REVIEW

Cellular therapy in Chagas' disease: potential applications in patients with chronic cardiomyopathy

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Nearly a century after its discovery, Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, remains a major health problem in Latin America. Although efforts in transmission control have contributed to a decrease in the number of new cases, approximately a third of chronic Chagasic individuals have or will develop the symptomatic forms of the disease, mainly cardiomyopathy. Chagas' disease is a progressively debilitating disease, which, at the final stages, there are no currently available treatments other than heart transplantation. In this scenario, cellular therapy is being tested as an alternative for millions of patients with heart dysfunction due to Chagas' disease. In this article, we review the studies of cellular therapy in animal models and in patients with Chagasic cardiomyopathy and the possible mechanisms by which cellular therapy may act in this disease.

Keywords: cardiomyopathy, cellular therapy, Chagas' disease, fibrosis, inflammation, stem cells

Chagas' disease

Infection with *Trypanosoma cruzi* causes Chagas' disease, also known as American trypanosomiasis. Chagas' disease is an important cause of cardiac lesions in Latin American countries. Its estimated cost is more than US$50 million per year for the care of 100 infected people, having a significant economic and social impact in endemic countries [1].

Chagas' disease is predominantly a rural endemy associated with bad habitational conditions. It is mainly transmitted to humans by the triatomine vectors, through transfusion of contaminated blood or organs, or congenitally [2]. A great effort has been undertaken in order to eradicate these vectors, and Brazil is an example of a country where this campaign has achieved great success. Transfusional transmission has been controlled in many Latin American countries by serological screening of blood. Although eradication of disease transmission can be achieved, millions of infected individuals will remain infected with the parasite for decades due to the lack of effective anti-*T. cruzi* drugs available.

T. cruzi is a hemoflagellate protozoan parasite capable of infecting different cell types in the mammalian host, with a preference to invade muscle cells, including cardiomyocytes [2]. In the acute phase of infection, parasites are easily found replicating in different tissues and organs, as well as circulating in the blood. As adaptive immune responses are stimulated, the high tissue parasitism is controlled, but not completely eradicated. States of immune suppression may bring about patent parasitism [3,4].

The acute phase is followed by a chronic phase, usually with absence of clinical symptoms, called the indeterminate form of Chagas' disease. Whereas most of the individuals will remain in this phase of the infection throughout the rest of their lives, approximately 30% develop a chronic symptomatic form of the disease after time periods ranging from a few months to decades [5]. The manifestations of chronic Chagas' disease may include cardiac, digestive and neurological disturbances [5].

The most prevalent form of chronic symptomatic disease is cardiomyopathy. One of the leading causes of heart failure in several Latin American countries, it is characterized by intense myocarditis, myocytolysis, replacement of lost cardiac tissue by fibrosis and subendocardic ischemia. These may lead to cardiomegaly, apical aneurism and arrythmias, causing, in concert, heart failure and death [2,5].

In addition to the intense inflammatory process related to the progression of heart lesions, irreversible damage to cardiac parasympathetic neurons induced by the infection during the acute phase may have cardiotoxic effects owing to the permanent and excessive sympathetic activation. As the disease develops, the hearts of patients with Chagas' disease undergo a process of cardiac remodeling. These mechanisms are activated when the area of segmental cardiac tissue damage is enough to impair the mechanical performance. The remodeling process occurs as an adaptive response by which the myocardium changes shape, size and function in reaction to increased mechanical and neurohumoral stress.

Cardiac remodeling is a maladaptive process fundamental to the progression of ischemic heart failure. The extent of remodeling is influenced by mechanical stress, inflammatory response and the activation of matrix metalloproteinases. The presence of nonfunctional myocardial segments overloads the remaining areas of the heart, induces cardiac enlargement and provokes the overexpression of biologically active molecules that could have cardiotoxic effects [6,7].

Benznidazole is the main antiparasitic drug available for the etiological treatment of Chagas' disease. This drug is effective in parasite eradication mainly in the acute phase of infection but not in the prevalent chronic stage of the disease [8]. Owing to its high toxicity and low efficacy in parasite eradication, the use of benznidazole during the chronic phase of infection has been highly debated, although a recent report indicates that it may decrease the cardiac alterations found in chronic cardiomyopathy [9].

