients had any clinical symptoms of systemic inflammation. For each patient, clinical informations were collected. Biological data were collected at the day of the MI event. Biological analyses were performed in the Laboratories of Clinical Biochemistry and of Haematology at the University Hospital of Nice which are certified by the National French Committee of Accreditation. For all assays, quality controls fell within predefined limits.

2.2. Monocyte isolation, culture and differentiation

Peripheral blood was drawn in EDTA tubes from all patients within the first 24 h after the MI occurrence (D0) or at 3 (D3) or 5 (D5) days after the event. 5 days correspond to the average hospitalization period after a MI event at the CHU of Nice.

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density gradient centrifugation as previously described [21], frozen and stored at −150 °C in RPMI medium supplemented with 40 % FCS and 10 % DMSO. For *in vitro* experiments, PBMC were thawed and *in vitro* cultured simultaneously, using a standardized protocol [22,23]. Monocytes were seeded at density of $1x10^6$ cells/well in Primaria 24 well plastic culture dishes (Corning) for 2 h at 37 °C. After this period, non-adherent lymphocytes were removed by PBS washes. Unpolarized macrophages, used as control, were obtained from adherent monocytes cultured for 6 days in RPMI 1640 medium containing gentamicin (40 μg/ml), glutamine (2 mM) (Sigma) and supplemented with 10 % human pooled serum (Dutscher, France). To induce pro-inflammatory M(IL-1β) or anti-inflammatory M(IL-4) macrophage phenotype, recombinant human IL-1β or IL-4 (15 ng/ml, both from Peprotech) was added at the beginning of differentiation, respectively [24].

2.3. Flow cytometry analysis

PBMC were thawed in pre-warmed complete RPMI. Cells were gently washed twice and resuspended in PBS 0.5 % FCS plus 2 mM EDTA. Stained cell preparations were analysed using a Cytek® Aurora flow cytometer (Cytek Biosciences B.V., Amsterdam, Europe). Miltenyi Biotec (Paris, France) antibodies were used. One antibody cocktail was used with the following antibodies: αCD14FITC, αCD16 PE Vio770, αCD3-APC and αCD19 Vioblue. Extracellular labelling was done after 2 washes with PBS 0.5 % FCS plus 2 mM EDTA. Our gating strategy to quantify different monocyte populations started with discrimination of cells by size (FSC-A by SSC-A). Then singlets were distinguished using FSC-H by FSC-A. Live cells were gated, excluding Propidium Iodide-positive cells (Miltenyi Biotec). Following this strategy, we discriminated monocytes versus CD3+CD19+ cells. At this stage, monocytes were gated as part of CD14++CD16-, CD14++CD16+, or CD14+CD16++ populations (Supplementary Fig. 1S).

2.4. RNA extraction and analysis

Total cellular RNA was isolated from differentiated macrophages using Trizol (Invitrogen), reverse transcribed and cDNAs were quantified by quantitative polymerase chain reaction (Q-PCR) on a StepOne apparatus (ThermoFisher) using specific primers (Supplementary Table 1S). The relative expression of each gene was calculated by the ΔCt method, where ΔCt is the value obtained by subtracting the Ct (threshold cycle) value of cyclophilin mRNA from the Ct value of the target gene [22]. The amount of target relative to the cyclophilin mRNA was expressed as $2^{-(\Delta Ct)}$ and normalized to the level of the unpolarized macrophages (used as control) set as 1 at each time point after MI.

