Desensitization of donor specific resistant positive lymphocyte cross-match by donor specific mesenchymal stem cell transfusion

Thesis

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بسم الله الرحمن الرحيم

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Abstract

Background:

Donor specific antibodies (DSA) and a positive cross-match in patients who are sensitized through previous blood transfusions, organ transplantation or pregnancy are an important obstacle in kidney transplantation. , trials of allograft transplantation across the HLA barrier have employed desensitization strategies, including the use of plasmapheresis, intravenous immunoglobulins, anti-B-cell monoclonal antibodies and splenectomy, associated with high-intensity immunosuppressive regimens. These measures have proven only partially successful in preventing or treating humoral rejection in high-risk patients, while causing a significant increase in the risk of severe infectious complications occurring after transplantation.

Mesenchymal stem cells (MSCs) have immunomodulatory capacity and are able to suppress allo-specific antibody production in vitro, and may therefore help overcome a positive cross-match in sensitized transplant recipients.

Aim of the Work:

To evaluate the effect of mesenchymal stem cell transfusion on desensitization of donor specific resistant positive lymphocyte cross-match and /or high titre Panel Reactive Antibody (PRA).

Material and Methods:

Ten patients with repeated positive lymphocyte cross match and /or high titre of Panel Reactive Antibody (PRA) were selected from renal transplantation outpatient clinic in Kasr Al Aini hospital. Ten age and sex matched patients with positive cross-match who turned negative spontaneously will be included as a control group.

Ninety ml bone marrow were aspirated from the iliac bone for separation of MSCs. Surface expression of CD271, CD29 and CD34 were analyzed using flowcytometry. Finally approximately 50 million MSCs were infused peripherally in two divided doses one week apart.

Results:

MSCs transfusion proved to be the only procedure which could achieve successful desensitization (negative cross match and PRA) before performing the renal transplantation owing to their immunosuppressive properties.

Conclusion:

The donor specific -MSCs is potential option for anti-HLA desensitization.

Key words: *mesenchymal stem cells, immunomodulatory properties, desensitisation protocols, renal transplantation*

