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Randomized, controlled trial of intramuscular or intracoronary injection of autologous bone marrow cells into scarred myocardium during CABG versus CABG alone

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SUMMARY

Background Studies of the transplantation of autologous bone marrow cells (BMCs) in patients with chronic ischemic heart disease have assessed effects on viable, peri-infarct tissue. We conducted a single-blinded, randomized, controlled study to investigate whether intramuscular or intracoronary administration of BMCs into nonviable scarred myocardium during CABG improves contractile function of scar segments compared with CABG alone.

Methods Elective CABG patients ($n = 63$), with established myocardial scars diagnosed as akinetic or dyskinetic segments by dobutamine stress echocardiography and confirmed at surgery, were randomly assigned CABG alone (control) or CABG with intramuscular or intracoronary administration of BMCs. The BMCs, which were obtained at the time of surgery, were injected into the mid-depth of the scar in the intramuscular group or via the graft conduit supplying the scar in the intracoronary group. Contractile function was assessed in scar segments by dobutamine stress echocardiography before and 6 months after treatment.

Results The proportion of patients showing improved wall motion in at least one scar segment after BMC treatment was not different to that observed in the control group ($P = 0.092$). Quantitatively, systolic fractional thickening in scar segments did not improve with BMC administration. Furthermore, BMCs did not improve scar transmurality, infarct volume, left ventricular volume, or ejection fraction.

Conclusion Injection of autologous BMCs directly into the scar or into the artery supplying the scar is safe but does not improve contractility of nonviable scarred myocardium, reduce scar size, or improve left ventricular function more than CABG alone.

KEYWORDS bone marrow cell therapy, myocardial infarction, revascularization, stem cells

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INTRODUCTION

The observation that stem cells can be used to repair injured human tissue has raised expectations for the treatment of failing organs, including the heart. Following encouraging animal studies, increasing attention has focused on the possible roles for autologous bone marrow cells (BMCs) in myocardial repair.[1,2] This type of stem cell is easy to harvest, simple to administer, ethically acceptable and does not require immunosuppression. Studies have shown global and regional functional improvements after BMCs have been injected into viable, peri-infarct areas of chronically ischemic myocardium.[3–5] The efficacy of BMCs in restoring function of scarred myocardium within an established infarct, however, has not been explored.

We have previously demonstrated the safety of injecting BMCs into myocardial scar tissue during surgical revascularization.[6] In the present study, we investigate whether the administration of BMCs, by either intramuscular or intracoronary injection, during CABG improves contractile function of nonviable scarred myocardium compared with CABG alone.

METHODS

This clinical trial is registered on Clinicaltrials.gov (registry number NCT00560742 assigned on 16 November 2007). The study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

Study population

Patients aged 18–80 years who were candidates for elective CABG were considered for the study. The inclusion criteria were the presence of at least one chronic, irreversible myocardial scar, defined as areas of akinesia or dyskinesia with no contractile reserve on dobutamine stress echocardiography (DSE)—identifiable more than 6 weeks after myocardial infarction and confirmed at surgery; and the availability of a graftable coronary artery

supplying the scarred segments. Exclusion criteria were valvular heart disease requiring concurrent valve surgery, cardiogenic shock (systolic blood pressure <80 mmHg, requiring intravenous inotropes or intra-aortic balloon pump), hepatic or renal failure, evidence of malignancy, preexisting bone marrow conditions, and contraindications to cardiac MRI.

All participants gave written informed consent at enrollment and received the same standard treatment and rehabilitation regimens available to other patients undergoing CABG.

Study design

All eligible patients underwent DSE for the identification of myocardial scarring.[7] During surgery, performed by a single surgeon (M Galiñanes), patient suitability was confirmed by the presence of epicardial scarring on visual inspection.