The pathological basis of chronic Chagasic cardiomyopathy has been a matter of intense debate over the last decades [10]. One hypothesis proposes that the pathogenic inflammatory response is exclusively directed to *T. cruzi* antigens at sites of parasite persistence [11,12]. The second hypothesis is that chronic Chagasic cardiomyopathy is an autoimmune disease triggered in some individuals by *T. cruzi* infection [13–15]. These two components are probably associated, since a decrease in parasite load also leads to a decrease in myocarditis and cardiac disturbances [9].

The only available treatment for patients with end-stage heart failure due to Chagas' disease is heart transplantation. However, this procedure is rarely performed, not only because of the high cost and the scarcity of organs, but also because the use of immunosuppressive drugs after transplantation may reactivate the latent infection. Thus, millions of Chagasic cardiopaths in Latin American countries will inexorably evolve to death with limited therapeutic options other than symptomatic treatment. Therefore, the development of new therapies for patients with chronic cardiomyopathy is of great social and economical relevance.

Cellular therapy in animal models of *T. cruzi* infection

The possibility of reconstructing the damaged heart has been the subject of intense investigation in the past decade. Different types of cells, including myoblasts [16] and embryonic stem cells [17–21], were tested in animal models of cardiac lesions caused by ischemia. Although improvement of heart function was observed after treatment with these cell types, their clinical use is currently impaired due to safety and ethical issues. Treatment with embryonic stem cells, besides all the ethical issues involved in its clinical application, caused the development of teratocarcinomas in animal models [22], whereas myoblasts caused arrhythmias in patients – most probably due to the lack of gap junctions between these cells and the resident cardiomyocytes [23,24]. Therefore, the search for other cell sources for immediate use has been an intense area of investigation.

Bone marrow became an attractive source of cells for tissue regeneration after the report of pluripotent cells in this tissue [25] opened the possibility of applying their plasticity to generate cardiomyocytes [26]. In addition, owing to previous clinical experience and easy accessibility, this is a readily available source for autologous application in therapy. In 2001, Orlic and colleagues published a report showing an extraordinary potential of bone marrow-derived cells (BMCs) in the regeneration of damaged myocardium [27]. Several reports demonstrating the repair of cardiac lesions as well as functional improvement of heart function in animal models were published shortly thereafter [28–31].

The possibility of recovering the damaged heart using adult bone marrow stem cells opened the perspective of its application to regenerate the myocardium and improve heart function in chronic Chagasic cardiomyopathy. The aim was not to treat the infection, but rather the sequelae caused by the chronic inflammation in the heart. Instead of replacing the heart of Chagasic cardiopaths, the repair of the heart with the patient's own cells became an attractive option to be investigated.

To test the effects of cellular therapy, we used a mouse model of chronic Chagas' disease caused by infection with the myotropic Colombian strain [32]. Mice chronically infected with this *T. cruzi* strain developed a cardiomyopathy with histopathological and functional characteristics similar to those observed in humans [33]. Chronically infected mice were treated intravenously with syngeneic BMCs obtained from normal littermates. Inflammation and fibrosis were evaluated at various times after treatment. A significant decrease in inflammation was observed 2 months after cellular therapy, an effect sustained up to 6 months after treatment [32]. The

efficacy of BMC therapy was dose-dependent and could be reproduced using BMCs obtained from chronically infected littermates. This last observation was relevant in proposing a translation to clinical studies using autologous BMCs to treat Chagasic cardiopaths.

Another effect of BMC therapy in chronic Chagasic mice was a significant reduction in fibrosis in the heart [32]. A reduction of approximately 60% was found after 2 months of treatment, compared with saline-treated controls, as assessed by morphometry in sirius red-strained heart sections. Fibrosis accumulation may lead to conduction disturbances and cardiac malfunction. Whether inflammation and fibrosis reduction after BMC therapy results in an improvement of contractile activity and a decrease in arrhythmias characteristic of the disease is an issue still to be determined in the mouse model.

