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Research article - Histology and cell biology

Dendritic cells: a candidate cell in injury response to myocardial infarction and a possible diagnostic tool for sudden death

Stefano Bacci[1,*], Martina Focardi[2], Laura Pieri[2], Beatrice Defraia[2], Vilma Pinchi[2], Francesca Scavone[1], Gaia Paroli[1], Aurelio Bonelli[2], Paolo Romagnoli[1], Gian Aristide Norelli[2]

Departments of [1]Clinical and Experimental Medicine, and [2]Health Sciences, University of Florence, Italy

Abstract

Dendritic cells and mast cells are involved in the organization of inflammatory cell infiltrates in general, and in vascular wall in particular. The behaviour of these cells in myocardial infarction is still to be studied in detail. Myocardial samples were taken at autopsy from the left ventricle of subjects respectively affected by (1) acute myocardial infarction, (2) previous myocardial infarction, (3) coronary artery disease and (4) traumatic death assumed as controls. Tissue sections were stained with haematoxylin and eosin and immunohistochemistry; organ sections were also stained with triphenyltetrazolium. Loss of acidophilia and disappearance of nuclei and intercalated disks were found in acute infarction. Massive infiltration of dendritic cells was found in acute and previous infarction, while mast cell numbers were similar to controls. Localized lack of reactivity with triphenyltetrazolium, indicating lack of viable tissue, was observed only when autopsy was conducted within 48 h from death. The results indicate that: dendritic cells react early to myocardial injury; they may be regulators of the inflammatory and scarring response in this tissue; their increase may be a useful marker of acute myocardial infarction.

Key words

Forensic science, cell infiltrate, immunohistochemistry, mast cells, morphometry, triphenyltetrazolium.

Key to abbreviations:

DC: dendritic cell(s)
MC: mast cells
MI: myocardial infarction
SCF: stem cell factor
TTC: triphenyltetrazolium

Introduction

Cardiovascular diseases are the most frequent causes of death all over the world (Alwan, 2014) and among them sudden cardiac death is the leading one (Adabag et al., 2010). Conversely, data from sudden adult death series indicate that the majority of cases,

* Corresponding author. E-mail: stefano.bacci@unifi.it

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up to 80%, is due to cardiovascular disease (Zipes and Wellens, 1998; Huikuri et al., 2001; Zheng et al., 2001). A task force of the European Society of Cardiology has estimated an incidence between 36 and 128 sudden cardiac deaths per 100,000 people per year; more than 60% of these are the results of coronary artery disease (Priori et al., 2001) and indeed the incidence of sudden cardiac death varies largely as a function of coronary artery disease prevalence. This condition is defined as narrowing of at least one of the three coronary arteries by 75% or more because of an atherosclerotic plaque or thrombosis.

As myocardial infarction (MI) due to coronary artery disease is the main cause of sudden adult death, the forensic pathologist needs to know how to make such diagnosis post mortem, as sudden death may also depend on other causes such as arrhythmia due to congenital defects in intracellular adhesion and communication molecules or in ion channels (Chockalingam et al., 2015; Cunha and Mohler, 2015; Te Riele and Hauer, 2015), or to myocardial scars causing re-entry tachycardia (Fineschi et al., 2006). The loss of contraction of a myocardial region is the first functional change following MI. The tissue in flaccid paralysis is subjected to intraventricular pressure, with consequent early cardiac wall and myocellular stretching, shown by prominent I bands demonstrated at light and electron microscopy (Fineschi et al., 2006). This in turn causes extravascular compression of intramural vessels, blockage of intramyocardial blood flow, secondary wall degeneration, and platelet-fibrin thrombosis which prevents rescue in case of late revascularization. Basic findings required for the diagnosis of ischemic heart disease are the presence of a coronary thrombus or the severity and extent of stenosing atheromata, signs of acute infarction and the possible presence of myocardial scars (Fineschi et al., 2006). Microscopically, the earliest histological findings of MI affect myocardiocytes within 30-60 min of infarction onset: mild myofibre eosinophilia and elongation of sarcomeres and nuclei, with peripheral clumping of chromatin. However these alterations are unspecific and may be found as agonic changes independent of the cause of death (Frangogiannis et al., 2008). Nuclei further undergo progressive fading until nuclei disappear within 10 to 15 days.

