

MAST CELL DEFICIENT W/W^v MICE HAVE LOWER SERUM IL-6 AND LESS CARDIAC TISSUE NECROSIS THAN THEIR NORMAL LITTERMATES FOLLOWING MYOCARDIAL ISCHEMIA-REPERFUSION

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Myocardial ischemia-reperfusion (IR) injury complicates all forms of coronary artery revascularization. Circulating interleukin-6 (IL-6) has been implicated in cell death following a variety of stimuli. Macrophages, platelets, neutrophils and the endothelium have been shown to release IL-6 after IR injury. Cardiac mast cells have been implicated in IR; however, their involvement has never been quantified. In this randomized, prospective study, we compared cardiac tissue susceptibility and serum IL-6 changes between mast cell deficient (W/W^v) mice and their normal littermates (+/+). Twenty-eight male W/W^v mice (n=14) and their +/+ littermates (n=14) were anaesthetized with 2.5% isoflurane. The left coronary artery (LCA) was ligated for 30 minutes or a sham procedure was performed. After 6 hours of reperfusion, the animals were sacrificed. The muscle viability was assessed on fresh whole-mount slices by nitroblue tetrazolium (NBT) histochemical assay and serum IL-6 concentrations measured by ELISA. Cardiac muscle viability was significantly higher in W/W^v mice than the +/+ mice. Serum IL-6 levels were higher in the +/+ sham mice (465 ± 32 pg/ml, n=6) than the W/W^v mice (185 ± 31 pg/ml, n=6), p < 0.001. The IL-6 levels increased significantly after reperfusion only in the +/+ mice (698 ± 41 pg/ml, n=8, p = 0.001), while it remained similar in the W/W^v mice (202 ± 48 pg/ml, n=8, p = 0.783). These results show that the absence of mast cells reduces the myocardial damage associated with IR injury. Furthermore, there is an attenuation in the inflammatory response, as measured by serum IL-6 levels, following this local insult. This finding entertains the prospect of developing prophylactic therapy - targeting selective inhibition of cardiac mast cell activation, in clinical situations involving medical or surgical myocardial revascularization.

Coronary Artery Disease (CAD) is the leading cause of morbidity and mortality in the United States; it accounts for 1.5 million of myocardial infarctions (MI) and 500,000 deaths each year. Unfortunately, in spite of earlier predictions, CAD

continues to be the leading cause of death globally. CAD is a clinical manifestation of atherosclerosis, which is now considered to be a chronic vascular inflammatory disorder (1). Angioplasty removes cholesterol plaques in CAD and allows reperfusion,

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which unfortunately is associated with a high rate of ischemia-reperfusion (IR) and restenosis (1). IR injury also occurs following thrombolysis, percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), and is associated with further tissue damage from the release of inflammatory mediators. These could be released from macrophages, platelets and neutrophils, as well as from the endothelium and resident mast cells. *In vivo* studies of animal models show evidence of the role of mast cell activation in IR injury. For instance, mast cell numbers increase in cardiac tissue during IR, while pretreatment with a mast cell stabilizer reduces the size of infarct (2). Mast cells are well known for their involvement in allergic reactions (3) and neuroinflammatory conditions that are precipitated or exacerbated by stress (4). Mast cells are not only a rich source of vasoactive and pro-arrhythmogenic molecules, such as histamine, but also many cytokines, including IL-6 (5). Increased numbers of activated cardiac mast cells are found in ventricles, the sinoatrial node and the shoulder region of the fibrous plaque in association with atherosclerosis (6-7). It has also been suggested that coronary inflammation may depend on mast cell-derived mediators (8).

IL-6 was originally identified in monocytes/macrophages, fibroblasts and endothelial cells (9). It is a pleiotropic cytokine that has multiple functions including: (a) stimulatory effects on the proliferation and differentiation of lymphocytes (9); (b) activation of the hypothalamic-pituitary adrenal (HPA) axis; (c) induction of the hepatic acute phase response proteins, particularly CRP; (d) increase of fatty lesions and increase of atherosclerosis in ApoE knockout mice. The importance of IL-6 in the pathophysiology of CAD has been documented in several studies (10-11) and it is probably the link between inflammation, stress and CAD (12). Both IL-6 and C reactive protein (CRP), are strong independent predictors of cardiovascular mortality. IL-6 can also be released from cardiomyocytes in response to hypoxia, IL-1 from mast cells (13) in acute stress.

To date, there is no published report evaluating the effects of IR in mast cell deficient W/W^v mice. Here, we report that IR-induced serum IL-6 increase, and significantly less myocardial damage in these

mice. These findings point to mast cell-derived IL-6 as a key player in the inflammation and damage during IR.