In the rat model of *T. cruzi* infection using a different therapeutic scheme, improvement of ejection fraction (EF%) was observed. Rats infected with *T. cruzi* usually control the infection and remain in an indeterminate form of the disease. In the studies carried out by Guarita-Souza and colleagues, chronically infected rats showing cardiac disturbances characteristic of Chagas' disease (EF <37%) were treated with autologous skeletal myoblasts cocultured for 14 days with bone marrow-derived mesenchymal stem cells. The cell preparation was injected directly into the left ventricle wall. A significant improvement of EF% was observed in the animals treated with the cells, whereas, in the control group, which received culture medium injection, no significant difference in EF% was observed. In addition, a decrease in left ventricle end-diastolic volume and left ventricle end-systolic volume were also observed after cellular therapy [34]. Histopathological analysis of the heart tissue indicated the presence of myogenesis and angiogenesis after treatment with myoblasts cocultured with mesenchymal stem cells [35]. Whether this therapy will cause conduction disturbances in Chagasic patients, as previously seen in patients transplanted with skeletal myoblasts, is still to be determined.

An alternative to BMC infusion for cardiac therapy is the use of cytokines able to mobilize BMCs, such as granulocyte colony-stimulating factor (G-CSF). This therapy is attractive since G-CSF is already used in clinical practice, has mild side effects and is a less invasive treatment than bone marrow aspiration and cell infusion into the coronaries. Using the mouse model of Chagas' disease, we tested the efficacy of G-CSF treatment. Groups of chronically infected mice received repeated injections of G-CSF by subcutaneous route. A significant decrease in inflammation and fibrosis was seen in hearts of Chagasic mice treated with multiple administrations of G-CSF, compared with saline-treated controls. In addition, Chagasic mice treated with BMCs followed by G-CSF showed a significant decrease in inflammation and fibrosis compared with animals treated with BMCs only (Santos *et al.* Unpublished Data). The results indicate that, in addition to the number of cells, the number of administrations should be taken into account while searching for a more effective cellular therapy for Chagas' disease.

A major concern in Chagasic patients is the residual parasite load found in individuals during the chronic infection. In the mouse model of Chagas' disease, we did not observe reactivation (appearance of patent parasitemia and intense tissue parasitism) of the infection after BMCs and/or G-CSF therapy by evaluation of blood samples and heart sections of treated mice.

Clinical studies in Chagasic patients

The results obtained in the aforementioned mouse model of chronic Chagasic cardiomyopathy led us to propose a Phase I clinical trial of autologous BMC therapy in patients with heart failure due to Chagas' disease. A total of 28 patients were included in this study, all having New York Heart Association (NYHA) functional class III or IV prior to treatment. These patients had a very bad prognosis unless heart transplantation could be performed. Due to difficulties related to organ supply and to the cost of the procedure, most patients in this condition would die within a few years without a therapeutic option.

BMCs were collected from each patient by multiple punctures of the iliac crests, and a mononuclear-enriched cell fraction was isolated by centrifugation in a ficoll-hypaque gradient. A mean of 235 million cells were resuspended in 5% human serum albumin saline solution and slowly injected into the coronary arteries using an angioplasty catheter. After cell injection, patients were sent to an intensive care unit for 24 h where electrocardiogram and cardiac enzyme levels were monitored, and then discharged to a ward for an additional 48 h before being sent home. Patients were evaluated before and after the

procedure to determine safety as well as potential benefits of the therapy. In addition to the cell infusion, patients received, on day 25 post-transplant, five daily administrations of G-CSF (5 µg/kg) as a therapy boost [36].

Observation of several clinical, biochemical and cardiological parameters during and after the procedure indicated that it was safe, since no complications could be directly related to the procedure. In addition, we demonstrated the feasibility of performing marrow puncture and coronary cell infusion in chronic Chagasic patients. A significant improvement on several parameters during a 60-day follow-up also suggested a potential benefit of the therapy. These included a decrease in the NYHA class (3.1 ± 0.3 to 1.8 ± 0.5; $p < 0.0001$) and in the Minnesota life quality questionnaire (50.9 ± 11.7 to 21.8 ± 13.4; $p < 0.0001$). An increase in the distance walked in 6 min (355 ± 136 to 443 ± 110 m; $p < 0.003$) and in left ventricular EF (20.1 ± 6.8 to $23.0 \pm 9.0\%$; $p = 0.02$) was also found. In addition, serum sodium levels, which are low in these patients (and are a bad prognostic indicator in patients with heart failure), were significantly increased after treatment.