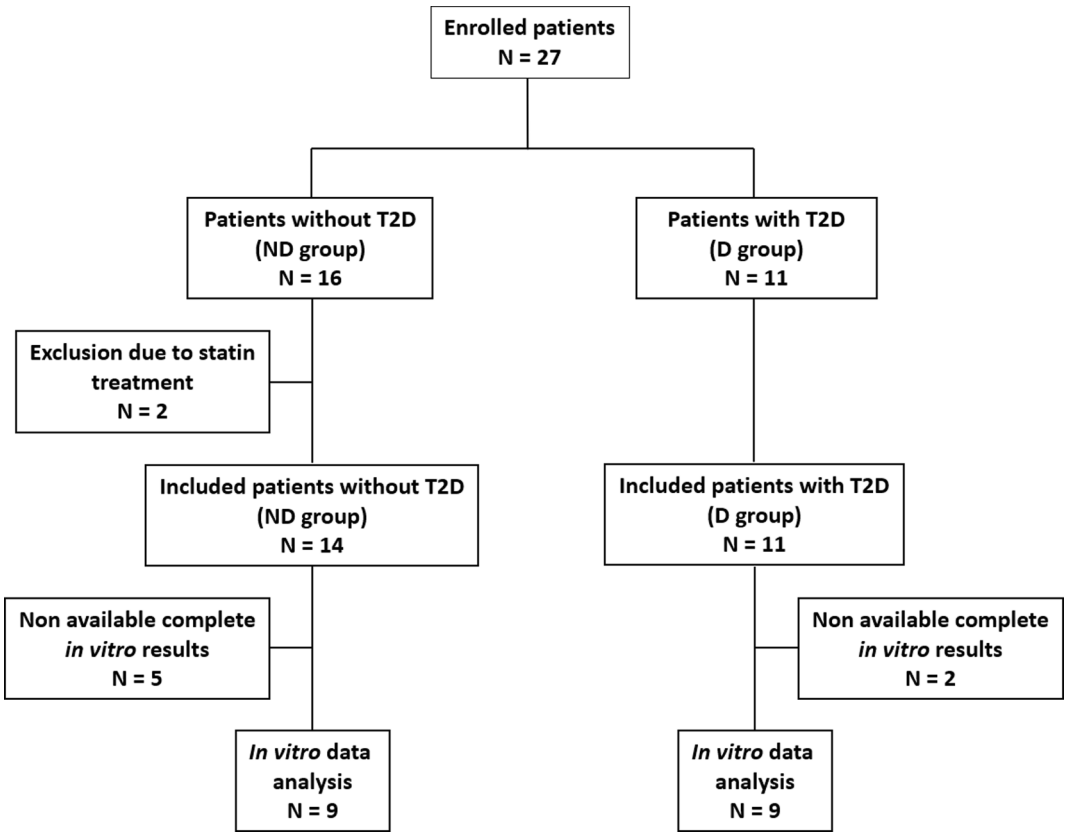

Fig. 1. Flow chart of the study.

2.5. Statistical analysis

Results were presented as median and interquartile ranges for quantitative variables and as relative frequencies for categorical variables. Baseline data were compared between groups using Fisher's exact test for categorical variables and the Student't test for continuous variables using SAS Enterprise Guide 7.1 software (SAS Institute, Inc. Cary, NC, USA).

The comparison of anti- and pro-inflammatory markers expression between group, at each time, were performed using two-way ANOVA with Bonferroni post-tests. For the flow cytometry data, two-way ANOVA was used with Tukey's multiple comparisons test. For the area under curve (AUC) representation of the flow cytometry data a Mann-Whitney test was used. All tests were two-sided, with a 5 % significance level. Statistical analyses were performed using Prism 9.5 software (Graphpad).

3. Results

3.1. Baseline characteristics of the population

Among the 27 patients included, cells from 9 subjects with T2D (D) and 9 subjects without T2D (ND) were analyzed *in vitro*. 2 ND subjects were excluded due to statin treatment, which can exert pleiotropic activities [25,26], and 5 ND and 2 D subjects were further excluded from *in vitro* analysis, due to an insufficient number of available cells at some time point (Fig. 1). Median age was 74 (68–77) (71 for ND (67–76) and 75 for D (70–81)), and median BMI was 25.2 kg/m^2 (24.3–26.9) (25.45 for ND (23.9–27) and 25.1 for D (24.3–26.9)). All patient baseline characteristics are shown in Table 1. No difference in systemic inflammation (based on CRP, monocytes, leukocytes or their ratio) was observed between the two groups of patients (Table 1). An in-hospital death occurred in the D group.