The pathological diagnosis of early MI is especially challenging within six hours of survival, both macroscopically and by routine histology. Triphenyltetrazolium chloride (TTC) has been used to visually detect the infarcted area post mortem. It is reduced by dehydrogenase enzymes to triphenyltetrazolium formazan, which imparts a brick-red colour to intact myocardium. On the other hand, infarcted tissue, in which the enzymes are inactivated or lacking, appears as an unstained pale zone (Csonka et al., 2010). Regarding the use of TTC to detect MI, effective analysis of forensic samples depends on the time between the critical insult and death and on that between death and autopsy. It has been reported that TTC hardly detects MI with ischemia since less than nine hours and the staining ability of TTC decreases logarithmically within 1,5 days after death. Therefore this staining method is most often considered of little help for the diagnosis of MI (Kakimoto et al., 2013). Alternatively Ouyang et al. (2009) have proposed to use Masson's trichrome stain associated to desmin and myoglobin immunohistochemistry to detect acute myocardial ischemia or infarction at autopsy.

Emphasis has been posed on the demonstration of complement component C5b-9 (Ortmann et al., 2000; Campobasso et al, 2008; Fracasso et al., 2011), the loss of its inhibitor CD59, dephosphorylation of connexin 43 and re-localization of the latter molecule to the cytoplasm (Beardslee et al., 2000; Turner et al., 2004; Hesketz et al., 2010; Kawamoto et al., 2014), but these analyses are not yet commonly accepted for pathological diagnosis.

Humoural factors, in particular myocardial cell proteins leaked into serum, may be of use in the clinics and might be useful also in forensic pathology, provided blood samples are available and not affected by post-mortem alterations of these factors (Väkevä et al., 1994; Jaffe, 2006; Meng et al., 2006; Jenkins et al., 2010; Remmer et al., 2013; Ilczuk et al., 2014).

The association of inflammation with MI has been recognized for a long time and is a bridge between acute injury, healing and ventricular remodelling (Frangogiannis, 2008, Nah and Rhee, 2009). Neutrophils accumulate in the first 24 hours and are quickly accompanied and followed by monocytes, which differentiate into macrophages to eliminate dead cells and matrix debris (Nahrendorf et al., 2010; Chilvers et al., 2015; Turillazzi et al., 2015). Macrophages, most but not perhaps all derived from recruited monocytes, also contribute to regulate remodelling and stimulate fibrosis (Hulsmans et al., 2015), functions that may be played also by lymphocytes, in particular regulatory T cells (Hofmann and Frantz, 2016; Ramos et al., 2016).

Mast cells (MC) may also come into action. Beta-tryptase is a neutral serine protease and is the most abundant mediator stored in MC granules. In the clinics, serum beta-tryptase measurements can be used to distinguish MC reactions to heart ischemia from other systemic disturbances such as cardiogenic shock, which can present with similar clinical manifestations. Platt et al. (1994) demonstrated that tryptase levels were significantly higher in cases of sudden infant death syndrome than controls. However a correlation between the level of tryptase measured in serum and the activation of MC has not always been confirmed for humans (Edston and van Hage-Hamsten, 1998; Kervinen et al., 2005).

