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ORIGINAL RESEARCH - PRECLINICAL

Deleterious Anti-Inflammatory Macrophage Recruitment in Early Post-Infarction Phase

Unraveling the IL-6/MCP-1/STAT3 Axis

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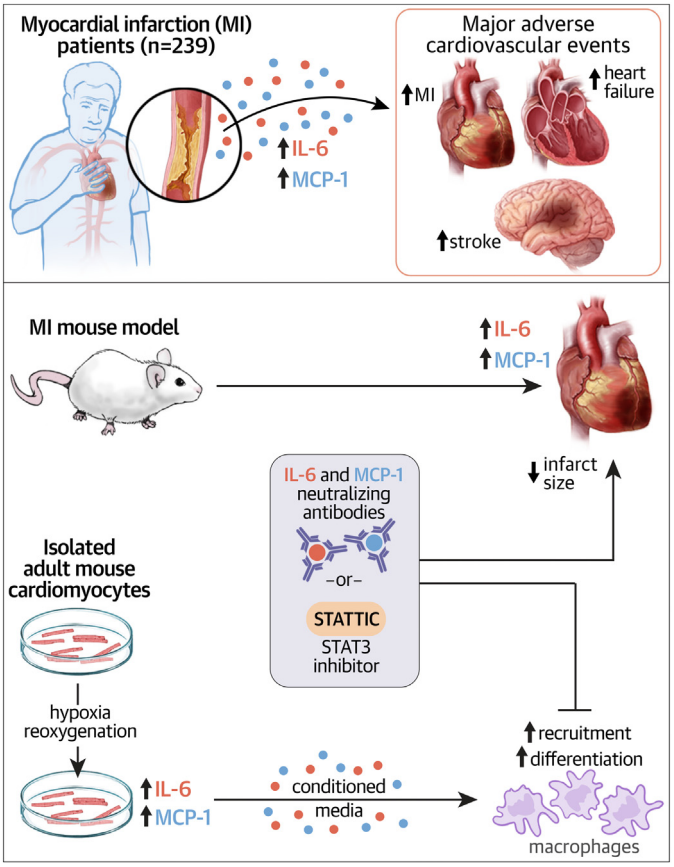
VISUAL ABSTRACT



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HIGHLIGHTS

- **MCP-1 and IL-6 blood levels peak at 24 hours in STEMI patients and are associated with an increased risk of MACE.**
- **Blood levels of MCP-1 and IL-6 peak at 3 hours in a mouse model of STEMI. The mRNA level of both cytokines was increased in the ischemic heart, suggesting a local production.**
- **Cardiac myocytes produce MCP-1 and IL-6 that play a synergistic role in macrophage recruitment and polarization via STAT3 signaling pathway.**
- **Use of anti-IL-6 and anti-MCP-1 neutralizing antibodies mix or a inhibitor of STAT3 pathway (STATTIC) at the onset of reperfusion reduces infarct size in a mouse model of STEMI.**
- **This study highlights the potential deleterious role of anti-inflammatory macrophages recruitment during the early phase of myocardial infarction.**

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ABBREVIATIONS AND ACRONYMS

CMA = adult cardiac myocyte

ELISA = enzyme-linked immunosorbent assay

HR = hypoxia-reoxygenation

IL = interleukin

IR = ischemia-reperfusion

JAK = Janus kinase

MACE = major adverse cardiovascular events

MCP = monocyte chemoattractant protein

MI = myocardial infarction

PCI = percutaneous coronary intervention

STAT3 = signal transducer and activator of transcription 3

STEMI = ST-segment elevation myocardial infarction

SUMMARY

Using a translational approach with an ST-segment myocardial infarction (STEMI) cohort and mouse model of myocardial infarction, we highlighted the role of the secreted IL-6 and MCP-1 cytokines and the STAT3 pathway in heart macrophage recruitment and activation. Cardiac myocytes secrete IL-6 and MCP-1 in response to hypoxic stress, leading to a recruitment and/or polarization of anti-inflammatory macrophages via the STAT3 pathway. In our preclinical model of myocardial infarction, neutralization of IL-6 and MCP-1 or STAT3 pathway reduced infarct size. Together, our data demonstrate that anti-inflammatory macrophages can be deleterious in the acute phase of STEMI. (J Am Coll Cardiol Basic Trans Science 2024;9:593–604)

Despite recent advancements in post-myocardial infarction (MI) care, many patients continue to experience complications such as heart failure or stroke in the following years. MI has reportedly triggered sterile inflammation, potentially contributing to adverse effects. Circulating cytokines have been broadly studied for their potential role as prognostic/diagnostic biomarkers post-MI[1-4] because some of them, such as interleukin (IL)-6 or monocyte chemoattractant protein (MCP)-1, have been demonstrated to be correlated with worsening post-MI recovery.[5-10]

Encouraging outcomes have emerged using the IL-6 receptor inhibitor tocilizumab, exhibiting improved myocardial salvage index in ST-segment elevation MI (STEMI) patients,[11] as well as attenuated inflammatory response, primarily percutaneous coronary intervention (PCI)-related troponin-T release in non-STEMI patients.[12] Despite these promising results, it is noteworthy that these clinical trials targeted the IL6-receptor rather than directly addressing the cytokine IL-6 itself. Thus, a comprehensive understanding of IL-6 and MCP-1 roles in sterile inflammation occurring during MI is imperative for optimizing therapeutic approaches.