Patients were randomly assigned in equal numbers to the control group (which received no BMCs or vehicle injection) or to groups administered BMCs via either intramuscular or intracoronary routes. The physicians treating the patients during the postoperative period and the investigators performing the examinations and interpreting the results were blind to which group patients had been assigned. At 6 months after intervention, all patients underwent clinical follow-up and DSE assessment. Cardiac MRI was available for the last 33 consecutive patients entering the study and was performed, on an equal sample from each group, before and 6 months following treatment to quantify myocardial scarring[8] as well as global left ventricular volumes and function.[9]

The primary end point was improvement in systolic function of scar segments 6 months after treatment. The secondary end points were reductions in infarct size, global end-diastolic volume and end-systolic volume, and improvement in stroke volume and left ventricular ejection fraction. Information was also collected regarding postoperative complications, troponin I levels within 24 h of surgery and clinical evaluation (assessment of functional status and adverse events) at 6 months.

Bone marrow cell preparation and administration

Before surgery, 50 ml blood was taken from each patient to obtain serum. After anesthesia but before CABG, 80 ml bone marrow was aspirated from the patient's iliac crest into preservative-free heparin (10 U/ml) and diluted with normal saline. The BMCs were isolated by density centrifugation with Lymphoprep® (AXIS-SHIELD PoC AS, Oslo, Norway), and the separated BMC layer was added to the autologous serum. The viability of BMCs after processing and immediately before administration was greater than 95%. To optimize cell contact and retention in patients receiving BMCs, 10 ml serum containing diluted BMC solution was injected into each scar segment on completion of coronary artery anastomoses, before the release of the aortic cross-clamp and while the heart was still arrested.[10] To cover the whole scar area evenly in the intramuscular group, 20 injections of 500 µl each were administered, approximately 1 cm apart, into the mid-depth of the scar under the guidance of transesophageal echocardiography; in the intracoronary group, the BMCs were delivered via the graft conduit supplying the scar.

Troponin I assessment

Troponin I levels were measured in venous blood samples taken within 24 h of surgery with a troponin I assay (Tosoh Bioscience, Tessenderlo, Belgium).

Dobutamine stress echocardiography

All patients underwent DSE before and 6 months following surgery. Calcium antagonists and β-blockers were discontinued 48 h before this investigation. Images were acquired with Philips Sonos 5500® and IE33® systems (Philips Medical Systems, Surrey, UK) before and during infusion of low-dose dobutamine (10–20 µg/kg/min). Cardiac stress was reversed with intravenous atenolol (2.5–5 mg). Digitized images were analyzed offline by an independent assessor (D Chin), who was unaware of the coronary anatomy and intervention performed.

Left ventricular segmental wall motion was qualitatively assessed at rest and during low-dose DSE according to the 16-segment model of the American Society of Echocardiography[11] and assigned to one of four grades (normokinesis, hypokinesis, akinesis, dyskinesis). To increase the specificity for the diagnosis of transmural fibrosis, a scar area without functional reserve was defined as an akinetic or dyskinetic segment, which demonstrated no improvement in wall motion during low-dose DSE. An improvement in wall motion after intervention was defined as a change in segmental function from either akinesis or dyskinesis to either hypokinesis

or normokinesis. A change in wall motion between akinesis and dyskinesis was not deemed functionally relevant.

In order to address the recognized concerns about the reproducibility of DSE,[12,13] percent systolic fractional thickening (%SFT) was calculated for all scar segments, both at rest and during low-dose DSE, before and after intervention. The midsegmental diastolic and systolic wall thicknesses were measured at each stage; the respective %SFT was calculated with the formula: %SFT = [(systolic thickness – diastolic thickness)/diastolic thickness] × 100%.

Cardiac MRI

Images were acquired on a 1.5 T Signa® scanner (General Electric, Slough, UK) using a phased-array cardiac coil during repeated 8 s breath-holds. A short-axis stack of left ventricular images was acquired using a steady state in free precession sequence (repetition time 3.0–3.8 ms, excitation time 1.0 ms, image matrix 224 × 224, field of view 36–42 cm, flip angle 45°) in sequential 8 mm slices (2 mm interslice gap) from the atrioventricular ring to the apex. Left ventricular volumes, ejection fraction, and mass (myocardial density = 1.05 g/cm^3) were quantified by planimetry of all short-axis steady state in free precession sequence cine images with MASS analysis software.[14] The observers (F Leyva, P Foley) were blinded to all other clinical details of the patients, including the outcome measures.

