Bone Marrow Cell Therapy Ameliorates and Reverses Chagasic Cardiomyopathy in a Mouse Model

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Chronic chagasic cardiomyopathy, which is caused by the protozoan Trypanosoma cruzi, is a major cause of heart failure in Latin America. It is a disease for which effective treatment in its advanced clinical forms is lacking. We have previously shown that bone marrow mononuclear cell (BMC) transplantation is effective in reducing inflammation and fibrosis in the mouse model of Chagas disease. The present study used magnetic resonance imaging to assess changes in the cardiac morphology of infected mice after therapy with BMCs. Serial imaging of the BMC-treated mice revealed regression of the right ventricular dilatation typically observed in the chagasic mouse model.

Infection with the protozoan Trypanosoma cruzi causes Chagas disease, which can result in acute myocarditis and a dilated cardiomyopathy. Although treatment with antiparasitic drugs may be beneficial in cases of acute infection, once chronic cardiomyopathy has been established these drugs are no longer of value. No specific treatment exists at this stage of the disease, and management is mainly based on supportive care. At this stage, patients can remain asymptomatic for decades (in the so-called indeterminate period of the disease). Approxi-
air administered via a nose cone). A set of Gould electrocardiogram (ECG) leads with custom silver electrodes were attached to the limbs for monitoring the ECG signal. The ECG signal was transmitted to a personal computer running Ponemah Physiology Suite software (version 3.12; Data Sciences International). The ECG signal was used to monitor the status of the mice and permitted us to adjust anesthesia levels to maintain a stable physiologic heart rate (500–550 bpm). Mice were positioned in a 40-mm homebuilt bird-cage MRI coil in a 9.4-T GE Omega vertical-bore imaging system. Body temperature was maintained by a water-heating system. Heart rate and ECG were monitored continuously. MRI experiments typically lasted ~1 h per mouse, after which time the mice were allowed to recover and then returned to their cages in the animal institute. The data were analyzed using SPSS; statistically significant differences ($P < .05$) between groups are indicated by asterisks in the figures.

**Results.** We performed serial in vivo MRI experiments on infected mice treated with BMCs. Figure 1 demonstrates images obtained from age-matched control mice (panel A), chronically infected mice that were left untreated (panel B), and chronically infected mice that were treated with BMCs (panel C). Infection resulted in marked dilatation of the RV cavity (compare panels A and B), a characteristic of this particular combination of mouse and parasite strain previously described by us [8, 9]. Treatment with the BMCs resulted in reduction of the RV dilation (3

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**Figure 1.** Bone marrow mononuclear cell (BMC) treatment of chronically infected mice. Shown are magnetic resonance images of an uninfected control mouse (A), an untreated chronically infected mouse (B), and a chronically infected mouse after BMC treatment (C) (note the enlarged right ventricle [RV] in panel B and the regression in panel C; white arrows and lines indicate the RV) as well as a graph (D) of the RV inner dimension (RVID) in control mice, untreated chronically infected mice (6 months after infection [MAI]), and chronically infected mice treated with BMCs for 1 (BMC-1M) through 4 (BMC-4M) months. Graphical data are presented as mean ± SE values. *$P < .05$ (statistically significant difference) for the comparison between the indicated groups.
months after treatment), as illustrated in panel C. In this study of chronic infection, infected mice were treated with BMCs 6 months after infection and were evaluated monthly by MRI to assess the dilatation of the RV (RV inner dimension [RVID], measured in millimeters), our marker for chagasic cardiomyopathy. The results are shown in the graph in figure 1D. Most notably, the dilation of the RV was significantly reduced after 3 months of treatment with BMCs, compared with that in untreated infected mice. These results demonstrate that BMCs can cause regression of RV dilation in infected mice that are treated during the chronic stage, when significant infection-associated cardiac remodeling has already taken place. BMC treatment of uninfected mice did not alter RV dimension (data not shown).