In the context of MI, it is reported that IL-6 and MCP-1 are released by cardiac fibroblasts or by resident, as well as circulating, immune cells, whereas cardiomyocytes are mainly described to release damage-associated molecular pattern molecules.[13] Although few studies have demonstrated the secretion of IL-6 and MCP-1 by adult cardiomyocytes (CMA) in normal conditions,[14] only 1 study using human atrial cardiomyocytes has shown IL-6 production.[15] However, no studies under hypoxia-reoxygenation (HR) conditions have investigated the secretion of IL-6 and MCP-1 by CMA. Both IL-6 and MCP-1 are recognized for attracting leukocytes to sites of damage or infection.[16-18] Among the downstream signaling pathways activated by MCP-1 and IL-6, the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway has been identified in nonischemic diseases[19,20] and serves as a key regulator of immune and inflammatory pathways.[21] JAK/STAT3 signaling has also been described to drive the polarization of macrophages. However, depending on the pathologies, either inhibition or activation of this pathway increases anti-inflammatory polarization.[22] Although a study on rats demonstrated that the inhibition of JAK/STAT3 leads to anti-inflammatory macrophage polarization in MI,[23] the role of this signaling pathway must be well understood. Macrophages' polarization is usually described as undergoing dynamic changes after reperfusion in MI, transitioning from a proinflammatory phase in the early days to a subsequent

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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anti-inflammatory phase starting around 3 to 4 days post-MI.[24,25] However, this dichotomic classification has been questioned by studies reporting that macrophage infiltration can be advantageous and deleterious depending on the kinetics of infiltration and on the activation states.[26,27]

In this study, we investigated whether high levels of IL-6 and MCP-1, along with the activation of their respective downstream signaling pathways, orchestrate macrophage recruitment and differentiation and exacerbate MI injuries. Initially, we establish a correlation between the circulating levels of IL-6 and MCP-1 and the occurrence of major adverse cardiovascular events (MACE) such as all-cause mortality, MI, hospitalization for heart failure or stroke within the first year post-STEMI. Using a murine model of MI and an in vitro coculture model, we showed that injured cardiomyocytes release IL-6 and MCP-1, consequently triggering the recruitment and differentiation of anti-inflammatory macrophages via the STAT3 signaling pathway. Finally, our study demonstrated that inhibiting IL-6/MCP-1/STAT3 pathways reduced the population of anti-inflammatory macrophages and decreased the infarct size.

METHODS

A HUMAN COHORT OF STEMI PATIENTS. Sera samples from patients of a previously described HIBISCUS cohort (coHort of patients to Identify Biological and Imaging markerS of CardiovascUlar outcomes in ST elevation myocardial infarction) was used.[1] Briefly, 239 patients were included with STEMI and underwent coronary angiography with a subsequent primary PCI for revascularization. The primary endpoint was all-cause of death, rehospitalization for heart failure, recurrent infarction, and stroke. MACE was assessed within the 12 months after the index hospitalization.

Clinical follow-up was prospectively recorded. MCP-1 and IL-6 concentrations were quantified using enzyme-linked immunosorbent assays (ELISA) and biochemical analyses (C-reactive protein and troponin I) were performed at the Hospices Civil de Lyon Laboratory. Further details are provided in the Supplemental Methods.

MOUSE MODEL OF ISCHEMIA-REPERFUSION IN VIVO AND TISSUE COLLECTION. All animal procedures were performed following the guidelines from Directive 2010/63/EU of the European Parliament of the protection of animals used for scientific purposes. The study was approved by the Lyon 1 Claude Bernard University ethics committee (N°20180322160064965). The in vivo model of ischemia-reperfusion (IR) developed in the laboratory was used to perform the study.[28] Details of the surgery procedure are described in the Supplemental Methods.

MOUSE CYTOKINES QUANTIFICATION. Circulating concentrations of IL-6 and MCP-1 in mouse sera stored at −80°C were quantified at different time points using ELISA according to the manufacturers' instructions (Invitrogen for IL-6 and R&D systems for MCP-1). The minimum detectable concentration is 0.666 pg/mL for MCP-1 and 4 pg/mL for IL-6.

HEART mRNA QUANTIFICATION. Mice (n = 8/group) were randomly assigned to 2 groups: a control group without ischemia (normoxia) and a second group corresponding to IR 1 hour. At T = 0 and T = 1 hour, mice were anesthetized and then euthanized. RNA extracted from hearts were used to quantify the expression of IL-6 and MCP-1 (Supplemental Methods).

IN VITRO HR ON CMA. The HR sequence was performed using a hypoxia incubator (Eppendorf Galaxy 48R). CMA were isolated from hearts as described in the Supplemental Methods. CMA were then incubated in 500 μL of hypoxia buffer (140 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, 10 mmol/L HEPES, 2 mmol/L $CaCl_2$) for 1.5 hours at 0.5% of oxygen. Following hypoxia, reoxygenation was performed by adding 1.5 mL of complete medium (DMEM F12, 10% fetal bovine serum, 1% penicillin-streptomycin). CMA then were placed in a normoxic incubator for 24 hours, and the conditioned medium was collected and directly frozen at −20°C until use.

IN VITRO MACROPHAGE PHENOTYPING. Macrophages isolated from bone marrow, as detailed in the Supplemental Methods, were cultured in 12-well plates at a concentration of 300,000 cells/mL for 7 days. Macrophages were stimulated with CMA conditioned medium diluted to one-half in complete medium for 24 hours. Macrophages were then harvested, and the cell suspension was stained for 30 minutes at 4°C with CD206 and CD86 (BioLegend, ref 141710 and 105043, respectively) at 1:100 in phosphate-buffered saline, 4% bovine serum albumin. Data were acquired on an LSR Fortessa X20 (BD Bioscience) and analyzed with FlowJo software (V10.8.0, Becton, Dickinson & Company).

Treatment with STATTIC inhibitor at 2.5 μmol/L (Sigma Aldrich, S7946)[29] or neutralizing antibodies against IL-6 at 0.3 μg/mL (R&D Biosystems, AF-406-NA) or against MCP-1 at 2.5 μg/mL (R&D Biosystems,

AF-479-NA) were performed at the same time as the stimulation with CMA conditioned medium.

IN VITRO MACROPHAGE RECRUITMENT BY CMA. For recruitment experiments, macrophages were cultured on Millicell hanging cell culture inserts (Merck, MCEP12H48) at 110,000 cells/mL for 7 days. After polarization in pro- or anti-inflammatory macrophages, recruitment experiments were conducted under a confocal microscope. Experiments are detailed in the Supplemental Methods.

STATISTICS. Continuous human data are expressed as median and 95% CI, median (IQR), or mean ± SD according to their distribution.

