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Monocyte subset accumulation in the human heart following acute myocardial infarction and the role of the spleen as monocyte reservoir

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Aims Monocytes are critical mediators of healing following acute myocardial infarction (AMI), making them an interesting target to improve myocardial repair. The purpose of this study was a gain of insight into the source and recruitment of monocytes following AMI in humans.

Methods and results Post-mortem tissue specimens of myocardium, spleen and bone marrow were collected from 28 patients who died at different time points after AMI. Twelve patients who died from other causes served as controls. The presence and localization of monocytes ($CD14^+$ cells), and their $CD14^+CD16^-$ and $CD14^+CD16^+$ subsets, were evaluated by immunohistochemical and immunofluorescence analyses. $CD14^+$ cells localized at distinct regions of the infarcted myocardium in different phases of healing following AMI. In the inflammatory phase after AMI, $CD14^+$ cells were predominantly located in the infarct border zone, adjacent to cardiomyocytes, and consisted for 85% (78–92%) of $CD14^+CD16^-$ cells. In contrast, in the subsequent post-AMI proliferative phase, massive accumulation of $CD14^+$ cells was observed in the infarct core, containing comparable proportions of both the $CD14^+CD16^-$ [60% (31–67%)] and $CD14^+CD16^+$ subsets [40% (33–69%)]. Importantly, in AMI patients, of the number of $CD14^+$ cells was decreased by 39% in the bone marrow and by 58% in the spleen, in comparison with control patients ($P = 0.02$ and <0.001, respectively).

Conclusions Overall, this study showed a unique spatiotemporal pattern of monocyte accumulation in the human myocardium following AMI that coincides with a marked depletion of monocytes from the spleen, suggesting that the human spleen contains an important reservoir function for monocytes.

Keywords Acute myocardial infarction • Inflammation • Monocytes • Spleen • Bone marrow

Introduction

In patients with acute myocardial infarction (AMI), an adequate healing response is crucial for preserving left ventricular (LV) function and geometry, and thus preventing adverse LV remodelling.[1,2] Infarct healing is a complex and dynamic process, consisting of a replacement of necrotic myocardium with scar tissue, and is critically mediated by monocytes.[3–5] Accordingly, monocytes have recently drawn considerable attention as a target to improve post-AMI repair.[6,7]

Human peripheral blood monocytes are a heterogeneous pool of cells, consisting of at least two subsets, the $CD14^+CD16^-$ and $CD14^+CD16^+$ monocytes, which have unique characteristics with regard to phenotype and function.[8] Although circulating monocytes, once infiltrated into the infarcted myocardium, are generally referred

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to as macrophages, results from mice studies indicate that monocytes may have distinct fates.[9] That is, infiltrated monocytes, while pursuing their functions, may eventually differentiate into macrophages, emigrate from the site of injury, or die by apoptosis or necrosis, and are rapidly replenished by newly recruited monocytes. It has been recently shown, after coronary ligation in mice, that a large proportion of these newly recruited monocytes is provided by the spleen, suggesting a unique extramedullary reservoir function for monocytes.[10]

While monocytes critically mediate infarct healing, there is a growing body of evidence, indicating that uncontrolled monocyte response may impair post-AMI healing and directly affect prognosis.[11,12] In AMI patients, blood levels of $CD14^+CD16^-$ monocytes peak at Day 3, whereas that of $CD14^+CD16^+$ monocytes peak on Day 5.[13] Several clinical studies reported that patients with high blood levels of $CD14^+CD16^-$ monocytes following AMI show poor functional outcome,[7,13] suggesting that excessive recruitment of particularly $CD14^+CD16^-$ monocytes may enhance post-AMI injury. However, it remains unclear whether the dynamic changes of monocyte subsets levels, observed in blood, reflect the cell's presence in the infarcted tissue.

So far, animal studies have provided important insights into the origin, source, recruitment, and functions of monocytes in post-AMI healing,[9,10,14,15] and clinical studies even provided a rationale for future therapeutic strategies.[7,13] However, histological data of patients are lacking. To address these issues, we performed detailed histological analyses of clinical autopsy materials to gain insights into the systemic monocyte response following AMI in patients.

METHODS

Patients, tissue collection, and processing

A total of 40 patients who were referred to the Department of Pathology, VU University Medical Centre (VUmc; Amsterdam, the Netherlands) for clinical autopsy were included in this study. Clinical autopsy was performed within 24 h after death. Twenty-eight patients were diagnosed at clinical autopsy with recent left ventricle AMI. All these patients showed macroscopical evidence for recent left ventricle AMI, as identified by lactate dehydrogenase (LDH) decolouration of the injured myocardial tissue (*Figure 1A*). Macroscopic heart slides were photographed, and the surface of the LDH decolourized area in relation to that of the left ventricle and septum was used to determine the percentage of infarct size. Furthermore, based on LDH decolouration, the infarct area was classified as subendocardial or transmural infarction. Twelve patients, who died from a cause not related to AMI, and thus, with macroscopic normal LDH staining, served as controls. All patients but one with evidence for sepsis, myocarditis, metastasized cancer, recent cerebrovascular accident, or recent pulmonary embolism were excluded, as these conditions are known to influence the number of monocytes in the myocardium, spleen, or bone marrow.[9,16–19] One patient died immediately after large pulmonary embolism in the proximal part of the pulmonary artery, indicative of sudden death, and was therefore included in the control group. Furthermore, lung tissue was examined for histopathological evidence of pneumonia at the time of death. The present study was conducted in accordance with the Declaration of Helsinki. The study protocol (CASIMIR) was approved by the Research Committee of the Department of Pathology of the VUmc. The use of autopsy material after completion of the diagnostic process is part of the patient contract in the VUmc.

Myocardial tissue specimens were obtained from the centre of the infarct area (left ventricle) in AMI patients, as identified by LDH decolouration (*Figure 1A*). In control patients, myocardial tissue specimens were obtained from the left ventricle. From each patient, also a tissue sample from a thoracic vertebral body (bone marrow) and from the subcapsular part of the spleen was collected, as this area was previously reported to be the site of a monocytic reservoir in mice.[10] Tissue specimens were formalin-fixed and paraffin-embedded for immunohistochemical analyses. Myocardial tissue specimens of 19 AMI patients were also snap frozen and stored at −196°C (liquid N2) for immunofluorescence analyses.