The presence of dendritic cells (DC) in the heart has long been known (Spencer and Fabre, 1990) and these cells are considered fundamental in rejection and tolerance upon transplantation (Startzl et al., 1993), in autoimmune heart disease (Marty and Eriksson, 2006) and in age-related fibrosis (Macri et al., 2012). These cells participate to injury response in the liver, arterial wall and skin (Gallè et al., 2001; Pieri et al., 2008; Bacci et al., 2014). The possible DC early response to an ischemic damage has yet to be analyzed in depth. Studies in man have shown that these cells infiltrate the myocardial tissue until the first day after MI, together with monocyte-macrophages and lymphocytes (Kretzschmar et al., 2012), and that the presence of DC rather than macrophages is correlated with correct tissue repair and reduction in the risk of aneurysmal evolution (Nagai et al., 2014). In experimental models, exosomes derived from DC may activate cardiac function upon infarction through activation of regulatory T lymphocytes (Liu et al., 2016; Wang et al., 2016).

Therefore, we have addressed the behaviour of DC in some selected cases as a step to understand their participation to injury response early upon myocardial infarction (no later than six hours after onset of symptoms) and to evaluate the possible use of analyses on these cells to recognize MI upon sudden death.

Material and methods

Cases

From the approximately 600 autopsies performed at the Service of Forensic Medicine of the University of Florence in years 2011–2014, the following cases were selected:

- 9 cases (3 females and 6 males; 34-74 year old, median 54.3) in which MI was indicated as cause of death. They were all cases with a well-defined clinical course (clinical symptoms, electrocardiographic and laboratory data), as proposed by clinical guidelines, in which the patients' survival time from first symptoms was less than 6 h and postmortem examination showed no macroscopic changes of heart muscle tissue nor other possible causes of death.
- 6 cases (1 female and 5 males; 40-84 years old, median 65) of traumatic death with macroscopic evidence of cardiac fibrosis (scarring). This group was designed to identify chronic changes due to previous infarction.
- 12 cases (4 females and 8 males; 61-94 year old, median 75.5) of traumatic death with evidence of coronary artery disease (acquired narrowing of at least one of the three coronary arteries by 75% or more). This group was designed to identify changes due to chronic coronary artery disease versus acute infarction.
- 10 cases (4 females and 6 males; 30-86 years old, median 50.4) of traumatic death without any sign of heart pathology, as healthy controls.

Triphenyltetrazolium staining

In each case, approximately 1 cm thick transverse sections of the heart, 1 to 2 cm below the atrioventricular sulcus, were taken for staining with 1% (w/v) 2,3,5-triphenyltetrazolium (Sigma, St. Louis, Mo) at 37°C for 40 min, while gently waving to obtain uniform staining.

Tissue specimens

The corpses were routinely kept at +4°C from arrival at the morgue until autopsy. The time interval between death and autopsy was usually within 48 h, average 6 h, while it was longer than 48 h in three cases of MI, two of coronary artery disease and five controls. In each case, tissue samples were routinely obtained from the left and right ventricles (anterior, lateral and posterior walls), septum (anterior and posterior half) and the first portion of coronary arteries; additional samples were taken from areas with macroscopic alterations.

Microscopy and histochemistry

In each group, some samples were fixed with 10% formaldehyde for more than 1 week and embedded in paraffin; 4-5 micron thick sections were stained with haematoxylin and eosin.

Other specimens were embedded in freezing tissue medium (Killik; BioOptica, Milan, Italy) and quick frozen at -80°C. Cryosections were post fixed in cold acetone and labelled (one section per staining) with primary antibodies against major histocompatibility complex II class molecules (MHC-II; mouse monoclonal, 1:100; Calbiochem, La Jolla, CA), as markers of putative DC. Fluorescein isothiocyanate labelled goat anti-mouse antibodies (1:32; Sigma) were used as secondary ones. Omission of the primary antibody and substitution with an irrelevant one were used as controls of immunohistochemical reactions. Rhodamine isothiocyanate conjugated avidin (1:400; Sigma) was used to stain MC (Tharp et al., 1985).

Morphometry and statistics

Adjacent, not overlapping microscopic fields were photographed separately with a x40 objective. To evaluate DC or MC, one photomicrograph from each heart specimen was taken from MHC-II or fluorescent avidin stained sections. DC, MC and granulocytes were counted and expressed as number per square millimetre of tissue section surface. For each parameter, the average values for each corpse was assumed as sample unit. All differences were subjected to one-way analysis of variance (ANOVA) among all experimental groups. When this gave significant results, the values for each time frame were compared with controls by two-tailed Student's *t* test for unpaired values. $P < 0.01$ was assumed as significant. Quantitative data are given as mean and standard error of the mean.