In a second MRI experiment, mice were infected and BMC treatment was initiated 1 month after infection to determine whether BMCs prevent dilatation of the RV in treated infected mice. RV dilation was already significant 1 month after infection and increased up to 3 months after infection in untreated infected mice, at which time it stabilized. BMC treatment 1 month after infection prevented RV dilation in the treated infected mice, as shown in figure 2. This effect persisted for as long as 5 months after infection, the longest time interval examined in the present study.

Discussion. The potential use of bone marrow stem cells in therapies for ischemic heart diseases has been widely investigated, but few studies of their use in experimental chronic chagasic cardiomyopathy have been published [7, 10]. We previously demonstrated that treatment with bone marrow mononuclear cells in a mouse model of chronic chagasic cardiomyopathy significantly reduced cardiac inflammation and fibrosis [7]. This was the same in our present study (data not shown); however, in the previous report no structural evaluation was conducted. Recently, Guarita-Souza et al. [10] reported improvement of cardiac function in a rat model of Chagas disease. In that model, skeletal myoblasts and mesenchymal stem cells were cotransplanted directly into the left ventricle of the rats. The ejection fraction (as measured by echocardiography) was significantly higher in the infected
rats that received the cotransplanted cells than in sham-treated infected control rats.

The present study is the first report of a serial in vivo MRI evaluation of BMC therapy in a mouse model of Chagas disease. It also reiterates that noninvasive cardiac testing can provide complementary information when the pathogenesis of dilated cardiomyopathy in experimental models of chronic Chagas disease is being studied. Previous MRI studies of mice infected with the Brazil strain of T. cruzi demonstrated remodeling of the RV. RVID increased during the acute phase, during the early stages of the chronic phase, and during the chronic phase of infection, thus indicating that enlargement of this chamber can be a marker for murine experimental chagasic cardiomyopathy [8, 9]. Similarly, a recent report by Nunes et al. [11] demonstrated for the first time that RV dysfunction predicts mortality in humans with chagasic cardiomyopathy.

In the present study, chronically infected mice underwent cardiac MRI 6 months after infection and then monthly (1–4 months) after BMC treatment. We compared the RVID of control, untreated infected, and BMC-treated infected mice (figure 1D). There were significant increases in the RVID in all infected mice, compared with that in the control mice. The dilation was significantly reduced 3 months after BMC treatment, compared with that in untreated infected mice. The regression of the dilatation observed in the chagasic mouse model is rather striking, and we do not have a mechanistic explanation at the moment. We speculate that injected cells home to the heart and may decrease wall stress, thus contributing to a “reverse” remodeling of the heart. Recent simulations by Wall et al. [12] have suggested that addition of noncontractile material to damaged hearts may have effects on cardiac mechanics, with the possible maintenance of stroke volume and reduction in end diastolic volumes after cell injection. We also treated mice that were infected for 1 month with BMCs. At this time point during the acute stage of infection there is a robust inflammatory response [8], which may provide an environment attractive for stem cell homing and adhesion.

In recent years, the idea of using stem cells for the repair of injured myocardium has been explored primarily in the setting of acute myocardial infarction. In that regard, chagasic cardiomyopathy represents an unusual challenge in that the damage—unlike the damage secondary to acute myocardial infarction—is diffuse and not focal. Our results indicate that BMCs prevents RV dilation (figure 2). Although the underlying mechanisms are not well understood, this observation suggests that BMC therapy can prevent the cardiac remodeling process that leads to dilated cardiomyopathy. Similar findings have been reported with the use of BMCs in models of myocardial infarction, but again, mechanistic insights are not yet available [13, 14].

Our present observations are novel and are particularly important to the treatment of dilated cardiomyopathies, for which prevention or reversion of cardiac dilatation is a major therapeutic goal. Moreover, our findings also provide further evidence for the beneficial effects of BMC therapies in chagasic cardiomyopathy. The results of a pilot human study using BMCs in chagasic cardiomyopathy have been reported [15]. A controversy regarding stem cell therapy for heart disease in general is whether the stem cells actually transform into functioning myocytes or serve as a delivery vehicle for certain growth factors and cytokines. We are currently using BMCs from transgenic mice under the control of a cardiac-specific promoter to investigate this question.

References