# Xenograft of Microencapsulated Sertoli Cells for the Cell Therapy of Type 2 Diabetes Mellitus in Spontaneously Diabetic Nonhuman Primates: Preliminary Data

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## ABSTRACT

Insulin resistance in type 2 diabetes mellitus (T2DM) may be due to a chronic inflammation of the visceral adipose tissue (VAT) leading to local and systemic increases in proinflammatory cytokines. Microencapsulated porcine Sertoli cells (MC-pSC), by provision of immunomodulatory and trophic factors, have been successfully used to reduce such inflammation in rodent animal models of type 1 diabetes with no complications or deleterious side effects. Herein, we have begun to investigate this novel and safe therapeutic approach in the spontaneously obese nonhuman primate with spontaneous, insulin-dependent T2DM. After MC-pSC intraperitoneal injection we have evaluated, throughout a 6-month follow-up period, daily ad libitum fed glucose levels, daily exogenous insulin supplementation, biweekly body weight measurements, periodic fasting blood glucose concentrations, glycated hemoglobin (HbA1c) levels, glucose tolerance tests (GTT), and fluorescence-activated cell sorting cytometry (FACS) assessment of peripheral blood mononuclear cells. Very preliminarily, we have observed a slight reduction in fasting (FPG) and mean nonfasting (NF) plasma glucose levels. We found minimal changes, only in 1 animal, in daily exogenous insulin requirements and HbA1c levels. Flow cytometric analysis was associated with decrease in $CD8^+$ cells only in 1 recipient with a reduction in mean regulatory T Cells (Treg), whereas interestingly, decrease of B lymphocytes was observed in both animals. These results may suggest that this novel MC-SC–based transplantation protocol might possibly impact the metabolic status of T2DM in higher mammals that are close to humans.

TYPE 2 diabetes mellitus (T2DM), a chronic metabolic disease characterized by insulin resistance and progressive insufficiency of insulin secretory patterns with hyperglycemia, is preceded by a long history of visceral obesity and insulin resistance, ultimately leading to severe functional decline of β cells. A major role in the pathogenesis of insulin resistance is played by chronic inflammation of the VAT due to tissue infiltration of activated macrophages (tumor necrosis factor [TNF]-α, interleukin [IL]-1β, and IL-6), $CD8^+$ and $CD4^+$ proinflammatory lymphocytes, interferon (IFN)-γ, and B lymphocytes [1]. Sertoli cells (SC) have been shown to be able to provide immunomodulatory and trophic factors that improve survival and development of different cell types. They have been proven to hold potent immunomodulatory properties in many diseases such as type 1 diabetes mellitus [2]. The aim of this work was to verify whether the

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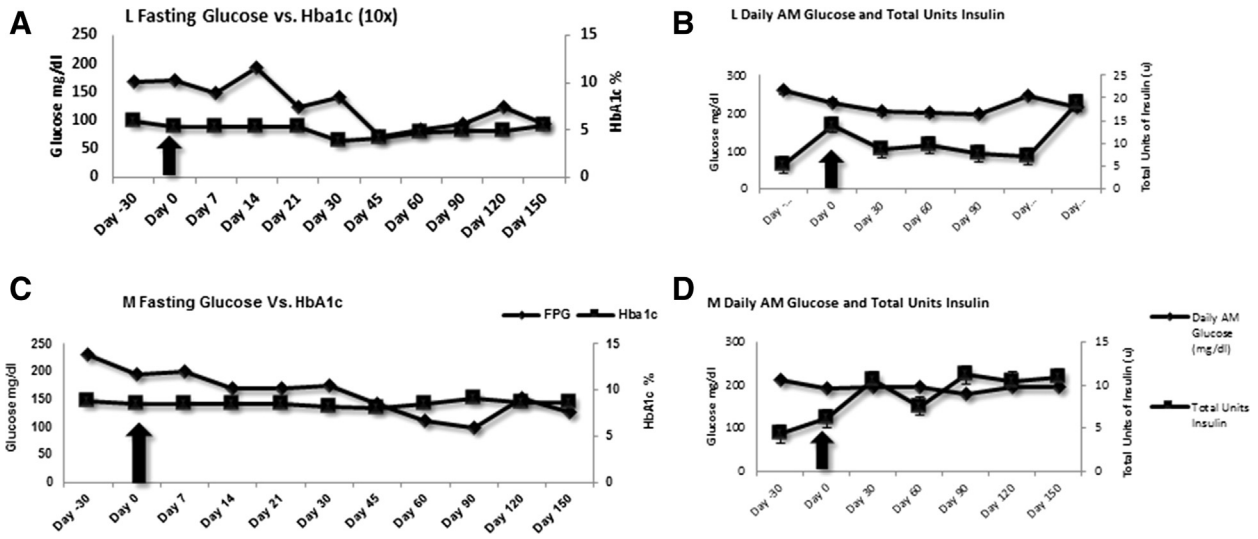



