The International Journal of Biochemistry & Cell Biology 42 (2010) 1893–1899

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Bone marrow cells are a source of undifferentiated cells to prevent Sjögren's syndrome and to preserve salivary glands function in the non-obese diabetic mice

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ARTICLE INFO

Article history:
Received 30 April 2010
Received in revised form 25 July 2010
Accepted 13 August 2010
Available online 21 August 2010

Keywords:
Sjögren's syndrome
Bone marrow cells
Cell therapy
Xerostomia
Salivary gland
Saliva

ABSTRACT

Non-obese diabetic (NOD) mice develop Sjögren's-like syndrome (Ss) and a gradual loss of saliva secretory function. Our previous study showed that injections of matched normal spleen cells with Complete Freund's Adjuvant (CFA) reversed salivary gland dysfunction in 14-week-old NOD mice, which had established Ss. The spleen and bone marrow are closely related organs, and both are among the first sites of hematopoiesis during gestation. Noticing a rapidly increasing number of clinical trials using bone marrow (BM) cells treatments for autoimmune diseases, we tested if BM cells can prevent Ss and restore salivary glands' function. We injected CFA and MHC class I-matched normal BM cells in 7-week-old NOD mice, which had not yet developed Ss. We found at week 52 post-treatment that all NOD mice receiving BM cells and CFA had a recovery of salivary flow and were protected from Ss and diabetes. BM cells-treated mice had their salivary function restored quantitatively and qualitatively. Saliva flow was higher ($p < 0.05$) in BM cells-transplanted mice when compared to control mice, which continued to deteriorate over time. Total proteins, epidermal growth factor, amylase, and electrolytes concentrations in saliva of BM cells-treated mice were not significantly changed at week 44 and 52 post-therapy when compared to pre-therapy (when the mice did not have Ss). Restoration of salivary flow could have resulted from a combination of rescue and paracrine effects from BM cells. This study suggests that a combined immuno- and cell-based therapy can permanently prevent Ss and restored salivary function in NOD mice.

1. Introduction

Sjögren's syndrome, which affects people with a frequency of 1 in 100, is characterized by an autoimmune destruction of the salivary and lacrimal glands. As a result, patients with Sjögren's syndrome suffer from dry mouth and dry eyes (Delaleu et al., 2005; Lee et al., 2009). Salivary glands have various cell types: acinar cells which are responsible for water and proteins secretion, ductal cells that principally regulate the composition of saliva, and myoepithelial cells surrounding the acini and ducts (Lombaert et al., 2008). In Sjögren's syndrome the immune system attacks the salivary glands, particularly the acinar cells. This leads to a loss of saliva secretion and the patients' quality of life is severely compromised due to xerostomia (dry mouth), severe dental caries, and oral infections (Fox and Speight, 1996; Delaleu et al., 2005; Lombaert et al., 2008; Lee et al., 2009; Nikolov and Illei, 2009). Unfortunately, there is no suitable treatment for Sjögren's syndrome, because the current pharmacological therapy that depends on the stimulation of residual acinar cells frequently fails, since in many cases all the salivary secretory tissue has already been lost (Tran et al., 2006). Regeneration of destroyed salivary glands and restoration of their function would greatly improve the quality of life for these patients.

Non-obese diabetic (NOD) mice, a frequently used animal model of Sjögren's syndrome and type 1 diabetes mellitus (T1DM), both exhibit infiltrates of lymphocytes in the salivary glands (sialadenitis) with a gradual loss of salivary function and in the pancreas (insulitis) (Jonsson et al., 2007; Kodama et al., 2003; Lee et al., 2009; Soyfoo et al., 2007a,b; Tran et al., 2007). The reduced saliva output is similar to what is observed in patients (Tran et al., 2007). Previous studies from our group and collaborators have shown that a two-limb intervention can permanently restore lost func-

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doi:10.1016/j.biocel.2010.08.008

tion in Sjögren's syndrome and T1DM in NOD mice (Kodama et al., 2003; Tran et al., 2007). This two-limb intervention consists of using complete Freund's adjuvant (CFA) as the first limb, and then combining this with matched Major Histocompatibility Complex (MHC) class I spleen cells, as the second limb. The rationale of this two-limb intervention is that cellular immunity (T lymphocytes) plays a major role in the pathophysiology of Sjögren's syndrome (Katsifis et al., 2007). NOD mice have a defect in the production of the low-molecular-weight protein 2, LMP2, leading to a problem in T cell selection. This results in the presence of autoreactive T cells and development of autoimmune diabetes and Sjögren's-like syndrome (Hayashi and Faustman, 1999). LMP2 is a catalytic subunit of the proteosomes, which are very large protein complexes inside all eukaryotes and their role is to degrade all proteins that the cell has no more use for. This proteasome defect also impairs the processing of nuclear factor κB (NF-κB), a transcription factor that stimulates the expression of genes involved in a wide variety of biological functions, including the protection from TNF-α induced apoptosis. The disruption of its function in antigen presenting cells results in the escape of autoreactive T cells from proper immune selection. NF-κB defect also increases the apoptosis of misselected T-cells by TNF-α-induced apoptosis (Ryu et al., 2001). Treatment of NOD mice with a TNF-α inducer such as CFA, promotes the apoptosis of autoreactive T-cells and eventually removes the autoimmunity (Kodama et al., 2003; Tran et al., 2007). Once the autoimmunity is removed, restoration of salivary glands' function was achieved.