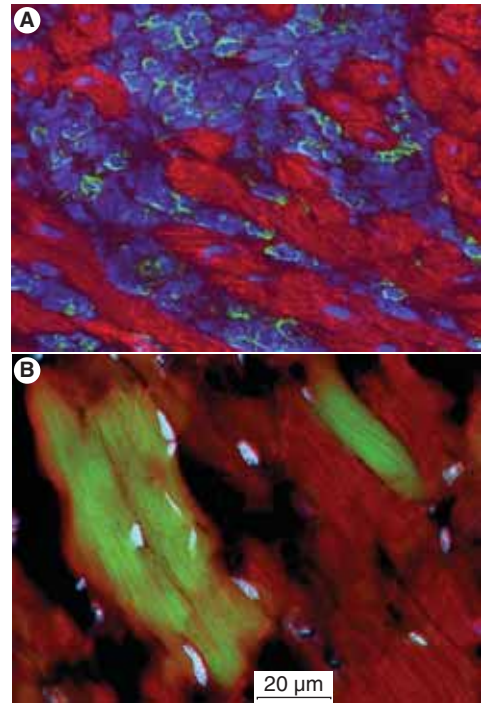
In order to demonstrate the efficacy of the procedure, a Phase II/III clinical trial sponsored by the Brazilian Ministry of Health is now underway involving 300 chronic Chagasic patients [37]. This multicenter, randomized, double-blind, placebo-controlled trial will allow a critical evaluation of the efficacy of BMC therapy in the setting of chronic Chagasic cardiomyopathy. The trial started enrolling patients in June 2005 and is expected to end in June 2008. In addition, based on our experimental results, we are also carrying out, as an alternative for cellular transplantation, a Phase I/II clinical trial of G-CSF therapy with multiple administrations. This is a randomized, double-blind and placebo-controlled trial.

Mechanisms of action

Using BMCs derived from green fluorescent protein (GFP)-transgenic mice, we observed the presence of transplanted cells in the hearts of Chagasic mice [32], suggesting that, after systemic injection of BMCs, chemotatic factors released by the inflamed heart are able to promote cell recruitment (Figure 1A). In addition, a few days after transplantation, we observed the appearance of GFP^+ cells co-expressing α-cardiac myosin in the hearts of cell-injected animals, indicating that some may have transdifferentiated into cardiomyocytes or fused with resident cells (Figure 1B). As in other models of cellular therapy in heart diseases, these few transdifferentiated/fused cells do not seem to account for the observed effects. In addition, it is possible that new cardiomyocytes are generated from endogenous stem cells or even mature proliferating resident cardiomyocytes. A growing body of evidence indicates that most effects of stem cell therapy in cardiac diseases are in fact due to paracrine effects [38,39], through secretion of cytokines that may act on resident mature cells and/or in tissue-specific stem cells.

To investigate the mechanisms by which inflammation is decreased after cell therapy in the mouse model of Chagas' disease, we evaluated the number of apoptotic cells in the hearts of chronic Chagasic mice both treated and not treated with BMCs by terminal transferase

Figure 1. Presence of bone marrow-derived cells in hearts of chronic Chagasic mice.



C57BL/6 mice were transplanted with green fluorescent protein $(GFP)^+$ bone marrow mononuclear cells during the chronic phase of infection by *Trypanosoma cruzi*. **(A)** GFP^+ cells (green) in heart section stained with anti α-cardiac myosin (red) and with DAPI nuclear staining (blue). **(B)** Confocal image of cells co-expressing cardiac myosin (red) and GFP (green) in a heart section stained with DAPI (blue).