Results

Triphenyltetrazolium staining

The hearts of control and coronary disease groups were completely stained vivid red (Fig. 1 A,B). In the MI group, when autopsy was performed within 48 h of cardiac arrest heart slices always showed pale areas indicating necrotic zones (Fig. 1 C). When autopsy was performed beyond 48 h from death, heart slices were completely stained either vivid red or brown, which indicates unspecific staining (Fig. 1 D). Old MI areas appeared white at TTC staining and had a fibrous aspect (Fig. 1 E).

Light and fluorescence microscopy

No differences were found depending on sex or age. The results are reported first for controls, in order to give a baseline to understand modifications occurring in the study groups.

Controls: by light microscopy cardiac myofibres had typical aspect and no inflammatory infiltrate was observed (Fig. 2 A). By fluorescence microscopy, MC were rare (Figs. 2 B, 6 A); interstitial MHC-II positive cells with branched profile, interpreted as DC, were also few (Figs. 2 C, 6 B)

Coronary artery disease: the findings were similar to those of controls, except for a slightly more frequent presence of MHC-II positive cells in the myocardium next to the arteries suffering from coronary disease, without significant differences from controls in the overall number density of these cells nor of other cell types (Figs. 3 A-C and 6 A,B).

Acute myocardial infarction: haematoxylin and eosin-stained sections showed elongation of sarcomeres and weakening of cardiomyocyte eosinophilia (Fig. 4 A). MC were few (Figs. 4 B, 6 A); in some instances they were in contact with MHC-II positive cells (Fig. 6 C). These latter cells on the contrary were significantly more numerous than in the controls (Fig. 6 B).

Old myocardial infarction: upon haematoxylin and eosin, cardiomyocytes were unstained or grossly altered and separated from each other by fibrous tissue; an inflammatory infiltrate was present between fibres (Fig. 5 A) while MC were as rare