Our previous report used spleen cells (Kodama et al., 2003). However, this population of cells is not easily obtained from patients (except from trauma cases). Bone marrow cells (BM cells) are clinically easier to harvest (either by needle aspiration or by mobilization to the blood). The spleen and bone marrow are closely related organs, and both are among the first sites of hematopoiesis during gestation. There are reports that BM cells have been used to treat autoimmune diseases (Bocelli-Tyndall et al., 2007; Burt et al., 2009; Lowenthal et al., 1993; Miniati et al., 2009; Oyama et al., 2005, 2007; Saccardi et al., 2006; Tran et al., 2003; Van Laar and Tyndall, 2006). BM cells have been suggested as a source of multipotent stem cells; particularly the marrow derived stromal cells, also known as mesenchymal stem cells (MSCs) with their ability to repair non-hematopoietic organs, including the salivary gland and pancreas (Couzin, 2006; Lombaert et al., 2006, 2008; Orlic et al., 2001; Urban et al., 2008). MSCs that can be isolated from a variety of tissues were shown to interact with all cells of the innate and adaptive immune system to modulate their function. Following systemic administration they home to injured tissues and can suppress the pro-inflammatory cytokines to help survival. In addition to immuno-modulation they can also regenerate bone, fat, cartilage and cells of other lineages (Uccelli and Pistoia, 2008; Zhao et al., 2009). The plasticity and immunosuppressive capability of MSCs have made them a novel therapeutic choice in autoimmune diseases (Aguayo-Mazzucato and Bonner-Weir, 2010; Zhao et al., 2009). MSCs can assist in the regeneration of the pancreas and salivary glands in mice (Lombaert et al., 2008; Urban et al., 2008). Transplantation of BM cells boosted levels of serum insulin and decreased blood sugar levels in diabetic mice by mechanisms such as the re-construction of β-cell islets (the insulin-secreting cells), secretion of growth factors to endothelial progenitor cells, or by direct cell differentiation (Aguayo-Mazzucato and Bonner-Weir, 2010; Uccelli and Pistoia, 2008). In summary, BM cells are capable to differentiate into other cell types, as well as to provide a beneficial effect by secreting cytokines and/or growth factors (Burt et al., 2009; Coppes et al., 2009; Uccelli and Pistoia, 2008). From all these reasons, the objective of this study was to assess whether the use of BM cells, instead of spleen cells, in our two-limb intervention (described above) could prevent Sjögren's syndrome and restore salivary glands' function in NOD mice. In addition, this study improved on our previous one (Tran et al., 2007) by: (a) providing therapy at an early time point (7-week-old NOD mice, as compared to our previous study using 14-week-old NOD mice with established Sjogren's syndrome), (b) following the mice daily for 52 weeks, (c) by including a group of NOD mice treated with CFA only (to allow comparison to the group of mice treated with CFA + BM cells), and (d) by assessing the composition/quality of saliva (as compared to its quantity only). These improvements all add new information to the current literature.

2. Materials and methods

2.1. Animals

Seven-week old female NOD mice (which had not yet developed Sjögren's syndrome; Taconic Farms, Germantown, NY) and aged-matched CByF1B6F1/J (CByB6F1) mice (Jackson Laboratory) were maintained under pathogen-free conditions in the Animal facility at McGill University. Female NOD mice were divided into three different groups (10 mice per group) and were followed for 52 weeks after treatments with either: (a) bone marrow cells transplant plus CFA (BM cells group), (b) only CFA injected (CFA group), or (c) no cell injection, no CFA, but daily injections of insulin to control blood sugar levels (Control group).

2.2. Cell transplantation

Bone marrow cells of male CByB6F1 mice (1×10^7 cells) were harvested and freshly injected into female NOD recipients (of the BM cells group) through the tail vein, twice a week for six consecutive weeks (Kodama et al., 2003; Tran et al., 2007; Urban et al., 2008; Zhao et al., 2009). No bone marrow cells were injected into NOD mice in the CFA group or Control groups. Complete Freund's adjuvant (CFA, Difco, Detroit, MI) was freshly mixed with an equal volume of physiological saline and then injected (50 μl) into each hind footpad simultaneously with the first bone marrow cells injection. CFA was also injected once in NOD mice of the CFA group. No CFA was injected in mice of the Control group.

2.3. Salivary flow rate

Secretory function of the salivary glands (salivary flow rate) was obtained by inducing mild gas anesthesia to NOD mice with 5% Isoflurane, and 1.5–1 L/min Oxygen (as per animal facility protocols at McGill University). Whole saliva was collected after stimulation of secretion using 0.5 mg Pilocarpine/kg body weight administered subcutaneously. Saliva was obtained from the oral cavity by micropipette, placed into pre-weighed 0.5-ml microcentrifuge tubes. Saliva was collected for a 10-min period and its volume determined gravimetrically. Salivary flow rate was determined at baseline (week 0, when NOD mice were 7-week old; before transplantation started) and at weeks 2, 12, 34, 38, and 52 post-transplantation.

2.4. Blood sugar

Blood glucose levels from NOD mice were monitored once a week (Accu-Check, Roche Diagnostics, Laval, QC, Canada). The mice were diagnosed with diabetes after observing two consecutive daily blood glucose concentrations of >200 mg/dl. These diabetic mice were injected with insulin on a daily basis (Humulin N, Lilly, ON, Canada).