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Roles of Notch and NF-κB Signaling in Allogeneic Responses

By

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Statement

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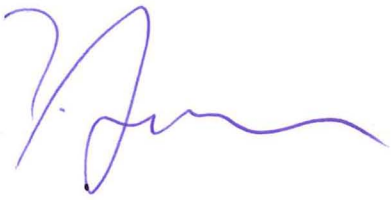

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List of abbreviations

ADCC	Antibody-Dependent Cytotoxicity
ANU	Australian National University
APC	Antigen Presenting Cell
BCR	B cell Receptor
bp	base pair(s)
CI-MPR	Cation-Independent Mannose-6-Phosphate Receptor
CLP	Common Lymphoid Precursor
CSL	CBF1/RBP-J kappa, Suppressor of Hairless, Lag-1
CTL	Cytotoxic T Lymphocyte
CTLA	Cytotoxic T Lymphocyte Antigen
DC	Dendritic Cell
Dll	Delta-Like Ligand
DNA	Deoxyribonucleic Acid
DTH	Delayed Type Hypersensitivity
EGF	Epidermal Growth Factor
FACS	Fluorescence Activated Cell Sorter
Foxp3	Forkhead box p3
GFP	Green Fluorescent Protein
GITR	Glucocorticoid Induced Tumor Necrosis Factor Receptor
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HT	High Titer
IC	Intracellular
ICAM	Intercellular Adhesion Molecule
IFN	Interferon
Ig	Immunoglobulin
IKK	IκB Kinase
IL	Interleukin
iNOS	inducible Nitric Oxide Synthase
i.p	intraperitoneal
i.v	intravenous
Jag	Jagged
JCSMR	John Curtin School of Medical Research
kDa	kilo Dalton
Lfng	Lunativc Fringe
LPS	Lipopolysaccharide
LT	Low Titer
MCP	Monocyte Chemotactic Protein

MFI	Mean Fluorescence Intensity
Mfng	Manic Fringe
MHC	Major Histocompatibility Complex
mRNA	Messenger Ribonucleic Acid
NCR	Notch Cytokine Response
NF-κB	Nuclear Factor-Kappa B
NIK	NF-κB-Inducing Kinase
NK	Natural Killer
ODN	Oligodeoxynucleotides
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
pDONR	donor vector
PDTC	pyrroclidine dithiocarbamate
PMA/I	phorbol myristate acetate/Ionophore
Rfng	Radical Fringe
TCR	T Cell Receptor
TGF	Transforming Growth Factor
Th	T helper
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor
UV	Ultraviolet
VCAM	Vascular Cell Adhesion Molecule

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Abstract

The induction of robust allograft tolerance is the ultimate goal for clinical transplantation. Although studies have identified that dendritic cells (DCs) are important for induction of antigen-specific tolerance, the requirements for generating tolerogenic DCs are yet to be elucidated. Recently, it has been demonstrated the modulation of two signaling pathways, Notch and nuclear factor κB (NF-κB) can render DCs tolerogenic. The studies documented here examine (1) whether immature DCs over-expressing Notch-related molecules (Jagged-1, Delta-like-1 (Dl1-l), Lunatic Fringe (Lfng), and Manic Fringe (Mfng)) act as immunoregulatory DCs and promote allograft survival; and (2) whether DCs deficient in NF-κB signaling inhibit the alloreactive T cell response and promote allograft survival.

The immature DC cell line (JAWS II cells ($H\text{-}2^b$)) was retrovirally transduced to over-express murine (m)Jagged-1, mDll-1, mLfng, or mMfng. JAWS II cells over-expressing Notch related molecules remained immature following transduction, however, these cells were unable to modulate an alloreactive T cell ($H\text{-}2^k$) response *in vitro*. Pretreatment of allogeneic CBA/H mice ($H\text{-}2^k$) with transduced JAWS II cells failed to promote C57BL/6 ($H\text{-}2^b$) thyroid allograft survival. Cellular transplantation of JAWS II cells over-expressing Notch related molecules were also acutely rejected in CBA/H mice suggesting lack of immunomodulation by genetically engineered JAWS II cells *in vivo*. In addition, cellular grafts of JAWS II cells to H-2-compatible mice ($H\text{-}2^b$) were chronically rejected, indicating that JAWS II cells and C57BL/6 mice differ at one or more minor histocompatibility loci.

The NF-κB inhibitor, BAY11-7082 (BAY), and cRel inhibitor, Pentoxifylline (Ptx), were used to prevent NF-κB signaling in C57BL/6 ($H\text{-}2^b$) splenocytes and bone marrow-derived DCs (BMDCs). BAY treatment abrogated the capacity of splenocytes (and to a lesser extent

BMDCs) to stimulate allogeneic (H-2^k) T cells. This effect correlated with reduced expression of costimulatory molecules and major histocompatibility complex (MHC) Class II molecules on the treated splenocyte and BMDC population. It was also shown that BAY-treated splenocytes did not produce inflammatory cytokines including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, IL-2 and IL-4, and produced less IL-5 compared to untreated splenocytes. Although allogeneic T cells did not proliferate in response to BAY-treated splenocytes, subsequent T cell proliferation in response to a secondary stimulus was observed *in vitro*. T cells previously exposed to BAY-treated splenocytes also failed to inhibit naïve T cell proliferation indicating lack of regulatory T cell differentiation.

Based on the finding that BAY treatment inhibited the capacity of APCs to induce alloreactive T cell proliferation *in vitro*, we examined whether BAY treatment of thyroid tissue or adult islets prior to transplantation inhibited the immunostimulatory capacity of donor passenger leukocytes to prime recipient T cells. Allografts precultured with BAY were rejected with similar kinetics to control cultured allografts, indicating that *in vitro* exposure to BAY was not sufficient to prevent alloreactive T cell activation by donor passenger leukocytes. However, CBA/H (H-2^k) mice which received BAY-treated splenocytes or BAY-treated BMDCs intravenously prior to implantation of C57BL/6 (H-2^b) thyroid tissue demonstrated prolonged allograft survival. This finding indicates that an *in vivo* environment provides additional signal(s) which are absent in the *in vitro* system and which are necessary for modulating alloresponses. The mechanism by which BAY splenocytes and BAY BMDCs prolong allograft survival requires further investigation.

Although the potential for Notch signaling to promote alloantigen-specific tolerance remains unresolved, these studies suggest that inhibition of NF-κB signaling in DCs represent a potential approach for promoting allograft survival.

Table of contents