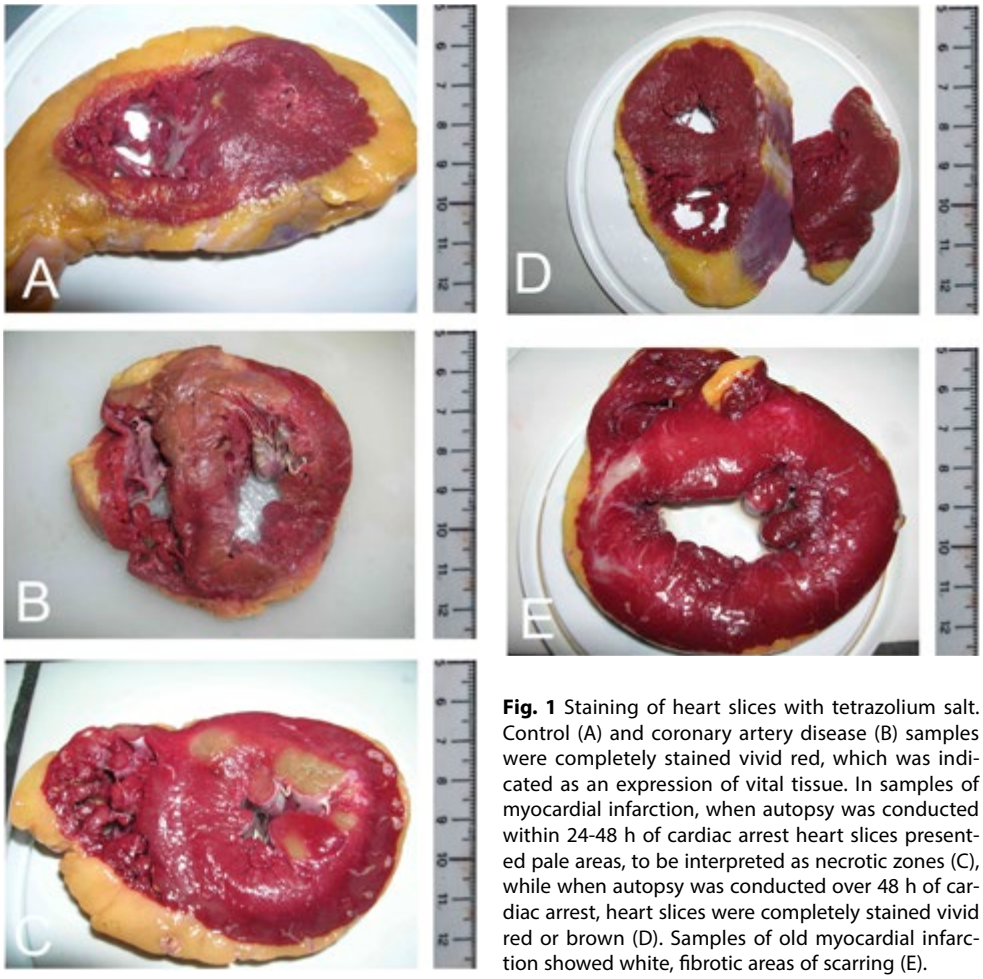

Fig. 1 Staining of heart slices with tetrazolium salt. Control (A) and coronary artery disease (B) samples were completely stained vivid red, which was indicated as an expression of vital tissue. In samples of myocardial infarction, when autopsy was conducted within 24-48 h of cardiac arrest heart slices presented pale areas, to be interpreted as necrotic zones (C), while when autopsy was conducted over 48 h of cardiac arrest, heart slices were completely stained vivid red or brown (D). Samples of old myocardial infarction showed white, fibrotic areas of scarring (E).

as in the controls (Figs. 5 B, 6 B). MHC-II positive cells were significantly increased over the controls (Figs. 5 C, 6 B)

Discussion

Myocardial infarction is the most common reason for cardiac injury and chronic heart failure (Priori et al., 2001). Although progress has been made in the prevention of cardiac death in certain groups of patients (Adabag et al., 2010), the pathologic assessment of early heart infarction is still a hard task despite attempts from different research groups (Turillazzi et al, 2014; Riezzo et al., 2015).

The present results suggest that even routine methods may be of use in case of positive results, while negative results are hampered by lack of sensitivity. Moreo-

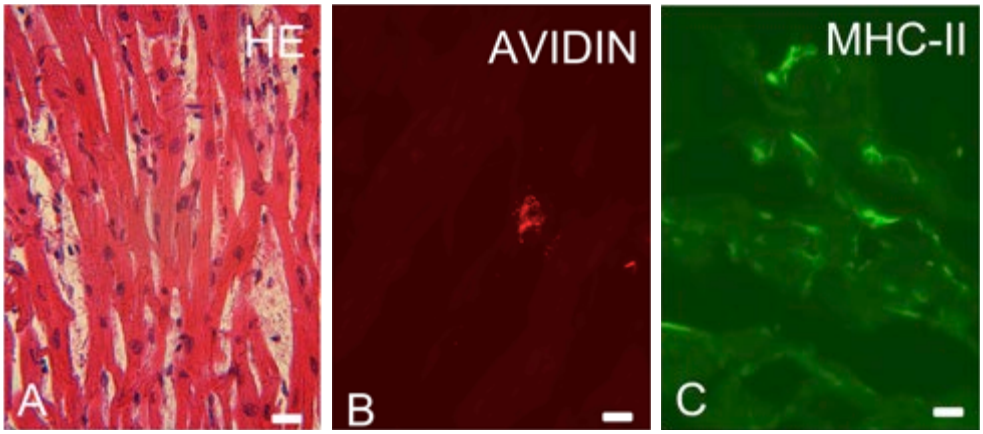

Fig. 2. Controls. A: Upon staining with haematoxylin and eosin the myofibres appeared normal and the stroma was not infiltrated. B: An isolated, partially degranulating mast cell is shown between muscle fibres upon staining with fluorescent avidin. C: Immunohistochemistry for MHC-II (MHC Class II). Scale bars = 10 µm.

