EXPRESSION AND FUNCTION OF TOLL-LIKE RECEPTORS IN LYMPHOCYTES FROM HUMAN NEONATES

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SUMMARY

Neonates have high global rates of morbidity and mortality due to infectious diseases; this susceptibility is attributed to the immaturity of the neonatal immune response. The immune system of neonates, compared to adults, has reduced function in several aspects of immunity and lacks the long-term memory response.

Toll-like receptors (TLR) are a family of pattern recognition receptors which bind various microbial components and alert the immune system to invading pathogens. Comparing TLR expression and function in neonatal lymphocytes and adult lymphocytes may reveal how TLR influence these immune cells in early life. Due to the immaturity of their immune responses, neonates may be more reliant on TLR for protection against infection.

The extracellular and intracellular expression of TLR1, TLR2, TLR3, TLR4, TLR6, TLR8 and TLR9 was examined on non-stimulated and stimulated T lymphocytes and B lymphocytes from cord blood, adult peripheral blood and tonsils from human subjects. B lymphocytes were categorised into subsets to see if differentiation stage of B lymphocytes affects TLR expression. The responses of purified B lymphocytes, from neonates, adults and tonsils, to ligands of TLR3, TLR4, TLR8 and TLR9 were compared. The functions measured were proliferation, levels of total Ig, IgG and IgM, and levels of IL-6 and IL-8. Tonsil B lymphocytes were tested for expression of activation markers (CD23, CD25, CD69 and HLA-DR), co-stimulatory molecules (CD40, CD80 and CD86), and CD21 and CD210.

The TLR expression patterns by T lymphocytes and B lymphocytes were similar between neonates and adults, and stimulation of lymphocytes had little effect on TLR expression. T lymphocytes from neonates and adults expressed TLR2, TLR3, TLR4, TLR8 and TLR9. B lymphocytes from neonates and adults expressed TLR1, TLR3, TLR4, TLR8 and TLR9. B lymphocytes from tonsils expressed TLR3, TLR4, TLR8 and TLR9. Cellular location of TLR was mostly consistent with the literature, except for detection of TLR4 in permeabilised cells and TLR8 on non-permeabilised cells. CpG ODN, TLR9 ligand, induced strong proliferation, secretion of total Ig, IgG, IgM and IL-6 from B lymphocytes from neonates, adults and tonsils. Adult B lymphocytes produced higher levels of total Ig, IgM and IL-6 in response to the other TLR ligands compared to neonatal and tonsil B lymphocytes. IL-8 levels were unaffected by TLR ligands in neonates, adults and tonsils.

Neonatal lymphocytes have adult-like capacity to "innately" recognise foreign pathogens. Neonatal B lymphocytes have reduced responses to TLR ligands compared to adult B lymphocytes. However, neonatal B lymphocytes respond to TLR ligands, especially CpG ODN, with increased functions. TLR agonists, particularly CpG ODN, are potentially strong candidates for future research in neonatal vaccinology.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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PUBLICATIONS ARISING FROM THIS PROJECT

(See Appendix)

PAPER FROM PRELIMINARY STUDY PRECEDING PHD STUDY

Dasari P, Nicholson IC, Hodge G, Dandie GW, Zola H. Expression of Toll-like receptors on B lymphocytes. Cell Immunol 2005;236(1-2):140-45.

PAPERS ARISING FROM PHD STUDY

Dasari P, Zola H, Nicholson IC. Expression of Toll-like receptors by neonatal leukocytes. Pediatr Allergy Immunol 2010 (in press)

Dasari P, Nicholson IC, Zola H. Toll-like receptors. J Biol Reg Homeost Agents 2008;22(1):17-26.