AMI patients were categorized into three phases of post-AMI healing based on microscopic criteria: the post-AMI early phase (macroscopically LDH decolouration but no extravasation of neutrophilic granulocytes in the infarct area; $n = 9$), the post-AMI inflammatory phase (extravasation

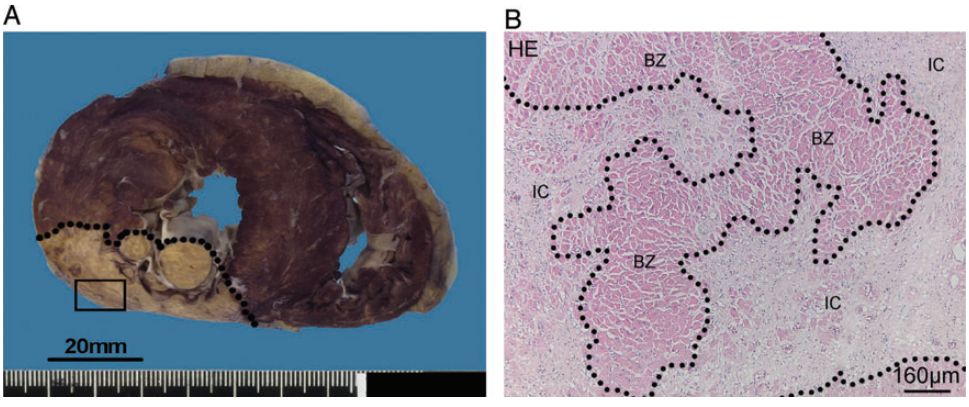

Figure 1 Identification of the infarct area by LDH decolouration, and the microscopical infarct core and border zone within the infarcted myocardial tissue. (*A*) Photograph of the LDH coloured myocardium. The dotted line outlines the LDH decoloured infarct area. The square indicates the centre of the infarct area (including the microscopical infarct core and border zone), wherefrom tissue was sampled. (*B*) Haematoxylin and eosin staining of infarcted myocardial tissue (post-acute myocardial infarction proliferative phase) was performed to identify two areas within the infarct area: the microscopical infarct core and border zone (×50 magnifications). BZ, border zone; IC, infarct core.

of neutrophilic granulocytes in the infarct area; $n = 9$), and the post-AMI proliferative phase (granulation tissue formation; $n = 10$), which correspond to an infarct age of ~3–12 h after AMI, 12 h–5 days after AMI, and 5–14 days after AMI, respectively.[20–22]

To identify multivessel disease, haematoxylin and eosin stainings of the three coronary arteries (left anterior descending artery, left circumflex artery, and right coronary artery) were used to microscopically determine the rate of stenosis in the artery. Patients who contained two or three coronary arteries with >50% stenosis were classified as containing multivessel disease.

Immunohistochemistry

Deparaffinized and rehydrated sections of the myocardium, spleen and bone marrow were incubated in methanol/H_2O_2 (0.3%) for 30 min to block endogenous peroxidases. Antigen retrieval was performed by heating in Tris–EDTA buffer (pH 9.0). Sections were then incubated with anti-human CD14 (1 : 40; clone 7, Novocastra, Newcastle Upon Tyne, UK). The immunostaining was revealed by using the EnVision Detection kit (Dako, Copenhagen, Denmark). Staining was visualized using 3,3′-diaminobenzidine (0.1 mg/mL, 0.02% H_2O_2), and sections were counterstained with haematoxylin, dehydrated, and covered. For the negative controls, the primary antibody was replaced by phosphate-buffered saline. These sections were all found to be negative.

Monocytes were identified as $CD14^+$ cells. Endothelial cells and neutrophils were found to stain negative for CD14. Stained myocardial tissue sections were scanned with a Mirax slide scanner system using a ×20 objective (3DHISTECH, Budapest, Hungary).[23] Numbers of $CD14^+$ cells were determined and equated for areas. Notably, in the infarct area of inflammatory phase infarcts and proliferative phase infarcts two areas can be identified. We defined the microscopical infarct core as the area consisting of necrotic tissue with infiltrating neutrophilic granulocytes in inflammatory phase infarcts and of granulation tissue in proliferative phase infarcts. The microscopical border zone was defined as the area adjacent to the microscopical infarct core, containing the viable cardiomyocytes (*Figure 1B*). In stained spleen tissue sections, numbers of $CD14^+$ cells were quantified per surface area, which was measured using Q-PRODIT (Leica, Cambridge, UK).[24] In stained bone marrow sections, numbers of $CD14^+$ cells were determined in a minimum of 10 high-power fields (×400 magnifications).

Immunofluorescence

Sections of frozen myocardial tissues were fixed in 3% paraformaldehyde (Sigma-Aldrich Co., St Louis, MO, USA), followed by incubation with 10% normal goat serum (Dako). Slides were incubated with the primary antibodies at 4°C: mouse anti-human CD14 [1 : 50; clone: M5E2 (IgG_{2a}); BD Pharmingen, San Diego, CA, USA], mouse anti-human CD16 [1 : 20; clone: 3G8 (IgG_1); Molecular Probes, Leiden, The Netherlands]; mouse anti-human α-actinin [1 : 100; clone: EA-53 (IgG_1), Sigma-Aldrich]; rabbit anti-human C3d (1 : 1000, Dako). Subsequently, slides were incubated with the appropriate secondary antibodies: goat anti-mouse IgG_{2a} Alexa Fluor 488; goat anti-mouse IgG_1 Alexa Fluor 647; goat anti-mouse IgG_1 Alexa Fluor 568; goat anti-rabbit IgG (H + L) Alexa Fluor 568 (all 1 : 100, Molecular Probes), and counterstained with Hoechst 33342 (1 : 1000, Molecular Probes).