3.2. Type 2 diabetes does not modify the phenotype of circulating monocytes after MI

Since a heterogeneity has been reported for circulating monocytes in humans after MI [8], our first objective was to study the phenotype of circulating monocytes after MI, in patients with or without type 2 diabetes (D and ND) by performing flow cytometry analysis on PBMC. The percentage of PBMC that were neither B (CD19+) or T (CD3+) cells at day of MI and 3 and 5 days after, was not different between groups (Fig. 2). Analysis of monocyte sub-populations, classical (CD14++CD16−), intermediate (CD14++CD16+), or non-classical (CD14+CD16++), gated on CD19-CD3- PBMC, on day of MI (0) and 3 and 5 days after, indicated that no significant differences were detected between the two groups of patients, nor between different time points after MI (Fig. 2). Nonetheless, analysis of results obtained by combining all time points after MI, indicated that the area under the curve (AUC) of non-classical CD14+CD16++ monocytes was significantly lower in ND compared to D patients (Supplementary Fig. 2S).

3.3. Type 2 diabetes does not influence the capacity of monocytes to differentiate toward an anti-inflammatory macrophage phenotype after MI

To determine the influence of donor phenotype on the ability of monocytes to differentiate toward anti-inflammatory macrophages after MI occurrence, monocytes from subjects with or without T2D were isolated during the first 24 h after the event (D0) or after 3 (D3) or 5 (D5) days and were then differentiated for 6 days in the presence of IL-4, a commonly used potent inducer of the macrophage anti-inflammatory phenotype [27]. Our results demonstrated that the gene expression of CD206, AMAC1, VEGF-A, CD200R, F13A1, and TGFβ, all established markers for the anti-inflammatory differentiation in humans [27], was induced compared to the unpolarized macrophages at each time point after MI, but no difference was observed between D and ND subjects, nor

Table 1
Clinical and blood parameters according to diabetic status of patients.

Characteristics	Total patients (n = 25)	Non diabetic patients (n = 9)	Diabetic patients (n = 9)
Age (years)	74 (68–77)	71 (67–76)	75 (70–81)
Arterial hypertension	12 (48 %)	7 (77.7 %)	5 (55.5 %)
Dyslipidemia	8 (32 %)	5 (55.5 %)	3 (33.3 %)
Smoking	5 (20 %)	3 (33.5 %)	2 (22.2 %)
Body mass index (kg/m^2)	25.2 (24.6–26.9)	25.45 (23.9–27.0)	25.1 (24.3–26.9)
Weight (kg)	76.0 (70–82)	78 (70–84)	75 (70–80)
Diastolic blood pressure (mmHg)	74.0 (64–86)	75 (64–94)	74 (63–83)
Systolic blood pressure (mmHg)	135 (112–147)	126.5 (111–144)	140 (114–160)
Left ventricular ejection fraction (%)	50 (45–55)	50 (45–55)	52.5 (45–55)
CRP (mg/L)	3.5 (2.3–6.3)	3.05 (0.6–7.1)	4.9 (1–54.9)
Fasting glycemia (mmol/L)	6.39 (5.71–7.36)	6.0 (5.69–6.92)	7.52 (2.66–14.27)
HbA1c (%)	6.25 (5.5–7.8)	5.6 (5.3–6.2)	7.7 (5.7–9.7)*
Triglycerides (mmol/L)	1.29 (1.02–2.02)	1.46 (0.67–2.4)	1.29 (0.96–4.21)
Total cholesterol (mmol/L)	4.52 (4.12–5.12)	5.13 (4.46–5.58)	4.31 (2.94–4.81)
LDL-cholesterol (mmol/L)	2.76 (2.22–3.35)	3.25 (1.96–3.93)	2.46 (1.05–3.14)
HDL-cholesterol (mmol/L)	1.17 (0.93–1.44)	1.23 (0.63–2.2)	1.02 (0.54–1.44)
Creatinine (μmol/L)	91 (75–99)	91 (68–111)	90 (58–114)
Leukocytes (G/L)	9.1 (6.7–10.5)	8.7 (5.5–12.9)	10.1 (4.2–16.0)
Monocytes (G/L)	0.7 (0.6–1.1)	0.8 (0.3–1.2)	0.9 (0.6–1.5)
Ratio monocytes / leukocytes * 100	8.43 (6.88–10.57)	7.82 (5.36–11.22)	9.80 (6.77–14.29)
MI classification:			
STEMI / NSTEMI		5 / 4	4 / 5
Metformin treatment (alone or in association)		0	6

