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Phenotypic and functional characterization of mesenchymal stromal cells isolated from pediatric patients with severe idiopathic nephrotic syndrome

NADIA STARC[1], MIN LI[2], MATTIA ALGERI[1], ANTONELLA CONFORTI[1], LUIGI TOMAO[1], ANGELA PITISCI[1], FRANCESCO EMMA[3], GIOVANNI MONTINI[4], PIERGIORGIO MESSA[5], FRANCO LOCATELLI[1,6], MARIA ESTER BERNARDO[1,*] & MARINA VIVARELLI[3,*]

[1]Department of Paediatric Haematology-Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Ospedale Pediatrico Bambino Gesù, Rome, Italy, [2]Renal Research Laboratory, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico di Milano, Milan, Italy, [3]Department of Pediatric Subspecialties, Division of Nephrology, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy, [4]Pediatric Nephrology and Dialysis Unit, Department of Clinical Sciences and Community Health, Fondazione IRCCS Cà Granda IRCCS Ospedale Maggiore Policlinico di Milano, Università degli studi di Milano, Milan, Italy, [5]Unit of Nephrology, Dialysis and Renal Transplant, Department of Medicine, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico di Milano, Università degli studi di Milano, Milan, Italy, and [6]Department of Paediatrics, University of Pavia, Pavia, Italy

Abstract

Background. Idiopathic nephrotic syndrome (INS) is one of the most common renal diseases in the pediatric population; considering the role of the immune system in its pathogenesis, corticosteroids are used as first-line immunosuppressive treatment. Due to its chronic nature and tendency to relapse, a significant proportion of children experience co-morbidity due to prolonged exposure to corticosteroids and concomitant immunosuppression with second-line, steroid-sparing agents. Mesenchymal stromal cells (MSCs) are multipotent cells that represent a key component of the bone marrow (BM) microenvironment; given their unique immunoregulatory properties, their clinical use may be exploited as an alternative therapeutic approach in INS treatment. *Methods.* In view of the possibility of exploiting their immunoregulatory properties, we performed a phenotypical and functional characterization of MSCs isolated from BM of five INS patients (INS-MSCs; median age, 13 years; range, 11–16 years) in comparison with MSCs isolated from eight healthy donors (HD-MSCs). MSCs were expanded *ex vivo* and then analyzed for their properties. *Results.* Morphology, proliferative capacity, immunophenotype and differentiation potential did not differ between INS-MSCs and HD-MSCs. In an allogeneic setting, INS-MSCs were able to prevent both T- and B-cell proliferation and plasma-cell differentiation. In an *in-vitro* model of experimental damage to podocytes, co-culture with INS-MSCs appeared to be protective. *Discussion.* Our results demonstrate that INS-MSCs maintain the main biological and functional properties typical of HD-MSCs; these data suggest that MSCs may be used in autologous cellular therapy approaches for INS treatment.

Key Words: *bone marrow niche, idiopathic nephrotic syndrome, immunomodulatory properties, mesenchymal stromal cells*

Introduction

Idiopathic nephrotic syndrome (INS) is the most frequent glomerular disease of childhood, affecting 3/100,000 children younger than the age of 16 years. Its main clinical features are edema with hypoalbuminemia and hyperlipidemia secondary to loss of the integrity of the glomerular filtration barrier (GFB) leading to intense proteinuria [1]. In children, first-line therapy with oral glucocorticoids leads to complete remission in about 80% of cases [2]. Those who do not respond to a standard course of steroid therapy are defined as steroid-resistant, and a significant percentage of these patients are subsequently found to harbor a mutation in genes coding for proteins involved in the integrity of the glomerular basal membrane (GBM) [2]. Generally, nongenetic forms of nephrotic syndrome respond either to first-line

*Co-last authors.
Correspondence: **Marina Vivarelli**, MD, Division of Nephrology, IRCCS Ospedale Pediatrico Bambino Gesù, Piazza S Onofrio 4, 00165, Rome, Italy. E-mail: marina.vivarelli@opbg.net