Stained myocardial sections were examined under the Leica DM6000 microscope (Leica Microsystems, Heidelberg, Germany). Monocytes were identified as $CD14^+$ cells. Two subsets were distinguished by the expression of CD16. The proportions of $CD14^+CD16^-$ and $CD14^+CD16^+$ cells per area were determined in 5–10 microscopic fields (×200 magnifications). These proportions combined with the absolute number of $CD14^+$ cells, as measured by immunohistochemical analyses, were used to estimate the numbers of $CD14^+CD16^-$ and $CD14^+CD16^+$ cells in distinct areas.

Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences software (SPSS 16.0 for Windows, SPSS Inc.). The Fisher's exact test and the Freeman–Halton extension of Fisher's exact test were used for testing associations between categorical data. To test for differences between groups, the Kruskal–Wallis test or Mann–Whitney *U* test was used for continuous data, unless indicated otherwise. Linear non-parametric correlation was calculated using the Spearman correlation. Results were considered statistically significant if the two-sided *P*-value was <0.05.

Results

Patients

Samples of myocardium, spleen, and bone marrow taken from 28 patients who died after AMI were studied. The characteristics of these patients are summarized in *Table 1*. Mean age was 66 ± 13 years, 75% was male. Thirteen patients had a previous AMI, 6 were diagnosed with diabetes mellitus Type II, 2 with chronic kidney disease, and 11 had multivessel disease.

Unique spatiotemporal pattern of $CD14^+$ cell accumulation following AMI

To investigate the sequential accumulation of monocytes following AMI and their regional distribution, we first performed detailed immunohistochemical analyses of $CD14^+$ cells in the infarct area of patients who died at different time points after AMI (*Figure 2A* and *C*). In the early phase after AMI, the presence of $CD14^+$ cells in the infarct area was comparable with the control myocardium [infarct area: 3.5 (1.9–5.4) cells/mm^2; control: 5.0 (3.2–8.9) cells/mm^2; $P = 0.11$], indicating an absence of additional influx of $CD14^+$ cells early after AMI. Thereafter, in the inflammatory phase after AMI, $CD14^+$ cells predominantly accumulated in the infarct border zone, adjacent and also adherent to cardiomyocytes (*Figure 2B*), and to a much lesser extent in the necrotic infarct core [border zone: 63.9 (33.6–90.2) cells/mm^2; infarct core: 13.9 (4.8–20.5) cells/mm^2; $P = 0.007$]. In contrast, in the proliferative phase after AMI, large numbers of $CD14^+$ cells were almost exclusively present in the infarct core, consisting of granulation tissue at this stage of healing after AMI [infarct core: 149.4 (103.1–501.8) cells/mm^2; border zone: 20.4 (12.0–50.4) cells/mm^2; $P < 0.001$]. These data reveal a distinct spatiotemporal pattern of monocyte accumulation following AMI.

Accumulation of $CD14^+CD16^-$ and $CD14^+CD16^+$ cells following AMI

Monocyte subsets have been attributed diverse functions in the post-AMI healing process.[15] Therefore, we subsequently analysed the proportions of $CD14^+CD16^-$ and $CD14^+CD16^+$ cells in the $CD14^+$ cell infiltrate in the border zone in the inflammatory phase after AMI, and in the infarct core in the post-AMI proliferative phase (*Figure 3*). In the inflammatory phase after AMI, 85% (78–92%) of the $CD14^+$ cells in the infarct border zone were $CD14^+CD16^-$ cells. In contrast, in the post-AMI proliferative phase, comparable proportions of $CD14^+CD16^-$ [60% (31–67%)] and $CD14^+CD16^+$ cells [40% (33–69%)] were observed in the infarct core (*Figure 4A*). In *Figure 4B*, the absolute numbers of $CD14^+CD16^-$ and $CD14^+CD16^+$ cells are depicted.

Table 1 Patient characteristics

	Control ($N = 12$)	AMI ($N = 28$)				AMI vs. control *P*-value
		Early phase ($n = 9$)	Inflammatory phase ($n = 9$)	Proliferative phase ($n = 10$)	*P*-value	
Age (years)	59 ± 16	69 ± 19	66 ± 7	62 ± 10	0.46*	0.20**
Male gender	8 (67)	6 (67)	8 (89)	7 (70)	0.64	0.70
Medical history						
Previous AMI	0 (0)	6 (67)	2 (22)	5 (50)	0.18	0.004
Diabetes mellitus Type II	0 (0)	2 (22)	1 (11)	3 (30)	0.85	0.15
Peripheral arterial disease	1 (8)	2 (22)	1 (11)	1 (10)	0.82	>0.99
Cerebral vascular accident	1 (8)	1 (11)	1 (11)	0 (0)	0.52	>0.99
Chronic kidney disease	0 (0)	0 (0)	0 (0)	2 (20)	0.31	>0.99
Chronic obstructive pulmonary disease	2 (17)	1 (11)	0 (0)	2 (20)	0.75	0.63
Cancer	1 (8)	3 (33)	0 (0)	3 (30)	0.19	0.65
Primary reason of death						
Acute myocardial infarction	–	9 (100)	9 (100)	10 (100)	–	–
Arrhythmia	–	4 (44)	2 (22)	3 (30)	0.69	–
Heart failure	–	1 (11)	3 (33)	4 (40)	0.45	–
Vital bleeding	–	1 (11)	2 (22)	1 (10)	0.82	–
Other/unknown	–	3 (33)	2 (22)	2 (22)	0.87	–
Arrhythmia (not associated with AMI)	2 (17)	–	–	–	–	–
Acute aortic rupture/dissection	5 (42)	–	–	–	–	–
Acute large pulmonary embolism	1 (8)	–	–	–	–	–
Chronic obstructive pulmonary disease	1 (8)	–	–	–	–	–
Trauma	3 (25)	–	–	–	–	–
Sudden cardiac death outside hospital	–	1 (11)	0 (0)	0 (0)	0.64	–
Out-of-hospital cardiac arrest followed by in-hospital care	–	3 (33)	3 (33)	6 (60)	0.44	–
In-hospital death	–	5 (56)	6 (67)	4 (40)	0.57	–
Successful reperfusion therapy	–	0 (0)	5 (56)	1 (10)	0.01	–
Multivessel coronary artery diseases	1 (8)[a]	3 (33)	3 (33)[b]	5 (50)[a]	0.21	0.16
Extent of infarction (%)	–	36 ± 23[a]	49 ± 20	57 ± 19[c]	0.22*	–
Transmural infarction	–	6 (67)[c]	8 (89)	6 (60)[d]	0.81	–
Pneumonia	4 (33)	4 (44)	2 (22)	3 (30)	0.69	>0.99