Continuous data are expressed as median (interquartile range) and categoric data as number (%). CRP: C reactive protein; HbA1c: glycated haemoglobin; LDL: low density lipoprotein; HDL: high density lipoprotein. STEMI: ST-segment elevation MI; NSTEMI: non-STEMI. Statistical significance differences are indicated. * $p < 0.05$ (Student *t* test).

between the different time points (D0, D3 and D5) after MI (Fig. 3). Expression of CD163, known to be reduced in IL-4 polarized macrophages at the gene expression level [27], was significantly different at D5, between D and ND subjects.

Moreover, VEGF-A expression results were significantly different only at D0, between ND and D patients. Expression of IL-10, an anti-inflammatory cytokine, was also measured. However, its expression was not significantly induced by IL-4, independently of the patient group or time point after MI (Fig. 3). Furthermore, under basal unpolarized conditions, the expression of these markers was not different between patients' groups, nor between time points (data not shown), suggesting that their expression was not preconditioned by the diabetic state.

3.4. Type 2 diabetes does not modify the capacity of monocytes to differentiate toward a pro-inflammatory macrophage phenotype after MI

Since our results showed that the presence of T2D did not influence the ability of monocytes to differentiate toward an anti-inflammatory macrophage phenotype, we wanted to determine whether T2D can lead to a more pronounced inflammatory macrophage response after MI occurrence. To this end, monocytes from subjects with or without T2D isolated during the first 24 h after the event (D0) and after 3 (D3) or 5 (D5) days, were differentiated for 6 days in the presence of IL-1β. Q-PCR analysis of the expression of pro-inflammatory genes (IL-1β, IL-8, CCL3 and MMP-9) [20], demonstrated that while the expression of these gene was induced compared to the relative unpolarized macrophages at each time points, no significant difference was observed between cells isolated from D or ND patients, at any time points after MI occurrence (Fig. 4). Expression of other pro-inflammatory markers (TNFα and CD86) was also evaluated. While their expression was not induced in our IL-1β driven polarization model, no significant difference was observed between groups of patients, nor between different time points after MI (Fig. 4). Perhaps the lack of induction of CD86 is due to the fact that this surface protein is mostly used as an M1 marker in flow cytometry and might be less suited as such by qPCR analysis. Moreover, the expression of these genes does not differ in macrophages under unpolarized basal conditions, between patients, nor between time points after MI (data not shown).

4. Discussion

After a MI event, circulating monocytes infiltrate the infarcted myocardium and differentiate toward pro- or anti-inflammatory macrophages in two sequential coordinated phases, thus allowing phagocytosis of tissue and cell debris as well as remodelling of extracellular matrix and angiogenesis, respectively, to ensure proper heart healing [5]. In addition, a shift from pro- to anti-inflammatory macrophages has been suggested after MI based on fatty acid synthesis and oxidative phosphorylation [15].

Different experimental observation in mice led to the hypothesis that shifting the macrophage phenotype from pro- to anti-inflammatory macrophages will protect the heart from adverse remodelling after MI occurrence. Indeed, reduced expression of the transcription factor IRF5 (interferon regulatory factor 5), involved in pro-inflammatory polarization, reduces the expression of inflammatory markers, accelerates heart healing and attenuates the development of heart failure after MI [28]. Regarding the role of anti-inflammatory macrophages, Trib1 kinase deficient mice, which exhibit a selective depletion of these macrophages after MI, display frequent cardiac rupture that can be completely rescued by an external supplementation of anti-inflammatory macrophages [29]. In line, MMP-28 deficient mice, showing reduced capacity of macrophages to differentiate toward an anti-inflammatory phenotype [30], have a higher mortality after MI due to increased heart rupture. However, the concept of macrophage polarization after MI has been recently challenged, at least in mice. In fact, mice with myeloid specific deletion of GATA3 (GATA-binding factor 3), a transcription factor involved in anti-inflammatory phenotype polarization, have improved cardiac functions in response to an acute MI, associated to the presence of a large number of pro-inflammatory Ly6C^{high} monocytes/macrophages and a reduced number of reparative Ly6C^{low} macrophages [31]. Indeed, this study also challenges the concept that the excessive presence of early pro-inflammatory monocytes/macrophages after MI would be deleterious.