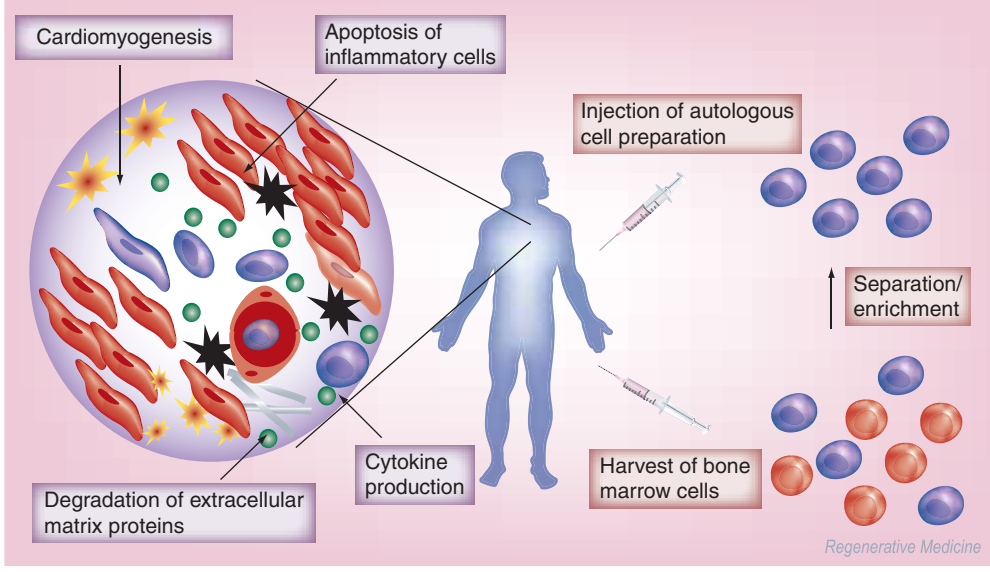
2´-deoxyuridine-5´-triphosphate nick-end labeling assay. BMC-treated hearts had an increased number of apoptotic inflammatory cells compared with untreated Chagasic controls, suggesting a local immunomodulatory action of the transplanted BMCs [32]. In fact, immunosuppressive effects of mesenchymal cells obtained from bone marrow have been recently demonstrated [40], which may contribute to the phenomenon observed in Chagasic hearts after BMC therapy. A complementary DNA-based microarray analysis, which is currently being carried out, may help clarify this phenomenon and other effects of BMC therapy. Importantly, we did not observe an increase of apoptotic cardiomyocytes after therapy, which does not appear to be a mechanism of cardiomyocyte death in Chagas' disease [41].

A marked effect of BMC therapy in the mouse model is fibrosis regression. A decrease in fibrosis after BMC therapy has been seen in models of other pathologies, such as liver fibrosis [42,43] and heart infarct [27,31]. Fibrosis results from an imbalance between collagen deposition and degradation, which is regulated by several factors with pro- and anti-fibrosant activities, such as metalloproteinases and their inhibitors, cytokines and other soluble mediators. An example is tumor necrosis factor (TNF), a cytokine associated with fibrosis deposition and heart dysfunction in various models of cardiomyopathy [44–46], which is increased in hearts of Chagasic mice (Santos *et al.* Unpublished Data) and in patients [47,48]. Since TNF is produced by different cell types – mainly by inflammatory cells – the fact that inflammation is decreased after cellular therapy suggests that this phenomenon may cause a decrease of TNF production. We are currently investigating whether this and other factors are indeed modulated after cellular therapy.

The degradation of extracellular matrix (ECM) components could contribute to a better interaction between the ECM and the cardiomyocytes, which is altered in Chagas' disease, causing myocardial compliance and disruption of synchronous contraction of the ventricles during systole, therefore contributing to ventricular dysfunction [49]. Thus, the decrease in cardiac fibrosis after cellular therapy may lead to an improvement in the contractile function of cardiomyocytes. In addition, the generation of new cardiomyocytes may also contribute to this process, which, in concert, may explain the improvement in EF% observed in Chagasic patients [36].

It is not known at the moment which cell population in the mononuclear fraction is responsible for the effects observed. The cell fraction used in the mouse model of chronic Chagasic cardiomyopathy is highly heterogeneous and contains mature cell populations as well as various types of stem and precursor cells. Therefore, it is possible that more than one population acts to improve the heart in this model. Although the identification of the relevant cell

Figure 2. Mechanisms by which bone marrow-derived cells may repair the heart of Chagasic patients.



population(s) may be important to improve therapy, it is of note that the methodology used is simpler and less expensive than purifying cell preparations and has allowed a fast translation to clinical studies.