Data are presented as mean ± SD or number (%) of patients.
AMI, acute myocardial infarction; h, hours.
[a]Analysis included six patients.
[b]Analysis included five patients.
[c]Analysis included seven patients.
[d]Analysis included eight patients.
*One-way ANOVA *P*-value.
**Student's *t*-test *P*-value.

CD14$^+$ cells in the spleen and bone marrow following AMI

Although monocytes originate in the bone marrow, recent experimental studies indicate that the majority of monocytes is recruited from the spleen following AMI in mice.[10] Because of this, we also investigated the numbers of CD14$^+$ cells in the bone marrow and the spleen. In the total group of AMI patients, we observed a 39% decrease of CD14$^+$ cells in the bone marrow and a 58% decrease of CD14$^+$ cells in the spleen, when compared with the control group ($P = 0.02$ and <0.001, respectively). *Figure 5A* and *B* shows the numbers of CD14$^+$ cells in the bone marrow and the spleen, stratified according to the three different phases of healing after AMI.

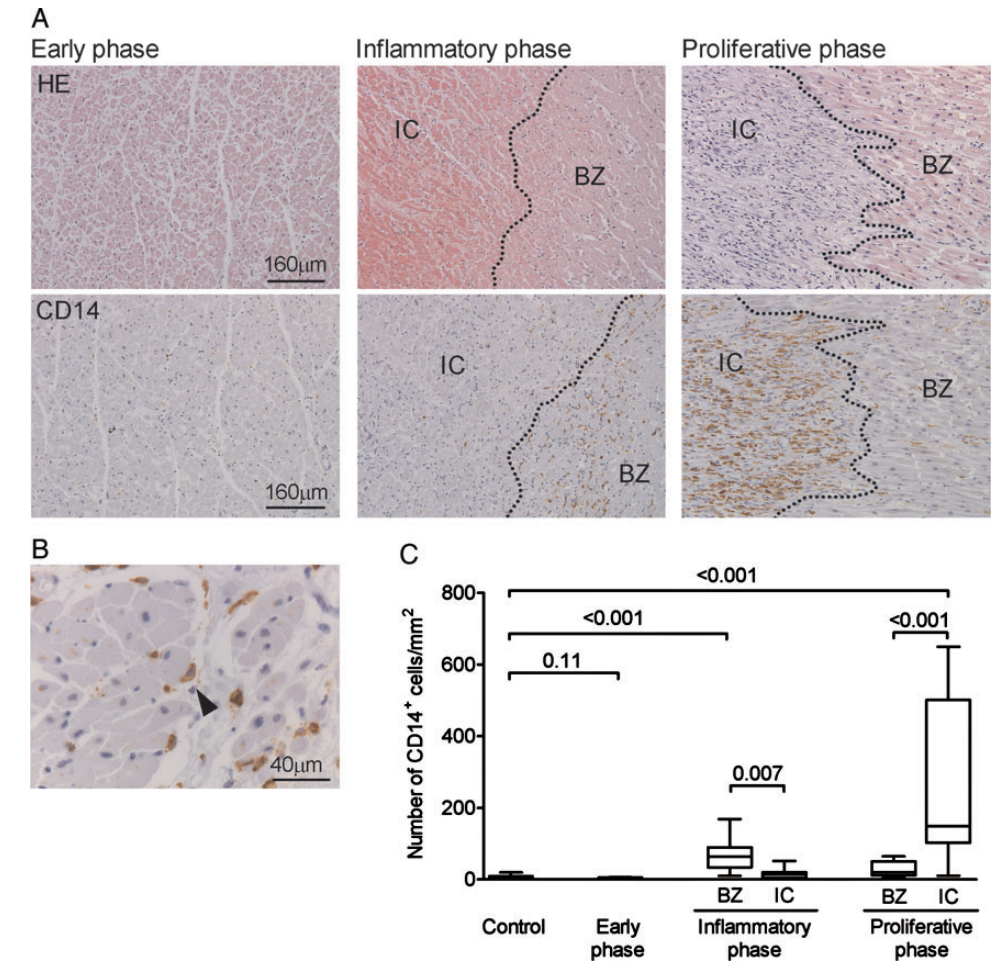

Figure 2 $CD14^+$ cells infiltrate distinct regions of the infarct area in different phases of healing after acute myocardial infarction. (*A*) Histology images of haematoxylin and eosin stainings (top row) and CD14 immunostainings (bottom row) of the infarcted area (×100 magnifications). In the inflammatory phase after acute myocardial infarction, $CD14^+$ cells predominantly infiltrate the infarct border zone, surrounding the necrotic infarct core. In the subsequent proliferative phase after acute myocardial infarction, $CD14^+$ cells are clustered in the infarct core, consisting of granulation tissue. (*B*) Magnification of the infarct border zone in the inflammatory phase after acute myocardial infarction (×400 magnifications), showing infiltrated $CD14^+$ cells adjacent and adherent to cardiomyocytes (arrow head). (*C*) Quantification of $CD14^+$ cells in different healing phases following acute myocardial infarction. Data are presented as box plot with median and 25th–75th percentiles (boxes) and 5th–95th percentiles (whiskers). BZ, border zone; h, hours; IC, infarct core.

Only in the spleen, the number of $CD14^+$ cells was significantly lower in all phases of healing after AMI, when compared with the control group, even in the early phase after AMI. Of note, no significant association was found between the extent of infarction and the number of $CD14^+$ cells in the spleen (Spearman's $r = 0.09$, $P = 0.69$) and the bone marrow (Spearman's $r = 0.02$, $P = 0.92$).