Within-group comparisons were performed using a paired *t*-test or Wilcoxon signed rank test, whereas differences between groups used the Wilcoxon rank sum test or unpaired *t*-test.

Kaplan-Meier methods (log-rank test) were used to compare MACE between groups. Cox proportional hazards models were used to obtain the HR and 95% CI for adjusted models predicting MACE.

For in vitro macrophage recruitment experiments, 2-way repeated measures analysis of variance with Sidak's post hoc test for multiple pairwise comparisons was performed. For in vitro macrophage activation experiments at 24 hours, 2-way repeated measures analysis of variance with Dunnett's post hoc test for multiple pairwise comparisons was performed. Linear regression was used to evaluate slope differences for infarct size. Statistical analyses were performed using GraphPad Prism 8.01 (GraphPad Software). A P value <0.05 was considered significant.

RESULTS

ELEVATED LEVELS OF IL-6 AND MCP-1 IN THE BLOOD POSITIVELY CORRELATE TO WORSE OUTCOMES AMONG STEMI PATIENTS. Supplemental Tables 1 and 2 provide an overview of the demographic characteristics of the study population (Supplemental Appendix). The mean age was 59 ± 12 years, and 79.5% of the cohort was composed of men. There were 160 patients with TIMI flow grades 0 or 1 at admission (66.9%), and 231 had TIMI flow grades ≥2 after PCI (96.7%).

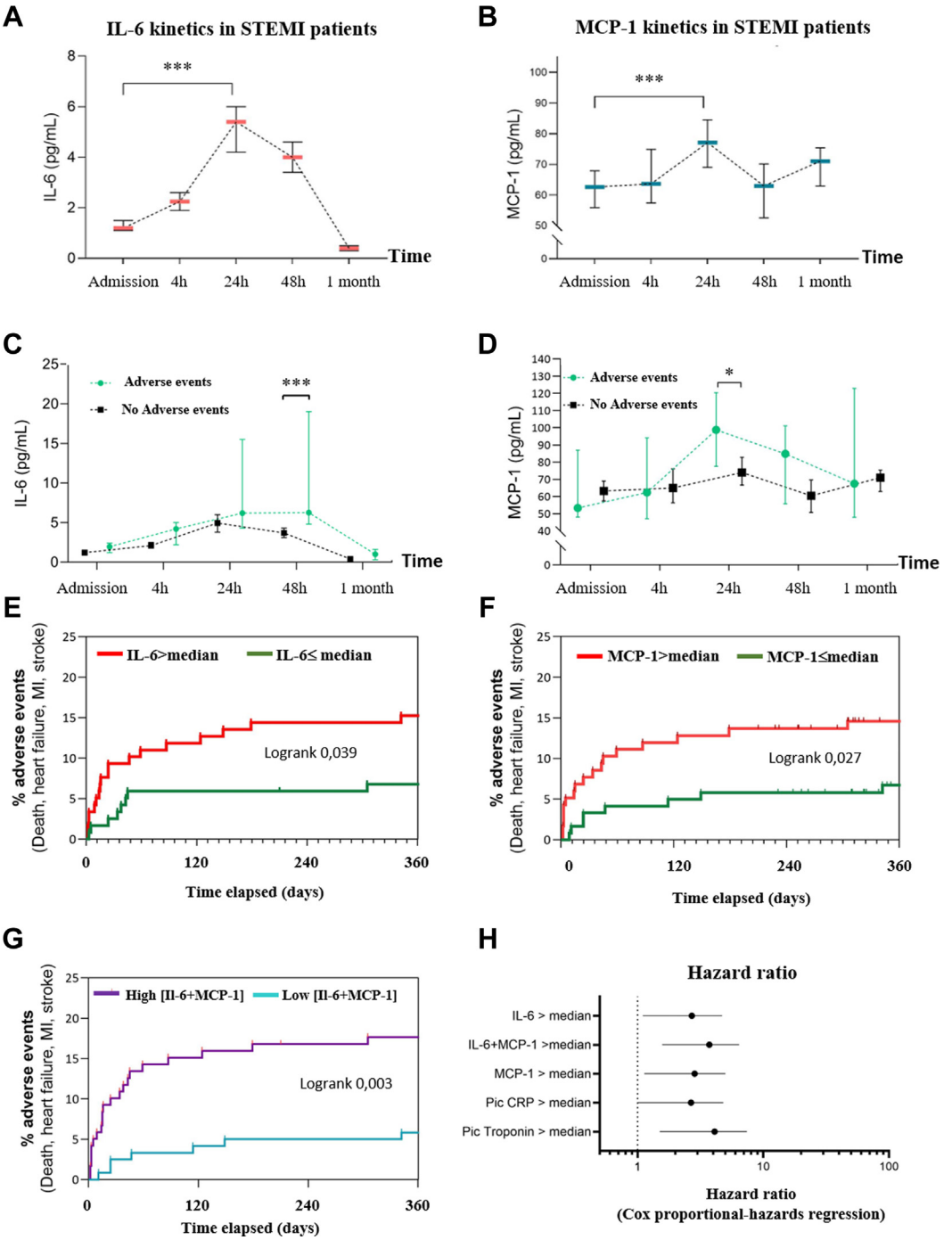
We first assessed the temporal variation of IL-6 and MCP-1 concentrations in STEMI patients' sera over the course of the first month. As illustrated in **Figure 1A**, the IL-6 level reached a peak at 24 hours, median value of 5.4 pg/mL (95% CI: 4.2-6.0 pg/mL), compared with the admission level value of 1.2 pg/mL (95% CI: 1.1-1.5 pg/mL; $P < 0.001$). Likewise, the circulating level of MCP-1 was also increased (**Figure 1B**) compared with the admission level with a median value of 77.1 pg/mL (95% CI 69-84.5 pg/mL; $P < 0.001$ at 24 hours).