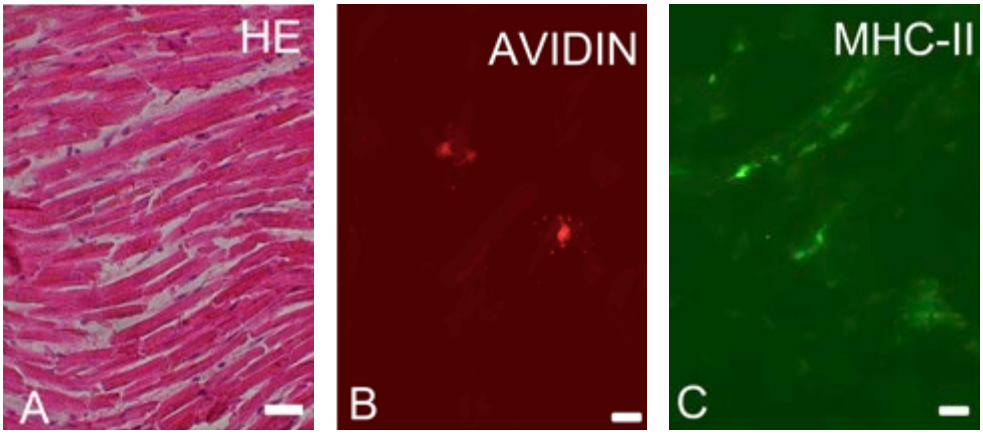

Fig. 3 Coronary artery disease. The panels depict tissue stained with haematoxylin and eosin (A), fluorescent avidin (B), and MHC-II antibody (C). In panel C there is a cluster of dendritic cells around a myofibre, but quantitative analyses did not show significant differences from controls. Scale bars = 10 µm.

ver, triphenyltetrazolium staining is strongly influenced by the time between death and autopsy and is meaningless if this time exceeds 48 h. By light microscopy, the samples of patients with MI died within 6 h from the onset of symptoms showed alterations of myocardial fibres and loss of acidophilia but no inflammatory infiltrate in the interstitium. Although in the present study such alterations of cardiomyocytes were not observed in the controls and in specimens from patient with coronary artery disease, previous studies indicate that their specificity may be low (Frangogiannis, 2008).

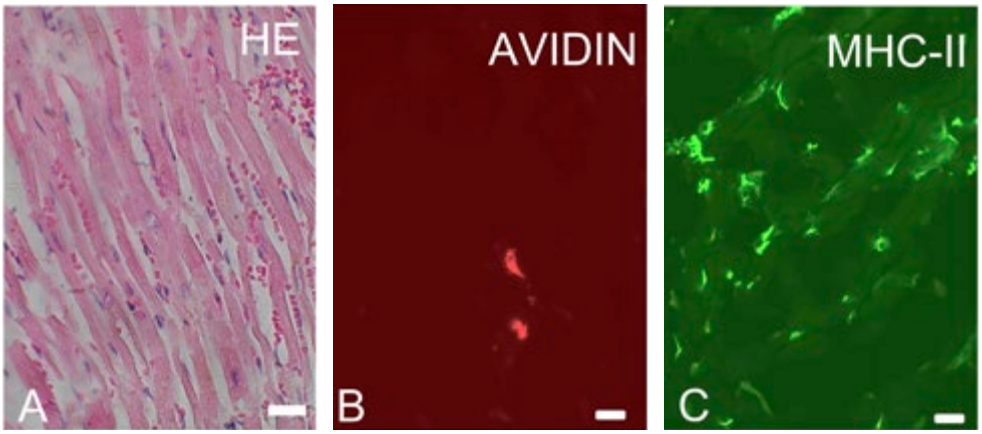

Fig. 4. Acute myocardial infarction. A: Mild myofibre thinning may be appreciated upon haematoxylin and eosin staining. B: Mast cells, stained with fluorescent avidin, are as few as in controls. C: Many MHC-II positive cells, including dendritic cells, surround myocardiocytes. Scale bars = 10 μm.