Discussion

Recent studies point to an important role of monocytes in post-AMI healing and adverse LV remodelling, making them an interesting target to improve myocardial repair.[6,7] To gain more insights into the source and recruitment of monocytes following AMI in patients, we conducted an autopsy study and found that $CD14^+$ cells accumulate in distinct regions of the infarcted myocardium in different phases of healing following AMI. We showed that $CD14^+$ cells, recruited in the post-AMI inflammatory phase (~12 h–5 days after AMI), predominantly accumulate in the infarct border zone and are located adjacent and also adherent to cardiomyocytes. In contrast, in the subsequent proliferative phase after AMI (~5–14 days after AMI), $CD14^+$ cells almost exclusively invade the infarct core, consisting of granulation tissue. Analysis of $CD14^+$ cell subsets showed an abundant presence of the $CD14^+CD16^-$ subset in the border zone in the inflammatory phase after AMI, whereas comparable proportions of $CD14^+CD16^-$ and $CD14^+CD16^+$ cells were present in the infarct core in the post-AMI proliferative phase. A decrease of $CD14^+$ cells was observed in bone marrow, but especially in the spleen following AMI, suggesting for the first time that the spleen may constitute an

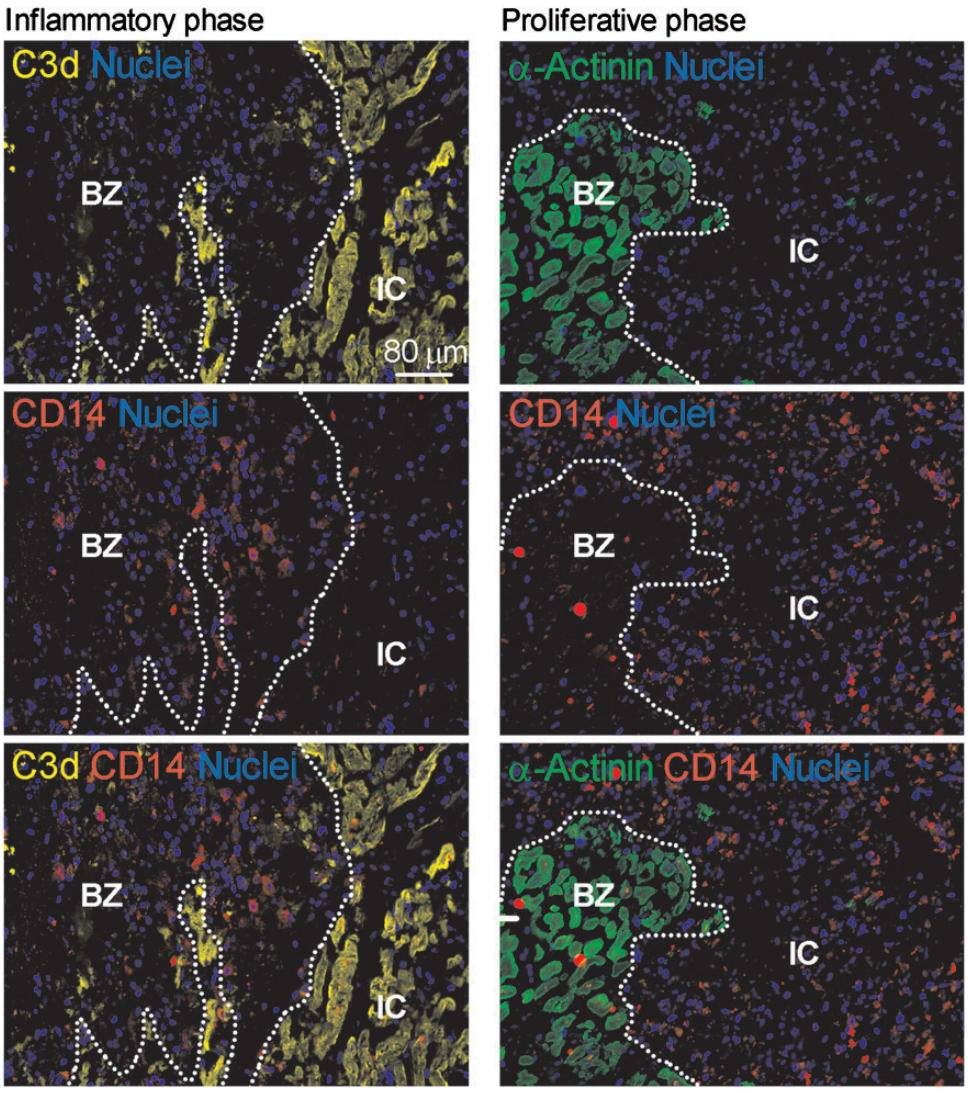

Figure 3 Co-localization of $CD14^+$ cells in different regions of the infarct area. In the inflammatory phase after acute myocardial infarction, $CD14^+$ cells (red) infiltrate the microscopical border zone, which is the area directly adjacent to the infarct core, consisting of the necrotic myocardium indicated by C3d (yellow). In the proliferative phase after acute myocardial infarction, $CD14^+$ cells (red) are located within the infarct core, consisting of granulation tissue, and surrounded by viable cardiomyocytes in the border zone, indicated by α-actinin (green). BZ, border zone; h, hours; IC, infarct core.

extramedullary reservoir of monocytes in humans. Taken together, these observations clarify the post-AMI monocyte response in patients and may provide new clues for treatment.