**Fig 1.** FPG and HbA1c (panels **A**–**C**), daily AM glucose and Total units in insulin (panels **B**–**D**).

intraperitoneal injection of microencapsulated porcine Sertoli cells (MC-pSC) would reverse hyperglycemia in 2 insulin-requiring, obese nonhuman primates with spontaneous T2DM.

## MATERIALS AND METHODS

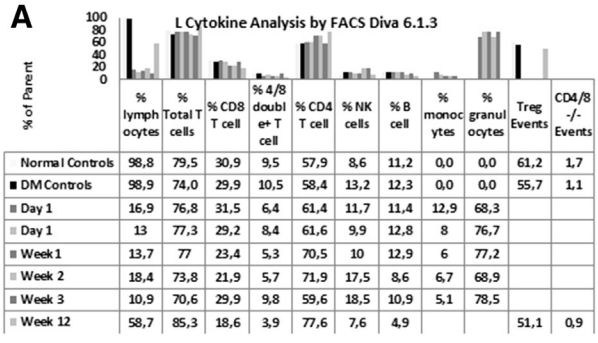
Two T2DM rhesus macaques, fed an ad libitum healthy diet, were studied. pSC, isolated according to previously established methods, modified in our laboratory [3,4], were enveloped in highly purified "clinical grade" alginate-based microcapsules [2] that were intraperitoneally injected using a 14-G catheter, under general anesthesia. To evaluate the dose response effects, we treated the 2 recipients using 2 different doses as follows: monkey 1 (M) received $0.3 \times 10^9$ cells/11.2kg, and monkey 2 (L) received $0.5 \times 10^9$ cells/10.8kg.

Post-transplantation, weights and blood samples were obtained at days 7, 14, 21, 28, 60, 90, 120, and 150.

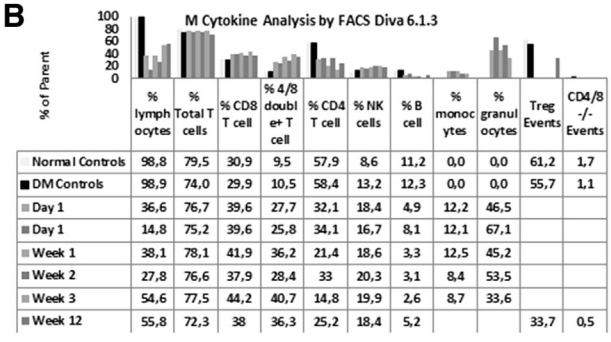
Blood samples were analyzed for fasting plasma glucose (FPG), HbA1c, and cytokines. Glucose tolerance tests (GTT) were assessed on days 21 and 45. FACS analysis was performed for the following antibodies: CD45 PerCp, CD3 Alexa 700, CD20 APC, CD4 PE, CD8 FITC, CD25 BD Horizon V450, and CD127 PeCy7. Data were anaylzed using BD FACSDiva 6.1.3 (BD Biosciences, San Jose, Calif).

## RESULTS

After MC-SC transplantation we observed a significant reduction in HbA1c levels and daily insulin dose requirements (baseline 5.4% vs 3.9% at 30 days [Fig 1A] and 14.1 vs 7.2 at 120 days [Fig 1B], respectively) in the animal (L) receiving the larger dose of SCs. Nevertheless, reduction in FPG (195 mg/dL vs 98 mg/dL [Fig 1C] and 171 mg/dL vs 94 mg/dL at 90 days [Fig 1A]) and decrease of mean nonfasting (NF) glucose levels (191 mg/dL vs 179 mg/dL [Fig 1D and 227 mg/dL vs 197 mg/dL [Fig 1B] at 90 days) were observed in both animals (L and M). FACS data in peripheral blood samples demonstrated a decrease, as compared with diabetic control animals, in CD $8^+$ T cells (29.9% vs 18.6% at 90 days [Fig 2A]) only in 1 recipient (L), whereas significant reduction in B cells (12.3% vs 4.9% and 12.3% vs 5.2% at 90 days [Fig 2A–2B], respectively) was observed in both animals. In addition, mean Treg cells value showed a slight decrease as compared with that of diabetic controls (33.7% vs 55.7% and 51.1% vs 55.7% [Fig 2B–2A], respectively). There were no major shifts in leukocyte populations and no apparent inflammatory response in either animal.