Across the entire cohort, a total of 32 patients encountered MACE during the 12-month follow-up. As shown in **Figure 1C**, patients who experienced at least 1 adverse cardiac event within the 12-month follow-up period exhibited elevated blood IL-6 levels in their blood (median value of 6.3 pg/mL; 95% CI: 4.8-19 pg/mL) at 48 hours compared with patients without clinical outcomes (median value of 3.7 pg/mL; 95% CI: 3.1-4.3 pg/mL; $P < 0.001$). Additionally, blood MCP-1 levels were higher in patients who experienced MACE (median value of 98.7 pg/mL; 95% CI: 77.6-120.4 pg/mL) at 24 hours than in patients without MACE (median value at 74.0 pg/mL; 95% CI: 66.6-82.8 pg/mL) at 24 hours; $P = 0.021$ (**Figure 1D**).

When patients were dichotomized according to the median value of IL-6 or MCP-1 concentration in blood at 24 hours, patients with an IL-6 value above the median had a significantly larger infarct size and a reduced left ventricular ejection fraction than those with a low level of IL-6 (Supplemental Figures 1A and 1B). Moreover, MCP-1 level was not associated with significant differences neither in infarct size (Supplemental Figure 1C) nor in left ventricular ejection fraction (Supplemental Figure 1D).

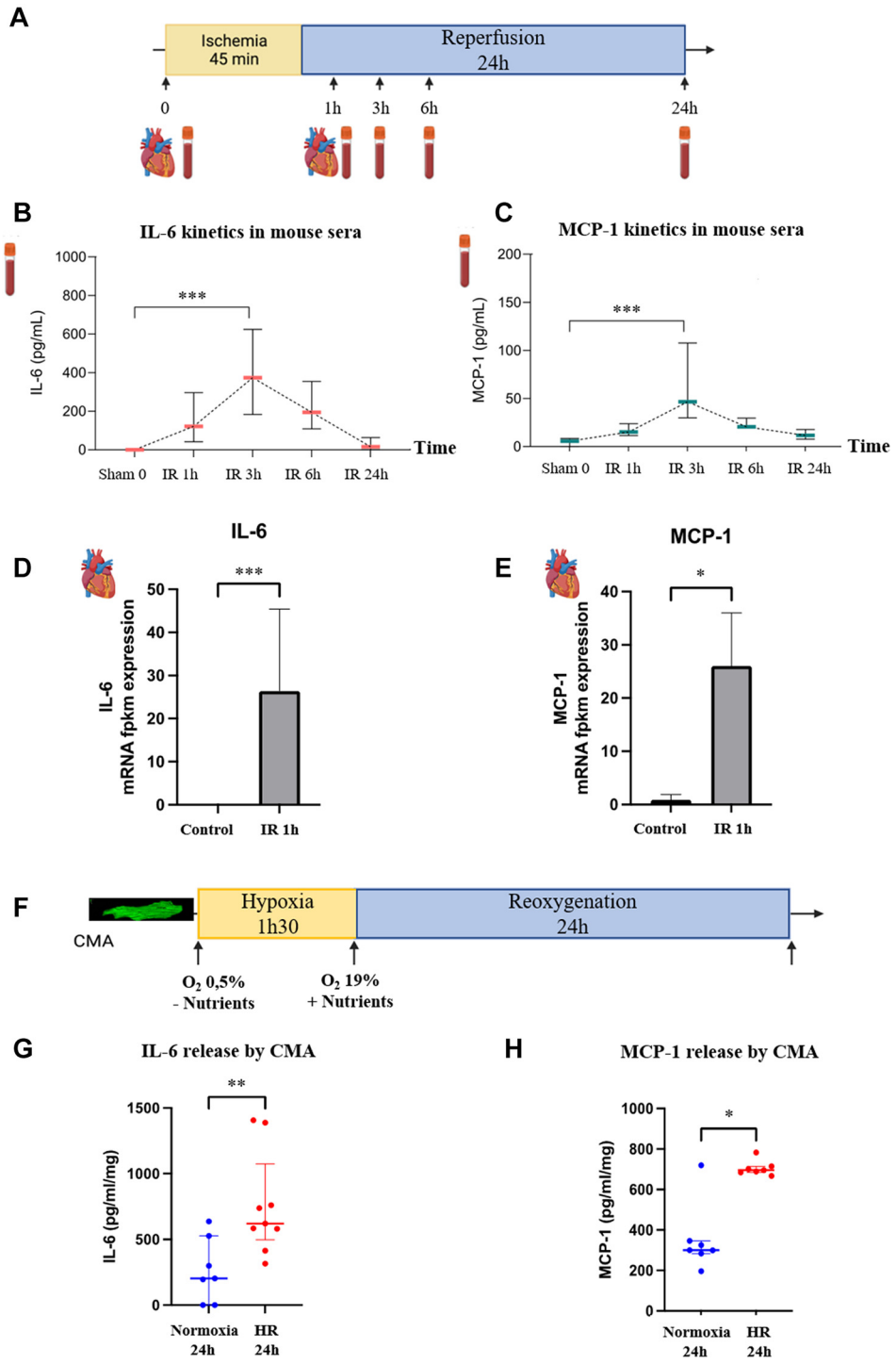
As displayed in **Figures 1E and 1F** for IL-6 and MCP-1, respectively, patients with IL-6 or MCP-1 levels that exceeded the median were observed to more likely experience MACE over the 12 months' follow-up (unadjusted HR: 2.3; 95% CI: 1.1-4.8; $P = 0.039$ and unadjusted HR: 2.4; 95% CI: 1.1-5.0; $P = 0.027$, respectively). With a Cox proportional hazards model incorporating IL-6 and MCP-1 levels at 24 hours, we successfully illustrated that patients with IL-6 or MCP-1 levels above the median were more at risk of experiencing an adverse cardiac event within the ensuing 2 years in contrast to patients with a level under the median value (HR: 3.2; 95% CI: 1.6-6.4; $P = 0.003$) as shown in **Figure 1G**. In a multivariable Cox regression model including age >65 years, sex, troponin peak level, TIMI flow grade >2 after PCI, ischemic time, and anterior MI, IL-6+MCP-1 serum level at 24 hours above the median value remained associated with an increased risk of experiencing the composite HR endpoint during the follow-up (adjusted HR: 5.3; 95% CI: 1.9-14.4; $P = 0.003$).