Quantification of myocardial scarring was undertaken in all cardiac MRI. Briefly, 0.1 mmol/kg gadolinium-diethylenetriamine penta-acetic acid was administered intravenously and images were acquired after 10 min with a segmented inversion-recovery technique in identical short-axis slices. Inversion times were adjusted to null normal myocardium (260–400 ms). Quantification of myocardial scarring was carried out by planimetry of hyperenhanced tissue on short-axis images. Infarct volume was calculated in cubic centimeters by multiplying the planimetered area in each segment by the slice thickness. The percent volume of myocardial scar was then derived by expressing infarct volume as a percentage of left ventricular myocardial volume in the diastolic phase.

Scar transmurality was assessed on contrast-enhanced images and matched to echocardiogram segments. Scar transmurality was used as a measure of the transmural extent of the scar expressed as a percentage of left ventricular wall thickness. A scar was considered to be transmural if its scar transmurality exceeded 51%.[8]

Power calculation and statistical analysis

As this was a novel study, we did not know the variability associated with the primary outcome or its minimal clinically relevant difference at the start of the study. Therefore, to determine the sample size, we specified that we wished to detect whether improvement was as much as 1 SD better in a treated group than in the control group.[15] Assuming 80% power and that $\alpha = 0.05$, 17 scar segments were required per group to show this difference. Allowing for 20% withdrawals and assuming each patient has at least one scar segment, we calculated that 63 patients had to be enrolled in total.

Continuous variables that were normally distributed are presented as mean ± SD. For baseline characteristics, analysis of variance was used to compare the means between the treatment groups. The effects of different interventions were analyzed with a generalized linear model that compared the mean differences between groups before and 6 months after treatment, adjusting for baseline values. Variables that were not normally distributed are expressed as medians (interquartile range) and were compared by the appropriate nonparametric tests, such as the Kruskal–Wallis test. Categorical variables were analyzed with the χ^2 or Fisher's exact test as appropriate.

For segmental data on %SFT and scar transmurality, the mean values were calculated for each patient. Summary measures were analyzed by the Generalized Linear Model to enable several simultaneous observations in the same patient (clustering). Scar segments within the BMC treatment groups that were revascularized but could not be treated by BMCs, such as septal segments that were inaccessible to BMC injection, were not included in the analysis. The χ^2 test was used to compare the proportion of patients with at least one scar segment that showed contractile improvement based on segmental wall motion assessment between treatment groups.

All tests were two-sided and $P<0.05$ was deemed significant. Analyses were performed with SPSS version 14®[16] or SAS® version 9.1.[17]

RESULTS

Baseline characteristics and operative data

A total of 63 patients were included in the study. One patient in the control group withdrew from the study before the surgery. Another patient

Table 1 Patient characteristics.

Characteristics	Study group			*P* value
	Control (*n* = 20)	IM BMCs (*n* = 21)	IC BMCs (*n* = 21)	
Demographics				
Mean (SD) age (years)	61.3 ± 8.3	64.7 ± 8.7	62.1 ± 8.7	0.415
Male	18 (90.0%)	15 (71.4%)	19 (90.5%)	0.188
Mean (SD) BMI (kg/m^2)	29.2 ± 4.4	27.2 ± 4.8	26.8 ± 3.1	0.140
Risk factors				
Hypertension	12 (60.0%)	9 (42.9%)	10 (47.6%)	0.528
Diabetes	6 (30.0%)	4 (19.0%)	5 (23.8%)	0.708
Hyperlipidemia	16 (80.0%)	14 (66.7%)	15 (71.4%)	0.680
Smoking history	15 (75.0%)	12 (57.1%)	12 (57.1%)	0.409
Preoperative medication				
Aspirin	17 (85.0%)	13 (61.9%)	17 (81.0%)	0.227
Clopidogrel	10 (50.0%)	10 (47.6%)	11 (52.4%)	0.953
β-Blockers	13 (65.0%)	15 (71.4%)	18 (85.7%)	0.298
ACE inhibitors	13 (65.0%)	13 (61.9%)	16 (76.2%)	0.582
Statins	17 (85.0%)	16 (76.2%)	18 (85.7%)	0.768
Diuretics	9 (45.0%)	9 (42.9%)	7 (33.3%)	0.717
Number of diseased coronary vessels[a]				
One	1	0	1	—
Two	6	7	9	—
Three	13	14	11	—
Operative details				
Mean (SD) duration on bypass (min)	97.6 ± 35.5	101.5 ± 21.0	110.5 ± 36.1	0.409
Mean (SD) cross-clamp time (min)	50.9 ± 20.7	55.0 ± 12.0	65.7 ± 23.4	0.051
Median (interquartile range) number of grafts	3 (2)	3 (3)	3 (3)	0.778