In humans, little is known regarding macrophage heterogeneity after MI. Human myocardium contains distinct subset of CCR2$^-$ and CCR2$^+$ macrophages. The latter, derived from monocyte recruitment, are associated with left ventricular remodelling and systolic function in heart failure patients [32]. Moreover, the presence of CD14$^+$CD16$^-$ cells has been identified in the border zone in the inflammatory phase after MI, surrounding the necrotic infarct core, and CD14$^+$CD16$^+$ cells in the infarct core in the subsequent post-MI proliferative phase [9].

MI occurrence is frequent in patients with T2D, that are characterized by worse clinical outcomes and increased mortality, compared to subjects without T2D [4]. Our hypothesis was that after MI, monocytes isolated from patients with T2D could have a reduced and/or delayed response toward an anti-inflammatory macrophage polarization, compared to cells isolated from patients without T2D, thus providing a potential mechanism for the worse clinical outcomes observed in patients with T2D after MI. However, our results show that monocytes isolated from patients with or without T2D, differentiate *in vitro* into an anti-inflammatory phenotype (induced by IL-4) in a similar manner.

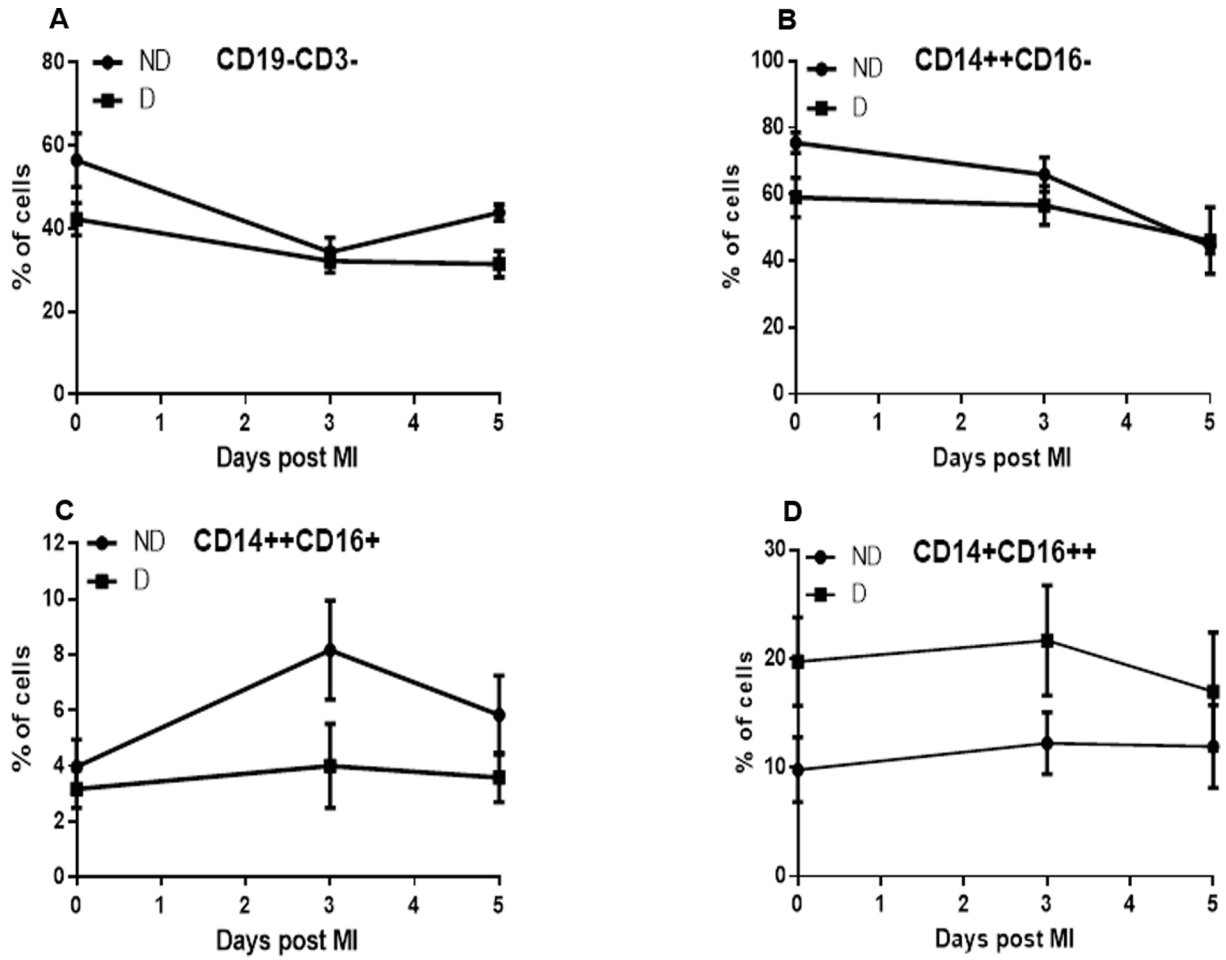

Fig. 2. Blood monocyte populations after myocardial infarction. A) Percentage of peripheral blood mononuclear cells (PBMC) that are neither B (CD19+) or T (CD3+) cells at day of myocardial infarction (MI) and 3 and 5 days after. B) Classical (CD14++CD16−), C) intermediate (CD14++CD16+), or D) non-classical (CD14+CD16++) monocytes, gated on CD19-CD3- PBMC, on day of MI and 3 and 5 days after. Data are presented as mean ± SEM. N = 8 for each group. ND = subjects without T2D, D = subjects with T2D.

Additionally, no differences were observed when blood was isolated immediately after the MI, nor 3 or 5 days after the event.

In the same line, polarization toward a pro-inflammatory phenotype, induced *in vitro* by the presence of IL-1β, was also similar in the monocytes isolated from both groups of patients at any time points. These results, even though obtained in a small group of patients (9 for each group), suggest that the pro- and anti-inflammatory macrophage polarization after MI, or the kinetics of the pro- and anti-inflammatory phases, seem