FIGURE 1 **IL-6 and MCP-1 Levels Peak 24 Hours After Admission for a STEMI and Are Associated With Adverse Clinical Outcomes**



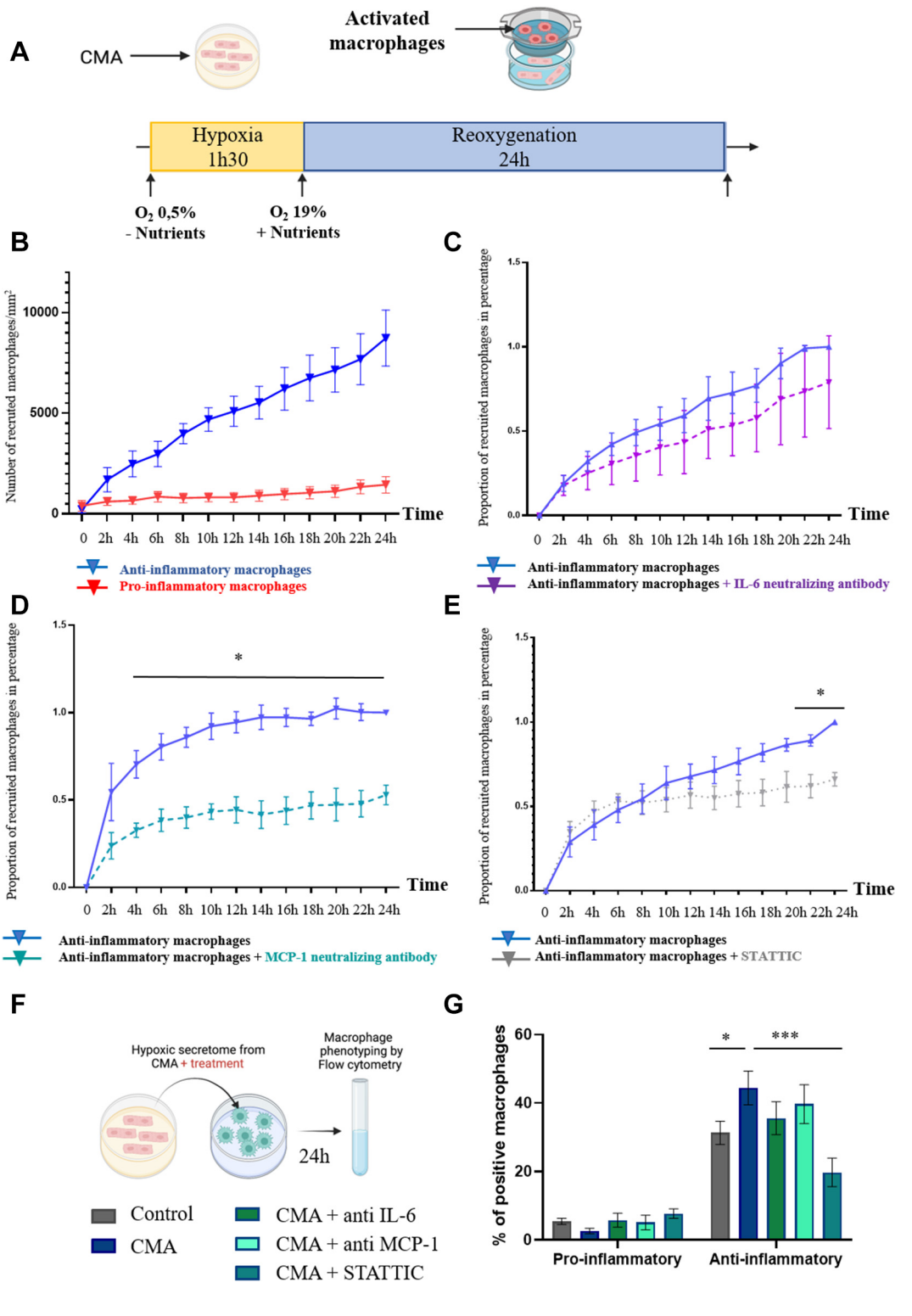
(A and B) Kinetics of IL-6 and MCP-1 concentrations in ST-segment elevation myocardial infarction (STEMI) patient sera measured by enzyme-link immunosorbent assay. (C and D) Major adverse cardiovascular events are presented according to IL-6 or MCP-1 serum levels. Data are presented as median with 95% CI. Wilcoxon rank sum test was used for statistical analysis. (E to G) Kaplan-Meier curves (log-rank test) are used to present adverse cardiac events according to the median concentration of IL-6 or MCP-1 independently or additionally at 24 hours. (H) Unadjusted HR and 95% CI for experiencing a composite endpoint during the median of 24 months of follow-up when having high IL-6 level (>median) or high MCP-1 (>median), both high IL-6 and MCP-1, elevated C-reactive protein (CRP >median), and high troponin peak (>median). The peak used is the maximum value of troponin, or CRP, measured for each patient individually. N = 239. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

FIGURE 2 **IL-6 and MCP-1 Levels Peak at 3 Hours in the Mouse STEMI Model**



(A) Experimental outline: peripheral blood and heart were collected at different time points of the ischemia-reperfusion (IR) sequence. (B and C) Kinetics of IL-6 and MCP-1 cytokine levels were quantified by enzyme-link immunosorbent assay from mouse sera after ischemia-reperfusion times; n = 3 to 8 mice. (D and E) IL-6 and MCP-1 mRNA expression expressed in fragments per kilobase million (fpkm) in the area at risk normalized by the healthy area. n = 3 to 8 mice. (F) Experimental outline, isolated cardiac myocytes adult (CMA) underwent in vitro hypoxia-reoxygenation (HR) sequence. Conditioned mediums were collected at 24 hours post-reperfusion. (G and H) IL-6 and MCP-1 levels in the conditioned medium were measured by enzyme-link immunosorbent assay under normoxia or HR. n = 7 to 9 independent experiments. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Data were represented as median + interquartile range. Wilcoxon rank sum test for unpaired data with Dunnett's post hoc test was used for statistical analysis. STEMI = ST-segment elevation myocardial infarction.

