T Cell Receptor Excision Circles (TRECs) as a Marker of Thymic Output

Thesis

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Abbrev.	Full term
AAAAI	American Academy of Allergy Asthma and Immunology
ADA	Adenosine deaminase deficiency
AR	Autosomal recessive
BCG	Bacille Calmette and Guerin
BCR	B-cell receptor
BM	Bone marrow
BMT	Bone marrow transplantation
bp	Base pairs
BTK	Bruton tyrosine kinase
°C	Degree Celcius
C	Constant
Cacl2	Calcium chloride
CBC	Complete blood picture
CD	Cluster of differentiation
cDNA	Complementary DNA
CID	Combined immunedificiency
CJ	Coding joint
CMV	Cytomegalovirus
Ct	Cycle Threshold
CVID	Common variable immune deficiency
D	Diversity

Abbrev.	Full term
DBS	Dried blood spot
DGS	DiGeorge syndrome
DNA	Deoxy ribonucleic acid
DNA-PKcs	DNA dependant protein kinase catalytic subunit
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbant assay
ESID	European Society for Immunodeficiencies
FACS	Flourescence-activated cell sorting
FHL	Familial haemophagocytic lymphohistiocytosis
GVHD	Graft versus host disease
HIV	Human immunedeficiency virus
HLA	Human leucocyte antigen
HMG	High mobility group proteins
HSCT	Haematopoietic stem cell transplantation
IDR	Immunedificiency related
IgH	Immunoglobulin heavy chain
IGH	Immunoglobulin heavy chain
IgK	Immunoglobulin kappa light chain
IGK	Immunoglobulin kappa light chain
IGKDEL	Immunoglobulin kappa deleting element or like
IgL	Immunoglobulin lambda light chain
IGL	Immunoglobulin lambda light chain
IQR	Interquartile range
IVIG	Intravenous immunoglobulin

Abbrev.	Full term
J	Joining
KRECs	Kappa deleting recombination excision circles
LB	Luria-Bertani
LOAF	Late onset antibody failure
Mg	Magnesium
MHC	Major histocompatibility complex
Mn	Manganese
NBS	Newborn screening
NHEJ	Non homologous end joining
NK	Natural killer
NTC	Non template control
OD	Optical density
OS	Overall survival
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PID	Primary immunodeficiency disease
PJP	Pneomocystis jiroveci pneumonia
PKU	Phenylketonuria
Pol	Polymerase
PTK	Protein tyrosine kinase
Q	Quartile
RAG	Recombination activation gene
RNA	Ribonucleic acid
Rpm	Rotation per minute

Abbrev.	Full term
RQ-PCR	Real time quantitative PCR
RSS	Recombination signal sequences
RSV	Respiratory syncytial virus
RTE	Recent thymic emigrants
SCID	Severe combined immunodeficiency
SCT	Stem cell transplantation
Sj	Signal joint
T21	Trisomy21
TCR	T cell receptor
TCR- γ (TCRG)	T cell receptor gamma chain
TCR- δ (TCRD)	T cell receptor delta chain
TCR-α (TCRA)	T cell receptor alpha chain
TCR-β (TCRB)	T cell receptor beta chain
TdT	Terminal deoxynucleotidyl transferase
TRA	T cell receptor alpha gene
TRAC	T cell receptor alpha constant gene
TRBV	T cell receptor beta variable chain
TRECs	T cell receptor excision circles
TRIS	Trisaminomethane
URD	Unrelated donor
V	Variable

Abbrev.	Full term
WAS	Wiskott Aldrich syndrome
WHO	World health organization
XLA	X linked agammaglobulineamia
XRCC4	X-ray repair cross-complementing protein 4
δRec	Delta recombination element

INTRODUCTION

Primary immunodeficiency diseases (PIDs) are inherited defects of the innate or adaptive arms of the immune system that lead to an increase in the incidence, frequency, or severity of infections. Major efforts are currently being undertaken to develop methods for detection of PIDs in the neonatal period ***(Uzzaman and Fuleihan, 2012).***

Severe combined immunodeficiency (SCID) in particular, is fatal in infancy unless affected infants can be diagnosed before the onset of devastating infections and provided with an immune system through allogenic hematopoietic cell transplantation, enzyme replacement, or gene therapy. Newborns with SCID typically appear normal at birth, lack a family history or any clinical clues before the onset of infections ***(Puck, 2011)***.

SCID which is one of the inborn errors of immune function represents a group of conditions characterized by blocks in T-cell development, which lead to functional deficiencies in both T-cells and B-cells ***(Baker et al ., 2010).***

T- and B-lymphocytes are unique in their ability to create a receptor by genomic rearrangement of their antigen receptor genes via V (D) J recombination. On one hand, DNA strand breakage

during the thymic and bone marrow maturation processes of the T cell receptor (TCR) α/β chains and the B cell receptor (BCR) light and heavy chains, respectively, creates functional receptors (i.e., the formation of coding joint recombination sites), while, on the other hand, it creates byproducts (i.e., the formation of signal joint recombination sites) termed TCR excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs), respectively ***(Ye and Kirschner , 2002).***

The DNA circles formed are stable and are maintained after cell division, but because they do not replicate, they become diluted as T cells proliferate through mitotic division ***(Puck, 2012).***

The determination of thymic output by quantification of TRECs is extensively used as an accurate measure of thymic function and T cell neogenesis, and this analysis was therefore suggested as a diagnostic tool for T cell immunodeficiency ***(Amariglio et al., 2010),*** for neonatal screen assay to detect SCID immediately after birth ***(Puck, 2007),*** and as being the most predictive factor for long-term T cell immune reconstitution after bone marrow transplantation (BMT) ***(Roifman et al., 2008).***

KRECs form the extra-chromosomal (episomal) excision product of the immunoglobulin gene rearrangement. Similar to TRECs, these episomal products cannot replicate in the cell. KRECs appear to be highly stable structures, which can persist for