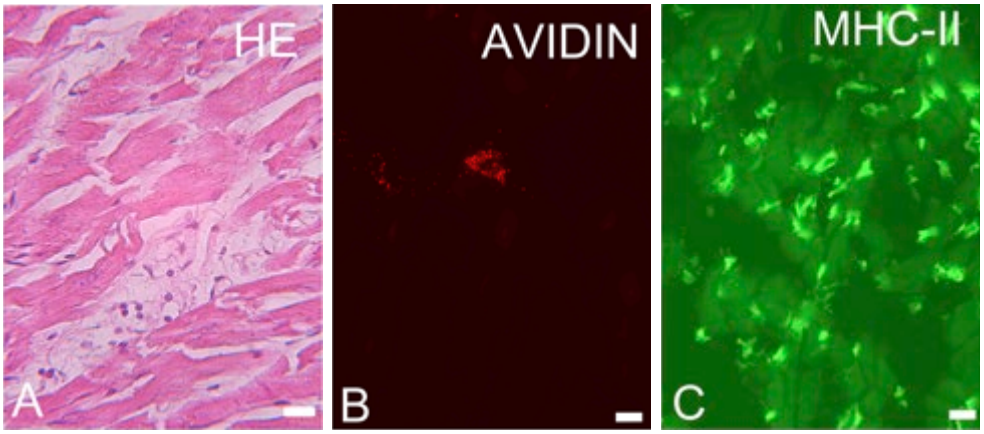

Fig. 5. Old myocardial infarction. A: Upon staining with haematoxylin and eosin myofibres appear intermingled with abundant connective tissue; a few inflammatory cells are present. B: An isolated, partially degranulating mast cell is shown between myofibres upon staining with fluorescent avidin. C: MHC-II positive cells (including dendritic cells) appear more numerous than in controls. Scale bars = 10 μm.

Since cardiac repair following MI depends on the activation of inflammatory pathways (Frangogiannis, 2012), the identification of early markers of inflammatory response upon MI may be relevant. DC play a crucial role in the activation of the immune response and seem to be involved in natural immunity for their ability to stimulate the recruitment of neutrophils and natural killer cells and for their secretion of type 1 interferon (Romani, 2006). Also, they may regulate cardiac scarring and regeneration by activating regulatory T cells (Liu et al., 2016; Wang et al., 2016).