FIGURE 3 CMA Release IL-6 and MCP-1 in Response to HR Stress to Recruit and Differentiate Macrophages



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Interestingly, the HR indicated that the combined association of MCP-1 and Il-6 served as a better predictor of secondary events than either cytokine considered independently, to a similar extent as the troponin peak (Figure 1H).

Together, these results demonstrate that IL-6 and MCP-1 levels collected at 24 hours after STEMI are associated with worse clinical outcomes. However, mechanistic explanations were lacking, prompting us to utilize preclinical models of MI to address the question.

TEMPORAL SERUM AND EXPRESSION PROFILES OF IL-6 AND MCP-1 IN A MURINE MI MODEL. In a mouse model of MI, blood samples were collected at different time points as displayed in Figure 2A. Both IL-6 and MCP-1 median blood levels peaked 3 hours after reperfusion (373.7 pg/mL; Q1-Q3: 183.3-624.5 pg/mL; $P = 0.001$ and 46.6 pg/mL; Q1-Q3: 30.1-107.8 pg/mL; $P = 0.001$, respectively) when compared with sham 0 control (Figures 2B and 2C).

Furthermore, both IL-6 and MCP-1 mRNA median expression was significantly increased in the ischemic compared with the sham hearts (26.4; Q1-Q3: 11.0-45.4 vs 0.04; Q1-Q3: 0.03-0.04; $P < 0.001$ and 26.0; Q1-Q3: 15.4-36.0 vs 0.9; Q1-Q3: 0.8-1.9; $P = 0.011$, respectively) (Figures 2D and 2E).

IL-6 AND MCP-1 RELEASED BY INJURED CMA TRIGGER MACROPHAGE RECRUITMENT AND DIFFERENTIATION MACROPHAGES VIA ACTIVATING THE STAT3 SIGNALING PATHWAY. Using an in vitro model of isolated CMA subjected to HR sequence (Figure 2F), we observed an increase in both IL-6 (Figure 2G) and MCP-1 (Figure 2H) concentrations, quantified by ELISA, in comparison to the normoxic condition ($P < 0.010$ for IL-6 and $P < 0.050$ for MCP-1).

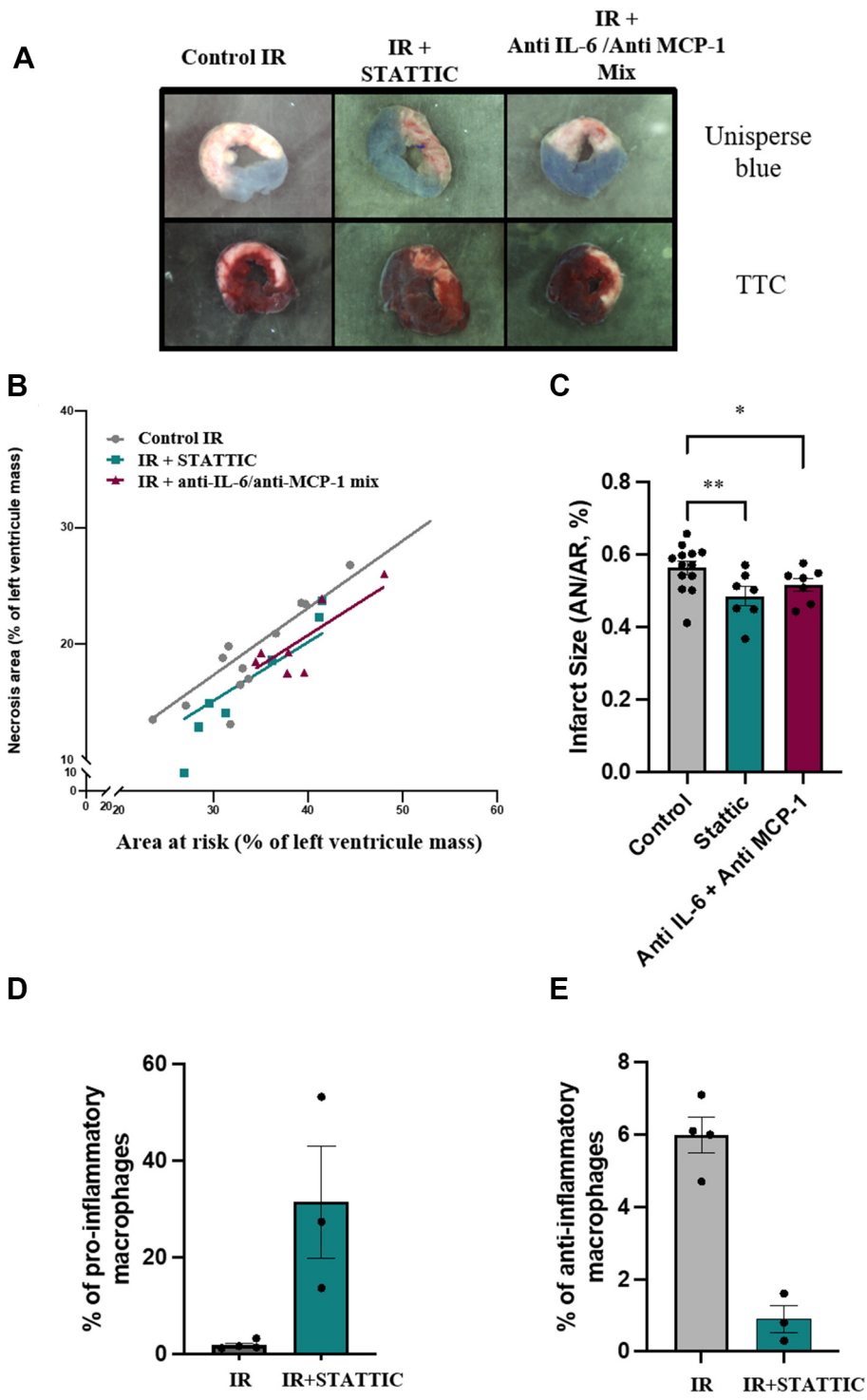
We tested whether IL-6 and MCP-1 could trigger macrophage recruitment using conditioned medium obtained from CMA exposed to 24 hours of HR in vitro (Figure 3A). We specifically measured the effect on pro- or anti-inflammatory macrophages following macrophages phenotype validation by flow cytometry (Supplemental Figure 2A). As illustrated in Figure 3B, in vitro injured CMA preferentially recruited anti-inflammatory macrophages (median 9,563 macrophages/mm^2; Q1-Q3: 5,240-11,737 macrophages/mm^2) compared with proinflammatory macrophages (median 1,519 macrophages/mm^2; Q1-Q3: 52-2,250 macrophages/mm^2; $P < 0.001$). This effect was partially inhibited by neutralizing antibodies against IL-6 or MCP-1. The IL-6 neutralizing antibody led to a 32.1% reduction of anti-inflammatory macrophage recruitment by CMA at 24 hours (Figure 3C), without impairing proinflammatory macrophage recruitment (Supplemental Figure 2B). Meanwhile, the MCP-1 neutralizing antibody led to a 44.2% reduction of anti-inflammatory macrophages ($P = 0.002$) and 29.8% reduction of proinflammatory macrophages ($P = 0.005$) (Figure 3D, Supplemental Figure 2C). The inhibition of the STAT3 signaling pathways by the STATTIC antagonist reduced anti-inflammatory macrophage recruitment at 24 hours ($P = 0.044$) (Figure 3E), whereas it had no significant effect on proinflammatory macrophage recruitment (Supplemental Figure 2D). These results suggest that IL-6- and MCP-1-mediated anti-inflammatory macrophage recruitment mostly relies on STAT3-dependent pathways.