| | % lymphocytes | % Total T cells | % CD8 T cell | % 4/8 double+ T cell | % CD4 T cell | % NK cells | % B cell | % monocytes | % granulocytes | Treg Events | CD4/8 -/- Events |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal Controls | 98,8 | 79,5 | 30,9 | 9,5 | 57,9 | 8,6 | 11,2 | 0,0 | 0,0 | 61,2 | 1,7 |
| DM Controls | 98,9 | 74,0 | 29,9 | 10,5 | 58,4 | 13,2 | 12,3 | 0,0 | 0,0 | 55,7 | 1,1 |
| Day 1 | 16,9 | 76,8 | 31,5 | 6,4 | 61,4 | 11,7 | 11,4 | 12,9 | 68,3 | | |
| Day 1 | 13 | 77,3 | 29,2 | 8,4 | 61,6 | 9,9 | 12,8 | 8 | 76,7 | | |
| Week 1 | 13,7 | 77 | 23,4 | 5,3 | 70,5 | 10 | 12,9 | 6 | 77,2 | | |
| Week 2 | 18,4 | 73,8 | 21,9 | 5,7 | 71,9 | 17,5 | 8,6 | 6,7 | 68,9 | | |
| Week 3 | 10,9 | 70,6 | 29,9 | 9,8 | 59,6 | 18,5 | 10,9 | 5,1 | 78,5 | | |
| Week 12 | 58,7 | 85,3 | 18,6 | 3,9 | 77,6 | 7,6 | 4,9 | | | 51,1 | 0,9 |




| | % lymphocytes | % Total T cells | % CD8 T cell | % 4/8 double+ T cell | % CD4 T cell | % NK cells | % B cell | % monocytes | % granulocytes | Treg Events | CD4/8 -/- Events |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal Controls | 98,8 | 79,5 | 30,9 | 9,5 | 57,9 | 8,6 | 11,2 | 0,0 | 0,0 | 61,2 | 1,7 |
| DM Controls | 98,9 | 74,0 | 29,9 | 10,5 | 58,4 | 13,2 | 12,3 | 0,0 | 0,0 | 55,7 | 1,1 |
| Day 1 | 36,6 | 76,7 | 39,6 | 27,7 | 32,1 | 18,4 | 4,9 | 12,2 | 46,5 | | |
| Day 1 | 14,8 | 75,2 | 39,6 | 25,8 | 34,1 | 16,7 | 8,1 | 12,1 | 67,1 | | |
| Week 1 | 38,1 | 78,1 | 41,9 | 36,2 | 21,4 | 18,6 | 3,3 | 12,5 | 45,2 | | |
| Week 2 | 27,8 | 76,6 | 37,9 | 28,4 | 33 | 20,3 | 3,1 | 8,4 | 53,5 | | |
| Week 3 | 54,6 | 77,5 | 44,2 | 40,7 | 14,8 | 19,9 | 2,6 | 8,7 | 33,6 | | |
| Week 12 | 55,8 | 72,3 | 38 | 36,3 | 25,2 | 18,4 | 5,2 | | | 33,7 | 0,5 |

**Fig 2.** Cytokines analysis (panels **A**–**B**) vs time FACS data in L (left) and M (right).

## DISCUSSION

These preliminary data showed that this novel cell transplantation treatment protocol based on xenogeneic pSC might positively impact the metabolic status of primates with spontaneous T2DM. This improvement took place together with an important reduction of B cells, thereby providing a new and interesting insight on the role of the adaptive immune system in this metabolic disorder. These effects, which were never reported before, using comparable treatment protocols, without complications, in nonhuman primates may open new experimental avenues for the future therapy of T2DM.

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