[a]$P = 0.785$ for χ^2 comparison. Abbreviations: ACE, angiotensin-converting enzyme; BMCs, bone marrow cells; IC, intracoronary; IM, intramuscular.

in the control group who underwent cardiac resynchronization therapy was deemed unsuitable for further follow-up. The preoperative and operative characteristics were similar in the three study groups (Table 1). From this cohort of patients, 154 scar segments were suitable for analysis (45 in the intramuscular BMC group, 59 in the intracoronary BMC group, and 50 in the control group).

Clinical outcomes

A mean of $84 \pm 56 \times 10^6$ and $115 \pm 73 \times 10^6$ BMCs ($P = 0.184$) and $142 \pm 166 \times 10^3$ and $245 \pm 254 \times 10^3$ CD34$^+$/CD117$^+$ cells ($P = 0.239$) were injected in the intramuscular and intracoronary groups, respectively. The indices for classification of physical activity for cardiac patients, given by the NYHA and the Canadian Cardiovascular Society, improved in all three groups following treatment (Table 2). No adverse events associated with BMC injections were observed throughout the study (Table 2).

Effect on myocardial scars

The %SFT values before and after surgery were negative for all segments and did not show systolic thickening at rest or during low-dose DSE, confirming absent contractile reserve and nonviability (Table 3). Moreover, there was no difference between the proportions of patients

with contractile improvement in at least one scar segment after intervention on the basis of segmental wall motion assessment at rest (control 47.1%, intramuscular 12.5% and intracoronary 29.4%, $P = 0.092$) and during low-dose DSE (control 47.1%, intramuscular 31.3% and intracoronary 41.2%, $P = 0.717$).

Scar transmurality was measured in 73 segments in the cohort of patients who underwent cardiac MRI. The mean transmurality before surgery in all groups was greater than 60%; values were not affected by BMC treatment (Table 3).

Effect on global left ventricular function

Of the 33 patients who underwent cardiac MRI, the images for 4, taken at one or more of the time points, were not suitable for accurate analysis. Comparison of percent volume of the myocardial scar could be accurately measured in only 22 patients. There were no differences between groups in the baseline functions, and the administration of BMCs did not affect the percent volume of the myocardial scar, end-systolic volume, end-diastolic volume, stroke volume, or ejection fraction (Table 4).

Table 2 Clinical outcomes.

Clinical parameters	Study group		
	Control ($n = 19$)	IM BMCs ($n = 21$)	IC BMCs ($n = 21$)
CCS angina class			
Patients with CCS angina class >2 before surgery	7 (35%)	7 (33%)	3 (14%)
Patients with CCS angina class >2 after surgery	0	0	0
NYHA class			
Patients with NYHA III–IV before surgery	3 (15%)	4 (19%)	3 (14%)
Patients with NYHA III–IV after surgery	0	0	0
Mean (SD) troponin I μg/l	1.4 ± 1.3	2.8 ± 2.6	1.7 ± 2.0
Complications			
New Q-wave myocardial infarction	0	0	0
Ventricular arrhythmia	0	0	0
IABP support	3 (16%)	0	0
Renal failure	1 (5%)	1 (5%)	0
Stroke	1 (5%)	0	0
Death within 30 days of treatment	1 (5%)	0	1 (5%)

Abbreviations: BMCs, bone marrow cells; CCS, Canadian Cardiovascular Society; IABP, intra-aortic balloon pump therapy; IC, intracoronary; IM, intramuscular.

DISCUSSION

This randomized, controlled study measured the effects of injecting BMCs into scarred myocardium on the regional contractility of these nonviable areas. In order to avoid the confounding effects of functional improvement in viable myocardium following revascularization, BMCs were injected directly into myocardial scars and the functional assessments were focused on these scar areas. Because all scars were revascularized, any observed changes in contractility could not be attributed to differences in perfusion.