In the past decades, substantial progress has been made in understanding the complex roles of monocytes in healing after AMI. Tsujioka *et al.* measured the levels of both the $CD14^+CD16^-$ and $CD14^+CD16^+$ cells in the blood of AMI patients and found that the $CD14^+CD16^-$ subset peaks at Day 3 after AMI, whereas the $CD14^+CD16^+$ subset peaks at Day 5. The same group reported that post-AMI myocardial salvage was decreased in patients with high peak levels of circulating $CD14^+CD16^-$ cells.[13] It has been suggested that excessive accumulation of $CD14^+CD16^-$ cells following AMI may enhance myocardial inflammation, leading to infarct expansion and adverse LV remodelling.[6]

Our data now reveal that $CD14^+CD16^-$ cells are not only increased in the blood, but also in the infarct area. More importantly, in the inflammatory phase after AMI, we show that $CD14^+CD16^-$ cells primarily accumulate in the border zone adjacent but also adhering to cardiomyocytes, surrounding the necrotic infarct core. Phagocytosis of dead cells and debris is considered the major function of $CD14^+CD16^-$ cells during the post-AMI healing process. Therefore, our finding raises several questions: (i) why do $CD14^+CD16^-$ cells primarily invade the infarct border zone (and not the necrotic infarct core), and (ii) what is their biological role in this area?

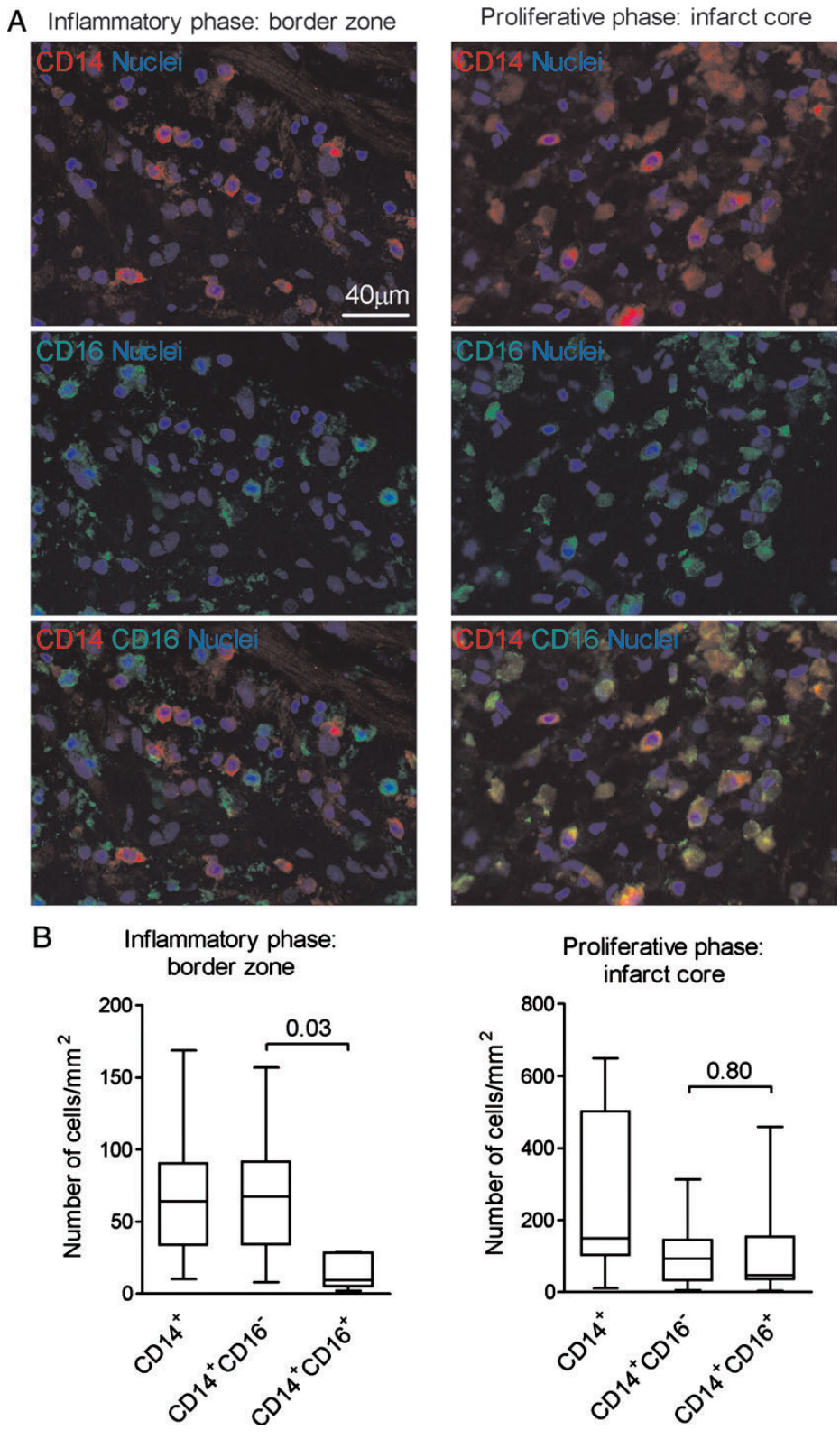

Figure 4 $CD14^+$ cell subsets in the myocardium following acute myocardial infarction. (*A*) Images of CD14 (red) and CD16 (green) double immunostainings of the infarcted myocardium at different phases of healing after acute myocardial infarction (× 400 magnifications). (*B*) Quantification of $CD14^+$, $CD14^+CD16^-$, and $CD14^+CD16^+$ cells in different phases of healing after acute myocardial infarction. Data are presented as box plot with median and 25th–75th percentiles (boxes) and 5th–95th percentiles (whiskers). H, hours.