not to be different in patients with T2D, compared to patients without T2D (ND) after MI event. One explanation, at least for the absence of difference in anti-inflammatory response, could be that the analyses have been performed too early after the MI, with a time point at maximal 5 days after the event. However, this cannot justify the absence of difference in the pro-inflammatory response, which is reported to develop, at least in mice, during the first days after the MI [5]. We previously reported that circulating blood cells maintain a patient-related phenotype once cultured *in vitro*.

Our data showed that heterogeneity of circulating monocytes reported in humans after MI, and based on the relative expression of CD14 and CD16, was not different between patients with and without T2D and did not change after MI. This could suggest that the absence of difference after *in vitro* macrophages polarization, reflects the unchanged circulating monocyte profile between the two groups of patients. Moreover, circulating monocyte phenotypes likely do not completely reflect the macrophage phenotypes once these cells infiltrate the heart, which can be influenced by microenvironmental cues. Unfortunately, we do not have access to heart samples to analyse the polarization status of infiltrated macrophages, the local cytokine content and to determine whether, in the context of damaged heart tissue, T2D has an impact. Indeed, in our experience, macrophage gene expression generally reflects well the functional phenotype [21,33].

This exploratory study, which is the first that followed *in vitro* macrophage polarization after MI performed in human samples, seems to exclude the involvement of macrophage polarization in the post-MI outcomes in patients with T2D. A possible limitation of our approach is that our conclusions are based on data from *ex vivo* experiments from a relative low number of patients and need to be confirmed in a larger cohort. Moreover, our study has been performed on male patients older than 70 years with high prevalence of arterial hypertension, two observations that could be considered as confounding factors. Given the increasing risk of MI in women, it could be also interesting in the future to perform similar studies in this population, to identify possible sex-related differences.