The results show that BMCs, whether delivered by intramuscular or intracoronary routes, did not improve contractile function in chronically scarred myocardium when compared with revascularization alone. Moreover, there was no reduction in scar transmurality or volume, or any significant improvement in global left ventricular function.

Our study differs from previously published randomized trials performed in patients with myocardial scarring where the BMCs were injected into the peri-infarct viable myocardium but not into nonviable scars. The two randomized studies that injected BMCs, enriched for $CD34^+$ or $CD133^+$ cells, into the peri-infarct zone concentrated on the impact on global left ventricular function[3,5]; only the study by Hendrikx *et al.*[4] looked at the effects of treating these areas with unselected BMCs on regional as well as global function. In that study, a modest improvement in regional systolic thickening was observed following BMC treatment. The cell isolation method was similar to ours in that unselected BMCs were used; however, those investigators cultivated the BMCs overnight before administration. We harvested and administered BMCs during surgery to minimize the patient's discomfort caused by aspiration and to avoid the theoretical risk of bacterial contamination from additional manipulation. Although the overnight cultivation step might be a contributory factor for the difference in results, another report[18] suggests that additional processing has little effect on the number, viability, and functional capacity of BMCs compared with freshly harvested BMCs. The regional improvements observed by Hendrikx *et al.* might be attributable to recovery of contractile function in the peri-infarct zone rather than the scar itself.

Table 3 Effects of bone marrow cell administration on scarred myocardium.

Summary measures	Number of scar segments assessed	Number of patients	Parameter at baseline and follow-up			*P* value
			Baseline	6 months after treatment	Mean difference (95% CI), adjusted for baseline	
%SFT at rest						
Control	42	15	–6.0 ± 4.1	–0.7 ± 7.2	4.3 (0.9 to 7.6)	0.256
IM BMCs	45	17	–3.5 ± 5.0	–2.9 ± 6.2	2.0 (–1.3 to 5.2)	
IC BMCs	52	17	–5.5 ± 5.3	–4.5 ± 5.8	0.4 (–2.7 to 3.6)	
%SFT during low-dose DSE						
Control	42	15	–4.5 ± 5.2	–1.0 ± 10.7	3.8 (–0.8 to 8.5)	0.920
IM BMCs	45	17	–4.7 ± 5.0	–2.3 ± 6.9	2.6 (–1.8 to 7.0)	
IC BMCs	48	16	–5.8 ± 5.4	–2.7 ± 9.8	2.7 (–1.8 to 7.3)	
Scar transmurality						
Control	26	6	68.9 ± 11.3	75.2 ± 10.3	6.4 (–6.5 to 19.4)	0.235
IM BMCs	16	6	60.7 ± 20.6	67.1 ± 25.2	5.9 (–7.3 to 19.0)	
IC BMCs	31	8	71.0 ± 22.8	56.8 ± 27.6	–14.0 (–25.3 to –2.6)	

Abbreviations: BMCs, bone marrow cells; DSE, dobutamine stress echocardiography; IC, intracoronary; IM, intramuscular; %SFT, percent systolic fractional thickening.

Although the main focus of our study was on the regional function of the scarred myocardium, we did not observe any substantial changes in global left ventricular parameters following BMC treatment. This finding is in agreement with that of Hendrikx *et al.*[4] but in contrast to those of other studies of BMCs enriched for $CD34^+$ or $CD133^+$ that reported improved left ventricular function.[3,5] $CD34^+$ and $CD133^+$ cells are capable of assuming endothelial phenotypes *in vitro* as well as contributing to neovascularization and improvement in cardiac function *in vivo*.[19–21] This feature might explain the improved global left ventricular function in patients who received BMCs enriched with these cells in the peri-infarct zone.[3,5] Whether the use of $CD34^+$- or $CD133^+$-enriched cells is beneficial when injected directly into the scar tissue in the clinical setting remains, however, to be seen. Such comparisons across trials should be interpreted with caution, as trial methods differ substantially, namely in the site of injection, the cell types injected, and the primary end points of the study.