MATERIALS AND METHODS

Ischemia-reperfusion

Fifteen male (30-35 g) W/W^v mast cell deficient mice (WBB6F1/J-W/W^v) and 15 normal +/+ littermates (Jackson Laboratories, Bar Harbor, ME) were allowed food and water *ad libitum* and were maintained in a 14:10 hr dark-light cycle. Animals were kept in the animal facility for at least one week before use. Each mouse was anesthetized with 2.5% isoflurane. The mice either had their left coronary artery (LCA) ligated 4 mm from the aorta for 30 minutes followed by a 6 hr reperfusion period, or were sham-operated. The animals were then sacrificed; blood was collected, allowed to clot at 37°C, centrifuged and the serum was removed and stored at -80°C for future IL-6 analysis. The heart was removed rapidly, fixed and stored. This investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

IL-6 measurements

Mouse serum samples were assayed for IL-6 with the quantitative sandwich enzyme immunoassay kit technique (Quantikine M Murine, Mouse IL-6, R&D Systems Inc., Minneapolis, MN).

Light microscopy

The heart was rapidly removed and fresh whole mount slices were cut using a Vibratome (Micro-cut H1200, E.M. Core, Chestnut Hill, MA). Cardiac sections were stained with nitroblue tetrazolium (NBT) to assess cardiac muscle viability as previously described (14). Two random sections from each heart were evaluated by two researchers, blinded to the experimental conditions, using a Diaphot inverted Nikon microscope (Don Santo, Natick, MA). Results are presented as percent cardiac tissue not stained.

Statistical analysis

The IL-6 values were subjected to the Levine's Test for the equality of variances (SPSS version 10.1, Chicago, IL). As the variances were not significantly different, each group data is presented as the mean±S.E.M. The cardiac tissue viability results were compared using non-parametric *U* test. One way ANOVA run by SigmaStat was used to compare the differences between the groups. A student's *t*-test was used to compare the treatments between each group of animals. A *p* value of less than

0.05 was considered statistically significant.

RESULTS

Serum IL-6 levels in wild-type sham-operated mice without IR were 465 ± 32 pg/ml and were significantly higher than the corresponding sham-operated W/W^v mice (185 ± 33 pg/ml, $p < 0.05$, Fig. 1). After reperfusion, the wild type mice showed a significant increase in IL-6 levels (698 ± 41 pg/ml, $p < 0.01$). However, the serum IL-6 concentration for the W/W^v mice did not increase (Fig. 1) following reperfusion (202 ± 48 pg/ml, $p < 0.783$). The difference in the serum IL-6 levels between wild type IR and W/W^v IR was also very significant ($p < 0.001$). There was no apparent difference in cardiac tissue viability

Table I. Effect of ischemia-reperfusion on cardiac viability.

Condition	Wild-type mice	W/W ^v mice
Sham-operated	+/-	None
IR	+++	None

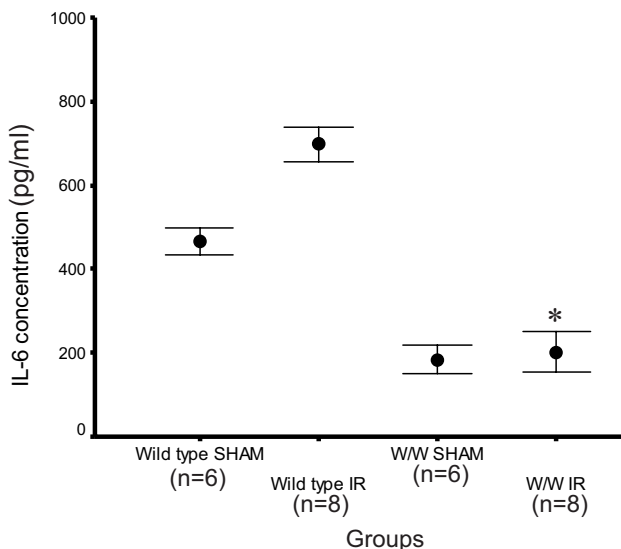


Fig. 1. Comparison of serum IL-6 levels in wild type controls ($n=15$) and W/W^v mast cell deficient ($n=15$) mice. Results are presented as mean \pm S.E.M. (\pm means least and +++ most tissue necrosis).

between sham-operated +/+ and W/W^v mice (Table I); however, cardiac tissue viability was strikingly higher in W/W^v mice than the +/+ mice after IR (Table I). In +/+ mice following IR, there was extensive cardiac necrosis, as seen with absence of nitroblue tetrazolium staining (Fig. 2).