Subsequently, we treated naive macrophages (M0) with conditioned medium obtained from injured CMA with or without neutralizing antibodies (anti-IL-6 and anti-MCP-1) or STATTIC (Figure 3F). The conditioned medium specifically stimulated the differentiation of anti-inflammatory (CD86$^-$, CD206$^+$) macrophages compared with the control medium (Figure 3G). This effect was slightly mitigated by the presence of the neutralizing antibodies (19.84%; $P = 0.1316$ for IL-6 and 11.3%; $P = 0.6611$ for MCP-1).

FIGURE 3 Continued

(A) Experimental outline of recruitment experiment. After hypoxia, a cell culture insert containing PKH-labeled macrophages was added to the CMA well for 24 hours. The dynamic recruitment of macrophages was followed and quantified by confocal microscopy. (B) Proinflammatory (blue line) vs anti-inflammatory (red line) macrophage recruitment by CMA in response to HR sequence. Data are expressed as numbers of macrophages/ mm^2. n = 10 independent experiments. (C to E) Effects of the presence of neutralizing antibody against IL-6 (C), MCP-1 (D) or STATTIC inhibitor on the recruitment of anti-inflammatory macrophages when they are added in the medium at reoxygenation time. n = 4 independent experiments. (F) Experimental outline of differentiation experiment. Neutralizing antibodies or STATTIC inhibitor was added at the time of macrophages stimulation with the conditioned medium. (G) Characterization of macrophage phenotype following the treatments by flow cytometry. Data are presented as median + SEM. For statistical analysis, 2-way repeated measures analysis of variance with Sidak's post hoc test (B to E) and Wilcoxon rank sum test for unpaired data with Dunnett's post hoc test (G). *$P < 0.05$, **$P < 0.01$, and ***$P <$ 0.001. Abbreviations as in Figure 2.

FIGURE 4 The Early Recruitment of Anti-Inflammatory Macrophages Is Deleterious in Vivo During the First Hours of MI



(A) Infarct size quantification using Unisperse blue to measure perfused area followed by triphenyl tetrazolium chloride (TTC) staining to measure necrosis area after treatment by STATTIC or the mix of IL-6 and MCP-1 neutralizing antibodies. (B and C) Effect of treatments on infarct size are represented by plotted necrosis area as a function of the area at risk (expressed as a percentage of left ventricular mass) or expressed by a ratio of necrosis area on area at risk in percentages. n = 8 to 10 per group. (D and E) Impact of STATTIC treatment on the phenotype of macrophages evaluated by flow cytometry; $P = 0.057$ (D and E). Data are presented as median + SEM. Mann-Whitney test with Sidak's post hoc test was used for statistical analysis *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Abbreviations as in Figure 2.

In comparison, STATTIC more efficiently reduced macrophage polarization (56%; $P < 0.001$) (Figure 3G).

REDUCING THE RECRUITMENT AND ACTIVATION OF ANTI-INFLAMMATORY MACROPHAGES AT THE ONSET OF REPERFUSION DECREASED INFARCTION SEVERITY. Combined anti-IL-6 and anti-MCP-1 neutralizing antibodies or STATTIC treatment were injected 5 minutes before reperfusion. Infarct size quantification was performed after 24 hours of reperfusion (Figure 4A). The distribution of infarct size as a function of the area at risk was slightly, but significantly, decreased in the groups treated with STATTIC or the combination of neutralizing antibodies compared to control IR ($P = 0.0061$ and $P = 0.0081$, respectively) (Figure 4B). Calculation of the infarct size as a ratio of area of necrosis over area at risk confirmed a significant effect of both STATTIC and neutralizing antibody treatment; $P = 0.0148$ and $P = 0.0484$, respectively (Figure 4C). These results demonstrate that IL-6, MCP-1, and the STAT3 signaling pathway are involved in worsening reperfusion injuries.

The isolation of immune cells from area at risk from ischemic hearts provided further validation that the STATTIC treatment seems to increase the proportion of proinflammatory macrophages while efficiently decreasing the number of anti-inflammatory macrophages (Figures 4D and 4E).

DISCUSSION

The present study demonstrates that IL-6 and MCP-1 blood levels positively correlate with worsened outcomes in STEMI patients. This cytokine release at a systemic level is partly due to the capacity of injured CMA to release IL-6 and MCP-1 in the acute phase of MI. Our study highlights the substantial contribution of IL-6, MCP-1, and the STAT3 pathway in both the recruitment and the activation of anti-inflammatory macrophages. Consequently, our findings unveil an unexpected perspective where rapid activation of anti-inflammatory macrophages is detrimental in the context of MI.