G-CSF treatment in ischemic cardiopathy is controversial but some authors claim it causes a repair of the myocardium and an improvement in heart function. This cytokine has been reported to play multiple roles, including recruitment of bone marrow-derived stem cells, stimulation of neovascularization [50], protection of cardiomyocytes from death by apoptosis, decrease in fibrosis [51,52] and even the generation of new cardiomyocytes [53,54]. In our model of chronic Chagasic cardiomyopathy, we observed a decrease in heart fibrosis. As areas of ischemia and cardiomyocyte apoptosis are also found in chronic Chagasic cardiomyopathy, we have yet to investigate this cytokine's mechanism of action in Chagasic mice.

A summary of possible effects of bone marrow-derived cellular therapy based on the observations in animal models is shown in Figure 2.

Future perspective

Although the preliminary results in clinical trials are encouraging, we are still a long way from being able to offer a therapy fully capable of reconstituting all of the lost tissue damaged by years or decades of inflammatory aggression to the heart. Nonetheless, a small gain in function seems to reflect in a significant improvement in life quality and, therefore, should not be ignored. In addition, the patients submitted to these first attempts of heart repair had very severe cardiomyopathy and, thus, the therapy may have been more efficient in initial or intermediate stages of the disease. As we are still taking the first steps towards the development of a new therapy, many issues, such as the best cell population, number of cells and administrations, and associations with cellular hormones, still need to be investigated in order to achieve a desired curative effect.

The fact that chronic Chagasic cardiomyopathy is characterized by the presence of diffuse inflammation may be of relevance when compared with other heart diseases, such as myocardial infarction and non-Chagasic dilated cardiomyopathy. In chronic lesions caused by infarction, as well as in many cases of non-Chagasic dilated cardiomyopathies, inflammation is mild or absent, and may not properly attract the cells to the lesion site. In addition, fibrosis deposition is spread throughout the heart, whereas

Executive summary

Need for cell-based therapy for chronic Chagasic cardiomyopathy

- Chronic Chagasic cardiomyopathy is one of the leading causes of death by heart failure in Latin American countries.
- Inflammation, fibrosis, arrythmias, cardiomegaly and parasite persistence are characteristic of chronic Chagasic cardiomyopathy.
- The only therapeutic option at end-stage chronic Chagasic cardiomyopathy is heart transplantation.
- The development of cell-based treatments to repair the damaged heart and to improve cardiac function in patients with chronic Chagasic cardiomyopathy is of great importance.

Effects of cellular therapies in models of chronic Chagasic cardiomyopathy

- Treatment with bone marrow-derived cells decreases inflammation and fibrosis.
- Bone marrow-derived cardiomyocytes are formed after cell transplantation.
- Injection of bone marrow-derived mesenchymal stem cells cocultured with myoblasts improves heart function.
- Treatment with granulocyte colony-stimulating factor causes a decrease in inflammation and fibrosis in the heart.

Effects of cellular therapies in patients with chronic Chagasic cardiomyopathy

- Treatment with bone marrow-derived cells improved cardiac function and serum sodium levels.
- A significant improvement in quality of life was reported by patients after therapy.
- The New York Heart Association class decreased after therapy, indicating an amelioration in cardiac function.
- No adverse events could be related to the therapy, thus indicating its safety.

Mechanisms of action of cell-based therapies in chronic Chagasic cardiomyopathy

- Apoptosis of inflammatory cells occurs after therapy with bone marrow-derived cells.
- Myogenesis and angiogenesis are stimulated after injection of bone marrow-derived mesenchymal stem cells cocultured with myoblasts.
- Granulocyte colony-stimulating factor treatment may induce neovascularization, rescue cardiomyocytes from apoptosis and recruit bone marrow-derived stem cells.
- Studies in other models of cardiac diseases suggest that stem cells act mainly by paracrine effects.

fibrosis after infarction is limited to the infarcted area, which may render it more difficult for the cells to penetrate and act in the damaged tissue.

The investigation of molecular and cellular mechanisms of action will certainly contribute to the development of new strategies for the treatment of this and, possibly, other diseases. If proven that the main effect of cellular therapy in heart diseases is the stimulation of endogenous stem cells, it may be possible to perform *ex vivo* expansion of these cells or to use cellular hormones to stimulate them to repair the damaged heart.

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