DISCUSSION

Our present data show that IR induces serum IL-6 release and myocardial damage absent in W/W^v mice, indicating that this process is mast cell-dependent. In other studies using W/W^v mast cell deficient mice, their immune system was otherwise found to be normal, thus excluding the possibility of defective monocyte/macrophage function that may otherwise explain our results (15). Our findings are important because mast cells have been considered as the link between inflammation and atherosclerosis (16).

The importance of cardiac mast cell activation to the onset and progression of CAD is increasingly recognized (17). Activated cardiac mast cells are found in the coronary arteries and the heart in association with atherosclerosis (6-7). We previously showed that acute stress induces cardiac mast cell activation (18), as well as release of histamine (19) and IL-6 (13), both of which were higher in ApoE k/o mice.

IL-6 is elevated in patients with ACS and is expressed in atherosclerotic lesions of both mice (20-21) and humans (22-24); intracoronary IL-6 release in patients with acute CAD is also documented (25). In fact, IL-6 is considered a possible link between inflammation, stress and CAD (12). IL-6 was shown to be a potent independent marker of increased mortality in unstable CAD (26). Mast cells can release many cytokines, including IL-6 (5), especially in response to the inflammatory cytokine IL-1 (27). Mast cell-derived IL-6 and TNF- α induce the expression of vascular adhesion molecules that participate in the pathophysiology of CAD (28).

Increasing evidence implicates focal inflammation in the pathophysiology of CAD (25, 29-30), and acute stress is well known to worsen (31-36) or precipitate cardiac ischemia especially in patients with CAD (31-35, 37). Neurogenic stimulation of cardiac mast cells could occur since adventitial mast cells were in contact with sensory nerve fibers

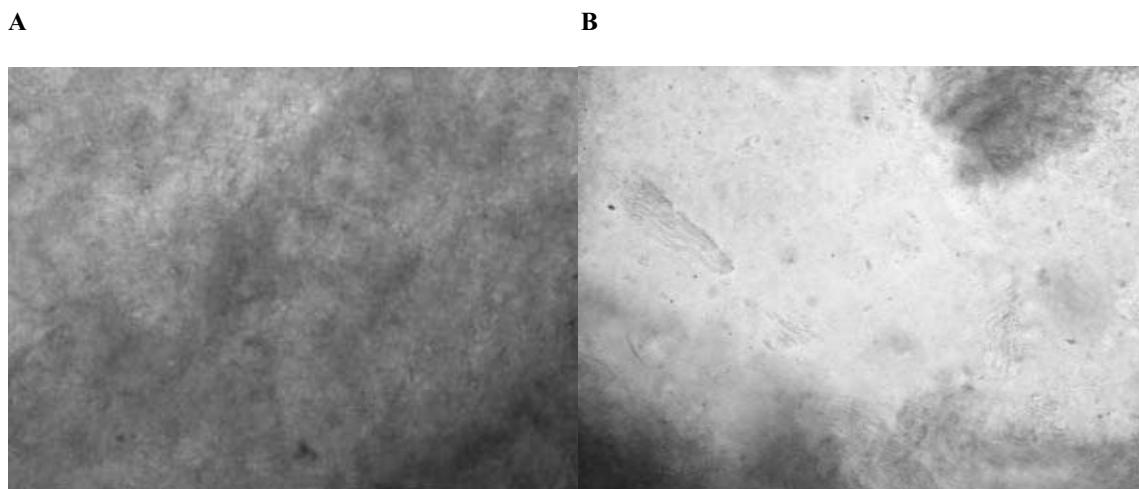


Fig. 2. Representative photographs of cardiac sections ($n=5$ in each case) after IR from (A) W/W^v and (B) $+/+$ wild-type hearts stained with tetrazolium blue chloride, which generates red/purple color when acted upon by dehydrogenases present in live cells. Note the extensive cardiac tissue necrosis apparent in the $+/+$ mouse.

in human atherosclerotic coronary arteries (38). A number of studies also suggested that mast cells may be involved in IR. An increased number of activated mast cells was reported in IR and they were noted to be degranulated with corresponding release of IL-6 and TNF- α . (2). Pretreatment with a mast cell stabilizer Iodoximide was reported to decrease the infarct size following IR in isolated rabbit hearts (39). Finally, *skeletal* muscle viability was reported to be higher in W/W^v mice (40).

It was recently shown that certain naturally occurring flavonoids, especially quercetin, could inhibit IL-6 release from human cultured mast cells (41). These findings entertain the prospect of developing prophylactic therapy by targeting selective inhibition of cardiac mast cell activation, in clinical situations involving medical or surgical myocardial revascularization.

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