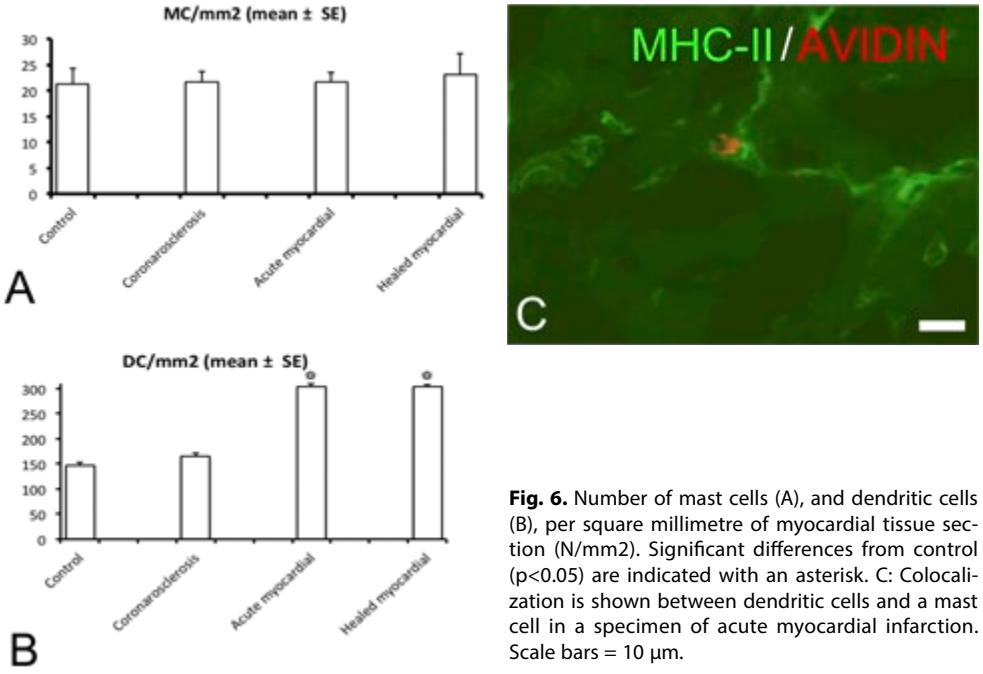

Fig. 6. Number of mast cells (A), and dendritic cells (B), per square millimetre of myocardial tissue section (N/mm2). Significant differences from control ($p<0.05$) are indicated with an asterisk. C: Colocalization is shown between dendritic cells and a mast cell in a specimen of acute myocardial infarction. Scale bars = 10 µm.

The observation of many MHC-II positive cells in MI, at variance with coronary artery disease and healthy myocardium, is in keeping with that of Yilmaz et al. (2010) of increased numbers of DC in the myocardium after sudden cardiac death. Therefore, it may be inferred that DC increase occurs very early and persists over time. The presence of several DC has been also demonstrated in several animal models of acute MI (Zhang et al., 1993; Naito et al., 2008; Takahashi et al., 2008). Kretzschmar et al. (2012) detected many CD209-positive immature DCs in cases of acute MI, which indicates that precursors of DCs are recruited soon after MI. An increase in DC number was also observed during the acute phase of skin wound healing (Bacci et al., 2014).

The human heart contains MC and these cells and their mediators are credited to play a role in acute myocardial ischemia (Kwon et al., 2011; Palmiere et al., 2014). In animal models, MC have been demonstrated to accumulate into the heart after MI. In a guinea pig model of ischemia/reperfusion, histamine was demonstrated to be released after the ischemic period (Masini et al., 1990). Mast cell distribution in the heart was investigated after induction of acute MI in rats, showing accumulation of MC in the infarcted region (Koike et al, 2005). Similarly, in a dog model, the number of MC was highly increased after induction of ischemia/reperfusion injury while macrophages in the infarcted myocardium were demonstrated to express stem cell factor (SCF), a potent MC chemoattractant. This led these authors to suggest that MC are recruited by macrophage-derived SCF (Frangogiannis et al., 1998, Dewald et al., 2004).

Our results cast doubts on an increase in MC density early after MI in humans. Rather, our results seem to be in agreement with those on sudden infant death syndrome, in which an increase of tryptase levels but not in the number of MC was

shown by some authors (Buckley et al., 2001) and no increase in tryptase concentration was even found by others (Nishio and Suzuki, 2004). The results on MC may depend on oscillations with time of the number of these cells upon MI, as happens in skin after wounding (Bacci et al., 2014). Although we acknowledge that a higher number of cases should be analyzed to draw conclusions on this issue, the present findings suggest suggest not to rely on MC counts to improve diagnosis of early MI. The observation of contacts between DC and MC found in MI may support the hypothesis of a role of MC in regulating the response of DC upon tissue ischemia, as already proposed for other circumstances (Carroll-Portillo et al., 2012).

In conclusion, on the basis of our data, we propose that DC may play a major role in MI since the very early phases of the tissue response to ischemia and that they deserve further attention as possible early indicators for the diagnosis of MI at autopsy in cases of unexplained death.

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Potential conflict of interest

One of the authors (P.R.) is also Editor of the Journal where this article is published.

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