Indeed, 1 study[9] has described that the time course of 3.5 days induced considerable variability concerning the kinetics we describe in the present study. Within our investigation, we observed that the peak of blood MCP-1 concentration occurred 24 hours post-MI and reverted to its basal level after 48 hours. It is worth noting that a single blood sampling within these first 3.5 days would have missed the variation in MCP-1 level. However, this study has also described that an elevated level of MCP-1 is associated with MACE.

Only 1 translational study assessed MCP-1 levels in both mice and 21 human MI samples.[14] This study showed that the heart produces MCP-1 in healthy states and post-MI conditions. However, the investigators chose an animal model of permanent ligation which, in the absence of reperfusion, was not representative of STEMI patients.

Regarding IL-6, a canine model of MI showed upregulation of IL-6 mRNA in the myocardium following MI.[30] In dobutamine stress echocardiography, IL-6 has been associated with an increased risk of cardiovascular events in patients with coronary artery disease,[31] and IL-6 is also correlated with hemodynamic impairment after injection of dobutamine in patients with chronic heart failure.[32] Elevated IL-6 levels have been described in unstable coronary artery disease and are associated with an increased risk of cardiac events and mortality.[33,34] Plasma levels of IL-6 was elevated after surgery in pulmonary venous effluents from 7 patients immediately and after uncomplicated coronary artery bypass grafting.[35] These studies show the elevation of IL-6 during a coronary artery disease.

The novelty of our study is presenting 5 sampling times to obtain the kinetics for each cytokine. Moreover, the study considers only STEMI patients, not all causes of coronary artery disease. We highlight that the combined association of MCP-1 and Il-6 served as a better predictor of secondary events than either cytokine considered independently, to a similar extent as the troponin peak. Our methodology and selected models enabled us to unravel the reasons and consequences of the burst of IL-6 and MCP-1 in STEMI patients.

Data from our in vitro model show that injured CMA release IL-6 and MCP-1 in response to IR. Although the primary characterization of CMA revolves around their contractile function, their role in cytokine production remains poorly described. A limited number of studies have shown that myocytes can release IL-6,[15,36] but none has investigated this mechanism under hypoxic conditions. Our study is the first to demonstrate that CMA secrete both MCP-1 and IL-6 in response to an IR stress. Besides, we show a synergistic interplay between these 2 cytokines in terms of macrophage recruitment and activation. Indeed, MCP-1, and to a lesser extent IL-6, drives the recruitment of macrophages by CMA. In parallel, IL-6 and STAT3 participate in differentiating anti-inflammatory macrophages by

CMA. Much like preceding studies, we could not confirm STAT3 involvement in MCP-1-mediated recruitment of anti-inflammatory macrophages. However, we successfully demonstrate its involvement in the polarization of macrophages,[37-39] In vivo, our results reveal that STAT3 inhibition by STATTIC proves comparably efficacious to the dual inhibition achieved by anti-IL-6 and anti-MCP-1 neutralizing antibodies. Consistent with our in vitro results, the in vivo results indicate that the reduction of anti-inflammatory macrophages in the area at risk is mainly attributed to the inhibition of the IL-6/MCP-1/STAT3 pathways.

Surprisingly, our results challenge the conventional notion that anti-inflammatory macrophages are inherently beneficial while proinflammatory ones are deleterious. Instead, the kinetics of intervention are likely the key. Indeed, it has been shown that a decrease in proinflammatory macrophages, paralleled by an increase of anti-inflammatory macrophages 6 days post-MI, reduced fibrosis and increased cardiac function.[12] This may suggest a dynamic shift in the role of the anti-inflammatory macrophages where their function during early reperfusion phase differs from their role during the subsequent remodeling phase.

STUDY STRENGTHS AND LIMITATIONS. The strength of the present study lies in its translational approach. We have used a cohort of 239 well-characterized STEMI patients with a clinical follow-up combined with in vitro and in vivo preclinical models in mouse.

However, we can see some limitations in our study. Even if we consider that the rate at 1 month for each cytokine could indicate a reference close to basal level, the study lacks a control population. Moreover, the phenotype of anti-inflammatory macrophage subtypes is complex and could benefit from more refined characterization in future studies.

CONCLUSIONS

Our study demonstrates the significance of timing of therapeutic targeting as pivotal factors in the success of clinical studies aiming to target inflammatory processes. Furthermore, our experimental findings propose that the simultaneous targeting of multiple functions, encompassing both recruitment and differentiation, could yield synergistic effects. These insights shed light on potential avenues for refining therapeutic strategies in the context of inflammation-related interventions.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Targeting inflammation in cardiovascular disease could pave a new way in therapeutic treatment, but a better understanding of the phenomena remains necessary. Our present study describes the early kinetics of 2 cytokines highly involved in MI response: IL-6 and MCP-1. We demonstrate the highly dynamic release of those 2 cytokines within the first month post-STEMI. Elevated levels of IL-6 and MCP-1 24 hours post-admission are correlated to MACE. Using a preclinical model of STEMI, we demonstrated that IL-6 and MCP-1 kinetics are similar in mice. With our in vitro model, we show that cytokines are released by injured cardiac myocytes and induce anti-inflammatory macrophage recruitment and differentiation via the STAT3 signaling pathway. Counterintuitively, we demonstrate that this anti-inflammatory signaling in the acute phase of MI can be deleterious, highlighting once more the complexity of targeting inflammation in cardiovascular diseases.

TRANSLATIONAL OUTLOOK: Two important points for future clinical trials can be highlighted in this study. First, the precise description of the kinetics of IL-6 and MCP-1 can help in the design of future studies targeting those cytokines to define the right time of treatment. Secondly, our preclinical data suggest that synergistic approaches targeting several cytokines could be of interest to improve treatment efficacy.

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KEY WORDS IL-6, inflammation, macrophages, MCP-1, myocardial infarction, STAT3

APPENDIX For an expanded Methods section and supplemental figures and tables, please see the online version of this paper.