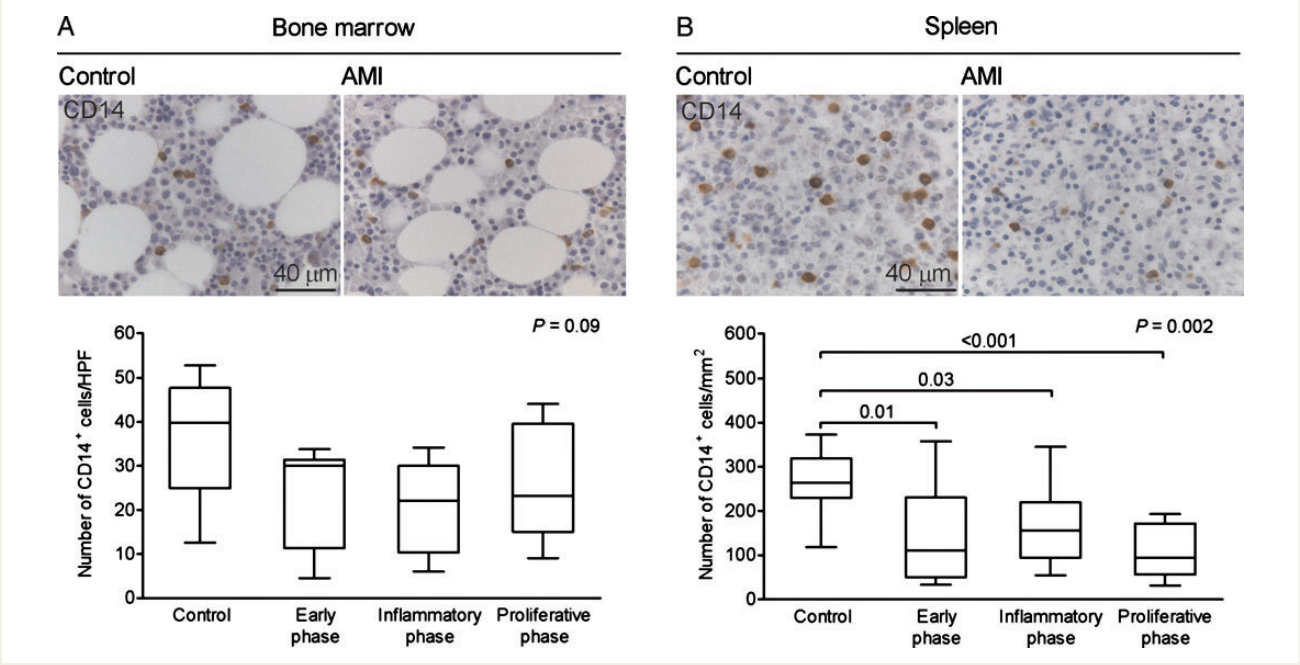

Figure 5 Presence of $CD14^+$ cells in the bone marrow and spleen after acute myocardial infarction. Histology images of CD14 immunostainings and quantification of $CD14^+$ cells in (*A*) bone marrow and (*B*) spleen in different phases of healing after acute myocardial infarction. Data are presented as box plot with median and 25th–75th percentiles (boxes) and 5th–95th percentiles (whiskers). HPS, high-power field; h, hours.

Particularly cardiomyocytes in the border zone are at risk for post-AMI apoptosis, due to inflammation.[25] In this regard, it is intriguing to speculate that $CD14^+CD16^-$ cells may contribute to the fate of the cardiomyocytes that survived the primary ischaemic period. As protection of viable cardiomyocytes following AMI is considered a 'holy grail' in cardiovascular medicine, future studies that unravel the role of $CD14^+CD16^-$ cells in the border zone are warranted.

The relevance of the $CD14^+CD16^+$ subset in infarct healing is less clear. $CD14^+CD16^+$ cells consist of multiple subpopulations with different characteristics.[26] No significant associations have been found between the blood level of $CD14^+CD16^+$ cells in AMI patients and parameters of functional outcome.[7,13] Our results show an abundant presence of $CD14^+CD16^+$ cells in the infarct core in the proliferative phase after AMI, at the same time as the previously reported increase of $CD14^+CD16^+$ cells in the blood.[13] Their location within the infarct core, more specific, in granulation tissue, supports a role for $CD14^+CD16^+$ cells in angiogenesis and subsequent scar formation. Of relevance, the proportion of $CD14^+CD16^+$ cells in the infarct core at this time after AMI of 40% (33–69%) is much higher than that of circulating $CD14^+CD16^+$ cells in the blood of ~10–15% reported in the clinical studies, [7,13] suggesting that $CD14^+CD16^+$ cells are selectively recruited, which may explain the lack of association between the level of $CD14^+CD16^+$ cells in blood and parameters of functional outcome in patients.

Monocytes were thought to originate in the bone marrow from haematopoietic stem cells and progenitor cells, and subsequently enter the circulation where they are made available to sites of tissue injury.[14,27] In support of this, we observed reduced numbers of $CD14^+$ cells in the bone marrow of AMI patients, indicating the release of monocytes in response to cardiac injury. Importantly, recent studies also point to the spleen as a major source of monocytes. In 2009, Swirski *et al.* showed that the murine spleen stores large amounts of monocytes, which can be recruited to sites of injury. In accordance with these studies in mice with coronary ligation, numbers of $CD14^+$ cells in the spleen of patients were profoundly decreased in all three phases of healing following AMI. Even in the early phase after AMI, numbers of splenic $CD14^+$ cells were already diminished, suggesting that the human spleen contains an important reservoir function for monocytes that can be rapidly deployed. This could also provide a possible explanation of why levels of monocytes in blood are already increased at hospital admission of patients with AMI or ischaemic stroke.[28] Interestingly, Swirski *et al.* also demonstrated in their preclinical studies that the release of splenic monocytes into the circulation is mediated by angiotensin II, and not the chemokine (C–C motif) receptor 2, which mediates the mobilization of monocytes from bone marrow,[10] suggesting that the mobilization of monocytes from the spleen depends on different cues compared with those from the bone marrow. Furthermore, recent studies have shown that the spleen not only stores and releases monocytes, but also supports post-AMI extramedullary monocyte production, further underscoring the role of the spleen in the monocyte response following AMI.[9,29] Hence, increased understanding of the mechanisms that regulate storage, production, and release of splenic monocytes in AMI patients may provide new perspectives for the development of therapeutic strategies that target the monocyte response to improve infarct healing.