Other T2D associated risk factors, such as hypertension, lower high density lipoprotein concentrations, or altered fasted glucose could be responsible for the clinical outcomes and higher mortality after MI in subjects with T2D. However, in our study population, these metabolic parameters were not statistically different between D and ND subjects. The only different parameter was the HbA1c, which was higher, as expected, in D subjects (Table 1). Different studies already reported that elevated HbA1c levels increase the risk of cardiac death, cardiovascular disease and stroke [34], and are associated with the severity of CAD

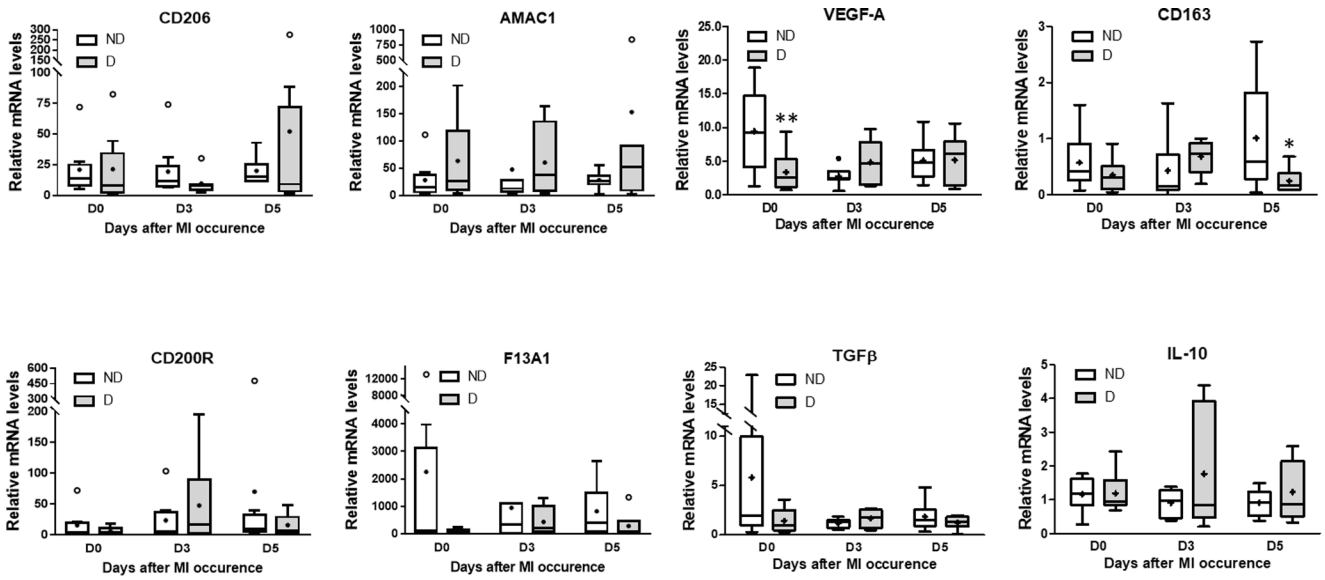

Fig. 3. Type 2 diabetes has no impact on the anti-inflammatory macrophage polarization after a MI event. PBMC were isolated from subjects without (ND) and with T2D (D), within the first 24 h (D0), and after 3 (D3) and 5 (D5) days after MI occurrence. Monocytes were differentiated in the absence or in the presence of IL-4 to obtain anti-inflammatory macrophages. The mRNA levels of anti-inflammatory markers (CD206, AMCA1, VEGF-A, CD163, CD200R, F13A1, TGFβ and IL-10) were measured by Q-PCR. The relative expression of each gene was calculated as described above, compared to the unpolarized macrophages set as 1 at each time point and represented in boxplots indicating the median (bar), the mean (dot) and the lower and the upper quartiles. Circles indicate outliners.*: $p \leq 0.05$; **: $p \leq 0.01$.