ABBREVIATIONS

7AAD:	7-amino-actinomycin
AF647:	Alexa Fluor® 647
AP-1:	Activating protein-1
APC:	Antigen-presenting cells
B1 cells:	$CD5^+$ B lymphocytes
BCR:	B cell receptor
BSA:	Bovine Serum Albumin
CBA:	Cytometric Bead Array
CD:	Clusters of differentiation
CL075:	A thiazoloquinolone compound – 3M002
CLR:	C-type lectin receptors
CpG ODN:	Oligodeoxynucleotides with unmethylated CpG dinucleotides
CRP:	C-reactive protein
DAPI:	4',6-diamidino-2-phenylindole
DC:	Dendritic cell
DDA-PE:	PE-conjugated anti-mouse Ig $F(ab)_2$
EC:	Extracellular
ELISA:	Enzyme-linked immunosorbent assay
ERK1:	Extracellular regulated kinase 1
Fab:	antibody fragment of single antigen-binding region
$F(ab)_2$:	antibody fragment of both antigen-binding regions
FBS:	Foetal bovine serum
Fc:	crystallisable antibody fragment with no antigen-binding region

FITC:	Fluorescein isothiocyanate
FSC:	Forward scatter
GC:	Germinal centre
HLA-DR:	Human leucocyte antigen DR-1
HLDA:	Human leucocyte differentiation antigen
HRP:	Horseradish peroxidase
HαMBi:	Biotinylated horse anti-mouse IgG antibody
IC:	Intracellular
IF:	Immunofluorescence
IFN:	Interferon
Ig:	Immunoglobulin
IgA:	Immunoglobulin with α heavy chains
IgD:	Immunoglobulin with δ heavy chains
IgE:	Immunoglobulin with ε heavy chains
IgG:	Immunoglobulin with γ heavy chains
IgM:	Immunoglobulin with μ heavy chains
IHC:	Immunohistochemistry
IL:	Interleukin
IRF:	Interferon-regulatory factor
LPS:	Lipopolysaccharide
LRR:	Leucine-rich repeats
LTA:	Lipotechoic acid
mAb:	Monoclonal antibody
MBL:	Mannan-binding lectin
MFI:	Median fluorescence intensity
MHC:	Major histocompatibility complex

mRNA:	messenger ribonucleic acid
MyD88:	Myeloid differentiation primary-response protein 88
NAIP:	Neuronal apoptosis inhibitor proteins
NALP:	NACHT-LRR-PYD containing proteins
NF-κB:	Nuclear factor-kappaB
NK:	Natural killer
NLR:	Nod-like receptor, NACHT proteins or NAIP receptors
NOD:	Nucleotide-binding oligodimerisation domain
N-S:	Non-Stimulated
OPD:	o-phenylenediamine dihydrochloride
p38-MAPK:	p38 Mitogen-activated protein kinase
PAMP:	Pathogen-associated molecular patterns
PBS/Azide:	PBS/0.02% Sodium azide
PBS:	Phosphate buffered saline
PCR:	Polymerase chain reaction
PE:	Phyco-erythrin
PerCP-Cy5.5:	Peridinin chlorophyll protein cyanine 5.5
PFA:	Paraformaldehyde
PolyI:C:	Polyinosinic-polycytidylic acid sodium salt
PRR:	Pathogen recognition receptors
RF10:	RF10 cell culture media
RNA:	Ribonucleic acid
RT-PCR:	Reverse transcriptase polymerase chain reaction
SA:	Streptavidin
SAP:	Serum amyloid protein
SSC:	Side scatter

T_C cell:	Cytotoxic T cell
TCR:	T cell receptor
T-D:	T cell-dependent response
T_H cell:	T helper cell
T_H1:	T helper cells type 1
T_H2:	T helper cells type 2
T-I:	T cell-independent response
T-I1:	T cell-independent type 1 response
T-I2:	T cell-independent type 2 response
TIR:	Toll/IL-1R
TIRAP:	TIR domain-containing adaptor protein
TLR:	Toll-like receptors
TNF:	Tumour necrosis factor
T_R cell:	Regulatory T cell
TRAM:	TRIF-related adaptor protein
TRIF:	TIR domain-containing adaptor protein inducing IFN-β
UV:	Ultraviolet
V_H region:	Variable region of antibody heavy chains
V_L region:	Variable region of antibody light chains
WCH:	Women's and Children's Hospital