Study limitations

There are several limitations to the present study. First, the results were solely observational in nature, and future clinical studies are necessary to confirm our findings, using advanced cell labelling and imaging techniques. Secondly, our sample size might be too small to detect small differences between groups. Thirdly, it cannot be excluded that findings of this autopsy study might be different in survivors of AMI, due to conditions/complications associated with death that may have influenced the systemic monocyte response. Fourthly, detailed pre-mortem clinical data were lacking. Fifthly, frozen tissue was only collected from the myocardium of 19 AMI patients, and not from the spleen and bone marrow. Notably, it was not possible to cut frozen bone marrow, because the bone needed to be decalcified. Therefore, $CD14^+$ cell subset analysis was performed only in the myocardium of AMI patients. Sixthly, in this study, it was not possible to distinguish monocytes from macrophages.

Conclusions

Overall, this study uncovers several novel aspects regarding the monocyte response following AMI in patients, such as the influx of $CD14^+CD16^-$ cells in the border zone in the inflammatory phase after AMI, surrounding the necrotic infarct core, and the presence of $CD14^+CD16^+$ cells in the granulation tissue (infarct core) in the subsequent post-AMI proliferative phase. Furthermore, this study for the first time suggests that monocyte recruitment to the site of infarction coincides with depletion of monocytes from the spleen, indicating that the human spleen contains an important reservoir function for monocytes, as previously shown in mice with AMI.[10] Future studies are needed to increase knowledge on the dynamics of monocyte subsets in the myocardium using advanced cell labelling and imaging techniques, and to increase understanding of their biological roles and the mechanisms that control monocyte recruitment following AMI in patients.

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Conflict of interest: J.J.P. is member of the medical advisory board of Abbott Vascular and consultant for Miracor.

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CARDIOVASCULAR FLASHLIGHT

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Huge left-ventricular pseudoaneurysm compressing coronary artery 10 weeks after stabbing attack

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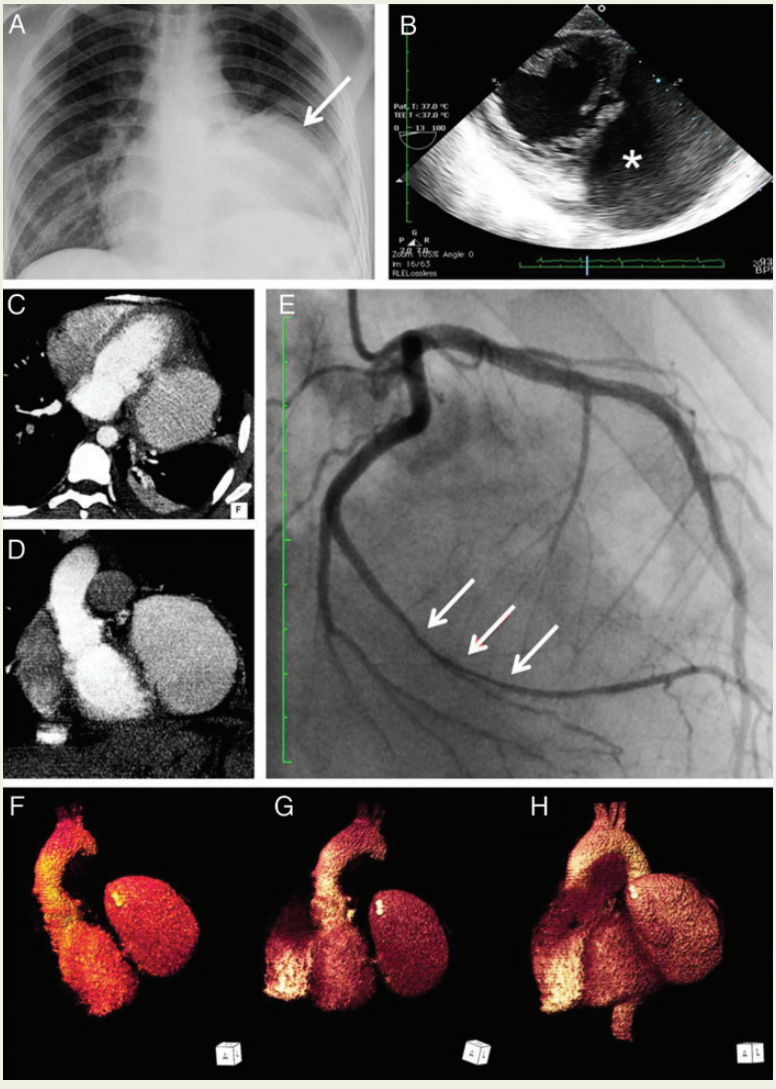

A 27-year-old male patient was referred 10 weeks following a stab wound to his left chest, for which he underwent an emergency-thoracotomy to repair a left lung-laceration and penetrating left-ventricular injury at the time. After an initial unremarkable recovery, he now re-presented with features of heart failure (NYHA III) and sepsis for which i.v. antibiotic therapy was initiated for a methicillin-resistant *Staphylococcus aureus* (MRSA) that was isolated from his blood cultures 1 week prior to referral. Chest X-ray (*Panel A*; arrow) and computed tomography (*Panels C* and *D* and *F–H*) revealed a 9.2 × 8.7 cm left-ventricular pseudoaneurysm. To delineate the coronary anatomy prior to aneurysm repair, coronary angiography (CA) was performed which excluded any traumatic coronary artery injury, but revealed displacement and compression of a large first obtuse marginal branch by the pseudoaneurysm (*Panel E*; arrows). Owing to ongoing sepsis, the patient was scheduled for urgent surgery. Intra-operative transoesophageal-echocardiography excluded any additional intra-cardiac pathology, confirming an impressive pseudoaneurysm (*Panel B*; asterisk). The right subclavian artery and femoral vein was cannulated and cardiopulmonary bypass instituted before sternotomy. The pseudoaneurysm was found to be infected, contained by the left lung and perfused via the old injury in the lateral left-ventricular wall (*Panels D*, *F*, and *G*). After thorough debridement, the neck was closed using interrupted mattress-sutures reinforced with teflon felt. The patient made an uneventful further recovery and completed 4 weeks of vancomycin for the MRSA that was also isolated from the intra-operative specimens. This rare case of left ventricular aneurysm formation after penetrating cardiac injury illustrates the importance and the necessity of serial CT or magnetic resonance imaging (MRI) follow-up imaging in such patients to prevent late cardiac complications.