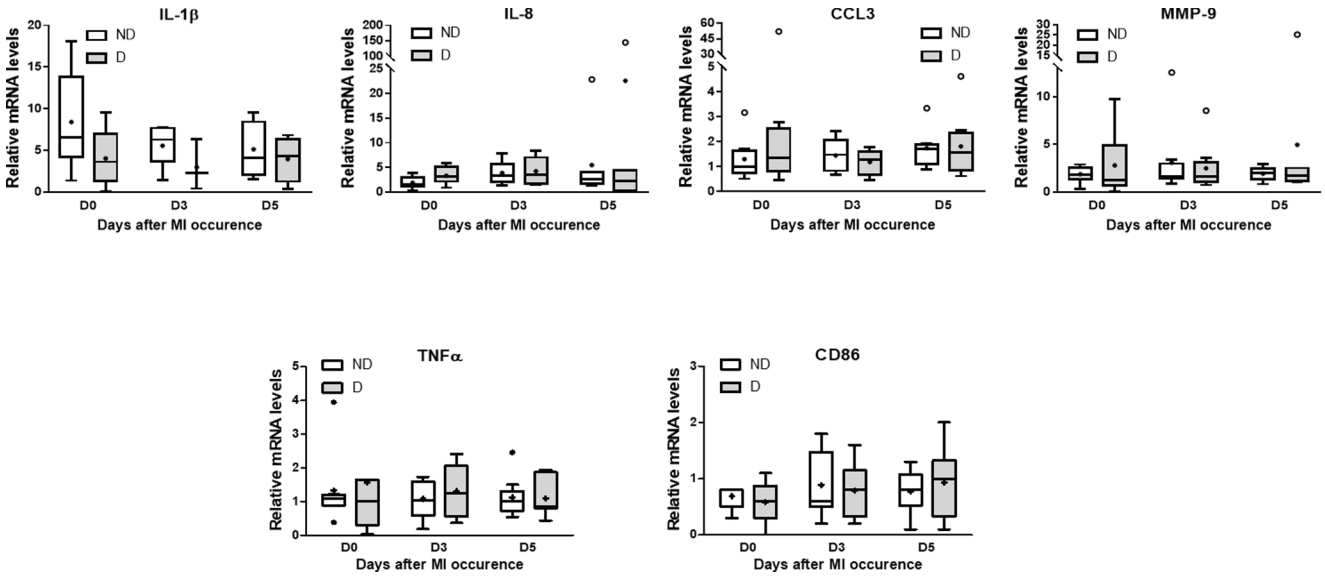

Fig. 4. Type 2 diabetes does not influence the pro-inflammatory macrophage polarization after a MI event. PBMC were isolated from subjects without (ND) and with T2D (D), within the first 24 h (D0), and after 3 (D3) and 5 (D5) days after MI occurrence. Monocytes were differentiated in the absence or in the presence of IL-1β to obtain pro-inflammatory macrophages. The mRNA levels of pro-inflammatory markers (IL-1β, IL-8, CCL3, MMP-9, TNFα, and CD86) were measured by Q-PCR. The relative expression of each gene was calculated as described above, compared to the unpolarized macrophages set as 1 at each time point and represented in boxplots indicating the median (bar), the mean (dot) and the lower and the upper quartiles. Circles indicate outliners.

[35].

Further studies will be necessary to understand the real contribution of macrophages after MI in humans, and to extend our results to a larger population of diabetic and non-diabetic subjects, thus allowing to fill the gap between current knowledge and immunotherapeutic strategies.

CRediT authorship contribution statement

Claudine Moratal: Investigation, Data curation, Writing – original draft. **Joseph Murdaca:** Investigation, Data curation. **Coralie Cruzel:** Formal analysis. **Amina Zamiti-Smondel:** Resources. **Nathan Heme:** Resources. **Florian Asarisi:** Resources. **Jaap G. Neels:** Funding acquisition, Data curation, Writing – review & editing. **Emile Ferrari:** Conceptualization, Resources, Writing – review & editing. **Giulia Chinetti:** Conceptualization, Funding acquisition, Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Registration number of clinical studies

The study, registered on the "clinicaltrials.gov" website under the number NCT02768935, was approved by the "Comité de Protection des Personnes Sud-Méditerranée", Ethics Committee (protocol 2016-A00793-48).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2023.101309.

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