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LIST OF ABBREVIATIONS

AD: …….Alzheimer's disease

AKI: ……Acute kidney injury

AMI: ……Acute myocardial infarction

AMR: ……..Antibody-mediated rejection

ARF: ……...Acute renal failure

ARMD: ……Age-related macular degeneration

AT: ……. Adipose tissue

ATG: ……Anti-thymocyte globulin

ATN: ……Acute tubular necrosis

BKV: ……BK polyomavirus

BM: ……. Bone marrow

BMSC: …… Bone marrow stromal progenitor cells

CB: …….. Cord blood

CDC: ……Complement dependent cytotoxicity

CFU-F: ……....Colony-forming unit-fibroblasts

CKD: ……Chronic kidney disease

CML: ……Chronic myelogenous leukemia

CMV: ……Cytomegalovirus

COX-2: ……Cyclooxygenase 2

CRF: …… .Chronic renal failure

CsA: ……..Cyclosporine

D.S: …….. Donor specific

DCs: ……..Dendritic cells

DSAs: …….Donor-specific antibodies

ES: ……..Embryonic stem cells

ESCs: ……...Embryonic stem cells

ESRD: ……..... End stage renal disease

FACS: ……..... Fluorescence-activated cell sorting

FCS: ……Fetal calf serum

FDA: ……Food and Drug Administration

FGF: ……Fibroblast growth factor

FITC: ……..Fluorescein isothiocyanate

GCSF: …… Granulocyte-colony stimulating factor

GVHD: ……...Graft versus host disease

HGF: ……...Hepatocyte growth factor

HGF-hucMSC: …... HGF modified human umbilical cord MSCs

HLA-G: …….....Histocompatibility leucocyte antigen-G

HLAs: ……....Human leukocyte antigens

hMSC : ……Human embryonic MSC

hOB: ……Human osteoblastic cells

HSCs: ……..Hematopoietic stem cells

Huc MSC: ……...Human umbilical cord-derived MSC

IA: ……...Immunoadsorption

ICAM: ……Intracellular adhesion molecules

IDO: ……..…Indoleamine 2,3dioxygenase

IFN-γ: …….....Interferon -γ

Ig: ……. ...Immunoglobulin

IGF-I: ……...Insulin-like growth factor I

IL-1β: ……...Interleukin-1β

iNOS: ……...Inducible nitric oxide synthase

IR: …….....Ischaemia-reperfusion

IRI: …….....Ischemia-reperfusion injury

IVIG: ……Intravenous immunoglobulin

LCM: …….....Lymphocyte cross match

MAbs: …….. Monoclonal antibodies

M-CSF: …….Macrophage colony-stimulating factor

MHC: ……Major histocompatibility complex

MMF: …….....Mycophenolate mofetil

MSCs: ……...Mesenchymal stem cells

NF: …….Nuclear factor

NK: ……...Natural killer cells

NO: ……Nitric oxide

OI: ……..Osteogenesisimperfecta

PB: …… Peripheral Blood

PBMC: ……..... peripheral blood mononuclear cells

PBS: ……...phosphate buffer saline

PECAM: ……... ….Platelet endothelial cell adhesion molecules

PGE2: …….prostaglandin E2

PLA: ……processedlipoaspirate

PP: ……..Plasmapheresis

PRA: ……..Panel Reactive Antibody

PRD: ……..... prednisolone

PTEC: …… proximal tubular epithelial cells

PTLD: ……post- transplant lymphoproliferative disease

R: …… ..AS Rescue

RCS: ..... Royal College of Surgeons

RGCs: ……Retinal ganglion cells

RPE: ……..Retinal pigment epithelium

SDF-1: ……..Stromal derived factor

SLE: ……..Systemic lupus erythematosus

SOT: ……..Solid Organ Transplant

STAT5: ……Signal transducer and activator of transcription-5

TGF- β: …….....Transforming growth factor-beta

TGF: ……..... Transforming growth factor

TGF-α: ……Transforming growth factor -α

TGP: ……Transplant glomerulopathy

Th.p: ……Third party

TNALP: ……Tissue non specific alkaline phosphatase

TNF-α: ……..Tumor necrosis factor-α

TX: ……Transplantation

UCB: …… Umbilical cord blood

VCAM: …….Vascular cell adhesion molecules

VEGF: ……..Vascular endothelial growth factor

VEGF-hMSC :……..VEGF-modified human embryonic MSC

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Introduction

Donor-specific anti-HLA antibodies (DSA) and a positive cross-match in patients who are sensitized through previous blood transfusions, organ transplantation or pregnancy are an important obstacle in kidney transplantation. Antibodies directed against alloantigens are implicated in the pathogenesis of several immune reactions complicating transplantation. In particular, in solid organ transplant (SOT) recipients, the role of humoral immunity in acute and chronic rejection has been highlighted by the recent histological characterization of antibody-mediated rejection and by the detection of donor-reactive antibodies with sensitive techniques ***(Moll S et al., 2005).***

In this latter setting, in an effort to expand the donor pool, trials of allograft transplantation across the HLA barrier have employed desensitization strategies, including the use of plasmapheresis, intravenous immunoglobulins, anti-B-cell monoclonal antibodies and splenectomy, associated with high-intensity immunosuppressive regimens ***(Snanoudj R et al., 2005 and Gloor JM et al.,2003).*** These measures have proven only partially successful in preventing or treating humoral rejection in high-risk patients, while causing a significant increase in the risk of severe infectious complications occurring after transplantation. Thus, the development of new therapeutic tools able to blunt alloantibody production with no risk of infection would be a welcome complementation to existing protocols. Mesenchymal stem cells (MSCs) play a central role in the development and differentiation of the lymphohematopoietic system ***(Noort WA et al., 2002).***

Stem cells are immature progenitor cells capable of self-renewal and multilineage differentiation through a process of asymmetric mitosis that

leads to the formation of two daughter cells, one identical to the stem cell and one capable of differentiation into more mature cells. This mechanism is a necessary physiological mechanism for the maintenance of the cellular composition of tissues and organs in the body ***(Mirzapour et al., 2011).***

Mesenchymal stem cells (MSCs) have immunomodulatory capacity and are able to suppress allo-specific antibody production in vitro, and may therefore help overcome a positive cross-match in sensitized transplant recipients ***(Patrizia Comoli et al., 2008).***

Mesenchymal stem cells (MSCs) are multi-potent progenitor cells that are isolated from the bone marrow and several adult organs and tissues. These cells possess remarkable immunosuppressive properties and can inhibit the proliferation and function of the major immune cell populations, including T cells, B cells and natural killer (NK) cells; modulate the activities of dendritic cells (DCs); and induce regulatory T cells both in vivo and in vitro. These unique properties make MSCs ideal candidates for clinical application as immunosuppressants. The immunomodulatory effect of MSCs is mediated by a non-specific anti-proliferative action of these cells, which is dependent on cell–cell contact or secreted soluble factors ***(M. Shi et al., 2011).***