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therapy or to second-line immunosuppression with calcineurin inhibitors, mycophenolate mofetil and, more recently, anti-CD20 monoclonal antibodies. They can have a more or less severe form of disease with periodic relapses, the frequency of which is used in the classification of INS severity [3]. For all forms that are nongenetic and responsive to systemic immunosuppression, the mechanism leading to the disruption of the GFB is unknown. Clinical data, including the response of the disease to drugs with immunosuppressive and anti-inflammatory properties, and the tendency of severe forms to recur in the engrafted kidney following transplantation suggest an immune-mediated pathogenesis, with involvement of one or more circulating factors [4]. Several lines of evidence have implicated different facets of immune system dysregulation in determining damage to the GFB. Since 1974, a T-cell abnormality has been suggested [5], and studies performed both in patients with INS and in murine models of the disease have suggested altered levels of T-cell subpopulations, as well as altered circulating cytokine levels [6,7]. More recently, the finding of a clear effectiveness of rituximab in treating steroid-dependent forms of INS has suggested a role for B cells in INS pathogenesis. In particular, the correlation between reappearance of memory B cells and relapse following rituximab infusion suggests a pathogenetic role for this B-cell subpopulation in INS [8].

In patients with severe forms of INS, the long-term burden of disease is significantly increased by treatment-associated toxicity [9,10]. Prolonged therapy with steroids is minimized by the use of one or more second-line agents, and, in this respect, the recent addition of anti-CD20 therapy has substantially improved the outlook for many patients [11]. However, even anti-CD20 therapy is not without risks, especially when repeated infusions are used in a pediatric setting [12]. Based on these considerations, there is an urgent need for safe and effective immunomodulation in INS.

Mesenchymal stromal cells (MSCs) are multipotent cells that can be isolated from several human tissues, including bone marrow (BM), and consistently expanded *ex vivo* for clinical use [13–15]. MSCs are endowed with unique immunomodulatory and anti-inflammatory properties directed toward cells involved in both the adaptive and innate immune responses [16,17]. Moreover, they are able to home to sites of injury and to promote tissue repair [18,19]. Because of these properties, their therapeutic use has been evaluated in a number of clinical settings, for example, to treat steroid-resistant acute graft-versus-host disease in the context of hematopoietic stem cell transplantation (HSCT) [20,21], to repair inflamed fistulas in Crohn's disease, to prevent chronic rejection in solid organ transplantation and in a variety of allo- and auto-immune disorders, including relapse of nephrotic syndrome following renal transplantation [15,22–25]. Moreover, many studies demonstrate that the use of autologous MSCs obtained from patient's tissues can be a feasible, safe and beneficial therapy in several diseases, including renal glomerulopathies [22,26–36].

The use of autologous MSCs may have some advantages over that of allogeneic MSCs, including the reduction of the risk of rejection, immunization and transmission of infectious agents, particularly in recipients who are not profoundly immunosuppressed. However, it is still debated whether the potency of autologous MSCs derived from patients bearing an immune system dysregulation is comparable with that of healthy donor (HD) MSCs. Therefore, before using patient-derived MSCs in any clinical context, it is mandatory to investigate whether these cells are endowed with the same full regenerative, differentiation and immunomodulatory properties [37,38].

In this pilot study, we have isolated and expanded *ex vivo* MSCs from the BM of five children with severe forms of INS with the aim to assess their phenotypic and functional characteristics, in view of their potential clinical application in immunomodulatory and regenerative cellular therapy approaches to the treatment of INS.

Patients and methods

INS patients and HDs

Five children (three males and two females; median age, 13 years; range, 11–16 years) diagnosed with INS at the Division of Nephrology and Dialysis, Bambino Gesù Children's Hospital, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy, between September 2013 and December 2015 were included in the study. Patient BM was collected, after obtaining parental informed consent, during diagnostic procedures (renal biopsy) according to the Bambino Gesù Children's Hospital–approved research protocol for MSC study in INS patients. As controls, we used MSCs isolated from residual cells of eight HDs (five males and three females; median age, 11 years; range, 5–18 years), who donated BM for transplantation to a sibling at the Bambino Gesù Children's Hospital. Patient and control legal representative signed a specific written informed consent. The study was conducted according to the Declaration of Helsinki. Table I depicts patients' characteristics including immunosuppressive treatments ongoing at time of BM and peripheral blood collection.

HD-derived peripheral blood mononuclear cells (PBMCs) were isolated from buffy-coats obtained from the Unit of Immuno-Hematology and Transfusion