Whether BMCs can restore myocardial function and, if so, what are the mechanisms of action involved, remain subjects of intense debate. Our study was not specifically designed to investigate the mechanism of action of BMCs, but the lack of functional improvement in injected scar segments and no substantial reduction of infarct size indicate an absence of meaningful myocardial regeneration by BMCs. Orlic *et al.*[2] reported that BMCs can regenerate up to 60% of the infarcted myocardium in mice; subsequent studies have not, however, replicated this finding.[22,23] Similarly, there are conflicting data regarding the role of BMCs in neovascularization.[19–21,24–26] Clearly, further studies are required to clarify the role of BMCs in the context of myocardial repair, in both the acute and the chronic phases.

Another possibility for the lack of improvement in scar function could be the failure of BMCs to engraft in the chronic ischemic myocardium. Although investigation of the engraftment potential would be difficult in the clinical settings, in a rat model of chronic myocardial ischemia, BMCs grafted well following intramuscular or intracoronary administration.[27] Despite engraftment, however, there was no clear evidence of differentiation. Skeletal myoblasts have also been shown to engraft in the myocardium after transplantation, but the Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial showed no functional improvement following administration of these cells into the myocardial scar.[28]

Table 4 Effect of bone marrow cells on myocardial scar volume and global function as assessed by cardiac MRI.

Parameter	Number of patients suitable for analysis	Parameter at baseline and follow-up			*P* value
		Baseline	6 months after treatment	Mean difference, adjusted for baseline (95% CI)	
Percent volume of myocardial scar					
Control	6	39.0 ± 15.8	41.0 ± 25.9	2.4 (–9.9 to 14.7)	0.713
IM BMCs	8	31.5 ± 18.1	28.2 ± 14.0	–3.5 (–14.0 to 7.1)	
IC BMCs	8	32.3 ± 18.1	33.5 ± 23.4	1.1 (–9.4 to 11.6)	
End systolic volume					
Control	7	198.6 ± 44.6	216.1 ± 54.7	17.9 (–10.2 to 46.1)	0.095
IM BMCs	10	178.5 ± 46.0	159.5 ± 50.5	–19.3 (–42.6 to 4.1)	
IC BMCs	8	181.6 ± 40.9	191.6 ± 59.9	9.9 (–16.1 to 35.9)	
End diastolic volume					
Control	7	249.0 ± 41.1	275.4 ± 56.2	27.0 (–6.8 to 60.8)	0.146
IM BMCs	10	237.3 ± 51.5	222.6 ± 54.2	–16.1 (–44.6 to 12.3)	
IC BMCs	8	252.7 ± 47.6	259.7 ± 62.4	8.3 (–23.4 to 40.0)	
Stroke volume					
Control	7	50.3 ± 18.2	59.3 ± 11.0	3.3 (–6.9 to 13.5)	0.990
IM BMCs	10	58.7 ± 16.8	63.5 ± 15.0	3.9 (–4.3 to 12.1)	
IC BMCs	8	71.1 ± 15.4	68.2 ± 15.3	3.1 (–6.6 to 12.8)	
Ejection fraction					
Control	7	20.9 ± 8.9	22.3 ± 5.8	0.7 (–3.2 to 4.5)	0.094
IM BMCs	10	25.4 ± 8.1	29.7 ± 9.1	4.3 (1.2 to 7.4)	
IC BMCs	8	28.5 ± 6.5	27.3 ± 7.7	–0.5 (–4.1 to 3.0)	

Abbreviations: BMCs, bone marrow cells; IC, intracoronary; IM, intramuscular.

Our data confirm those from previous studies[5,6,29–32] showing the safety of both routes of BMC administration. Neither intramuscular nor intracoronary BMC injections led to myocardial damage, as shown by the lack of substantial elevation in postoperative troponin I levels and the absence of new Q-wave infarction. Furthermore, neither route of administration triggered severe ventricular arrhythmia, which had been a concern of clinical trials of myoblast transplantation in the heart.[33]

In conclusion, we have shown that BMCs, administered via intramuscular or intracoronary routes, even though safe, do not restore contractile function in chronically scarred myocardium. This study only targeted chronically scarred nonviable myocardium and therefore our findings are not transferable to other clinical settings such as acute myocardial infarction or chronically ischemic but viable myocardium.

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Competing interests
The authors declared no competing interests.

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