eBioscience Toll-Like Receptor Guide

OVERVIEW

Toll-like receptor (TLR) family members, first discovered in Drosophila, are responsible for the recognition of many pathogen-associated molecular patterns (PAMPs) expressed by a wide spectrum of infectious agents. Monocytes/macrophages and neutrophils phagocytose microbial pathogens and trigger the cytokine network resulting in the development of the innate or natural immunity, inflammatory response and mediating an effective adaptive immunity.

The various TLRs exhibit different patterns of expression. Most TLRs are believed to act as homodimers, although heterodimers do exist. The TLR proteins have significant homology in their cytoplasmic domain to the IL-1 receptor Type I. To date, over thirteen Toll-like receptors have been reported in human and mouse. Studies with several TLRs have shown that they activate the NF-κB pathway, which regulates cytokine expression through several adaptor molecules including MyD88, TIRAP/Mal and TRIF. Activation of the NF-κB pathway leads to the initiation of the adaptive immune response by production of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α, IL-12, chemokines and induction of costimulatory molecules such as CD80, CD86, and CD40. In addition to induction of the cytokine network, MyD88 binds FADD and triggers apoptosis through the Caspase cascade. Hence, activation of the apoptosis pathway via TLRs appears to contribute to the repertoire of defense mechanisms utilized by the innate immune response. Several transcript variants encoding different isoforms of many TLR gene products have been reported.

The ligands for several TLRs have been reported. TLR2, in association with TLR1 and/or TLR6, recognizes and signals bacterial lipoproteins (BLP) and peptidoglycans from Gram-positive bacteria. TLR3 recognizes dsRNA. LPS from Gram-negative bacteria signals through TLR4. Bacterial flagellin binds to TLR5 while small anti-viral compounds/immune response modifiers, imiquimod/resiquimod, are agonists for TLR7. Cellular response to the CpG motif of bacterial DNA is mediated by TLR9 and TLR11 is involved in host recognition of protozoan pathogens. Recent reports suggest the involvement of several endogenous proteins generally present at the site of inflammation as TLR agonists.

In addition to the innate immune response, evidence implicates the involvement of the TLR family in a spectrum of systemic disorders following bacterial infections including sepsis, cardiac ischemia, peridontitis, and cerebral palsy. The rapid pace of research in the TLR family shall elucidate their functions and potential therapeutic interventions.

Name	Chromosome	Distribution (mRNA/protein)	Function/Comments
TLR1 (CD281, TIL, Rsc786)	human: 4 mouse: 5	protein: low level monocytes	associates with and regulates TLR2 response
TLR2 (CD282, TIL4, Ly105)	human: 4 mouse: 3	protein: monocytes, granulocytes; upregulated on macrophages	interacts with microbial lipoproteins and peptidoglycans, CD14-dependent and -independent responses, NF-κB pathway
TLR3 (CD283)	human: 4 mouse: 8	mRNA: DC, upregulated on endoth/epith protein: low level fibroblasts	interacts with dsRNA; activates NF-κB pathway; induces production of type I interferons; MyD88-dependent and -independent response to poly (I:C)
TLR4 (CD284, hToll, Lps, TOLL, Ly87, Rasl2-8, Ran/M1)	human: 9 mouse: 4 rat: 5	protein: monocytes, upregulated on endothelium	interacts with microbial lipoproteins, CD14-dependent response to LPS, NF-κB pathway, different isoforms
TLR5 (TIL3)	human: 1 mouse: 1	mRNA: leukocytes, prostate, liver, lung	interacts with bacterial flagellin, NF-κB pathway, response to Salmonella
TLR6	human: 4 mouse: 5	mRNA: leukocytes, ovary, lung	interacts with microbial lipoproteins, protein sequence most similar to hTLR1; associates with and regulates TLR2 response
TLR7	human: X mouse: X	mRNA: spleen, lung, placenta; upregulated on macrophages	low similarity to other TLR family members, interacts with small anti-viral compounds (imiquimod/Resiquimod)
TLR8	human: X mouse: X	mRNA: leukocytes, lung	interacts with ssRNA, 2 isoforms
TLR9 (CD289)	human: 3 mouse: 9	mRNA: leukocytes protein: pDC, B cells (intracellular, low)	receptor for CpG bacterial DNA; weakly similar to TLR3, different isoforms
TLR10	human: 4 mouse: N/A	mRNA: lymphoid tissues, B cells, pDC, DC subset	homodimer or heterodimer with TLR1 & 2
TLR11	human: ? mouse: 14	mRNA: macrophage, liver, kidney	host recognition of protozoan pathogens
TLR12	human: ? mouse: 4	-	function unknown
TLR13	human: ? mouse: X	-	function unknown
CD14	human: 5 mouse:	Myeloid lineage	binds to LBP/LPS
RP105 (CD180, Ly-78)	human: 5 mouse: 13	protein: mature B cells	B cell activation, LPS recognition in B cells, radioprotective 105kDa
MD-1 (Ly86)	human: 6 mouse: 13	protein: mature B cells	associates and regulates surface expression of RP105
MD-2 (Ly96)	human: 8 mouse: 1	macrophages, also secreted	associates and regulates surface expression of TLR4, signals LPS presence
IRAK4 (NY-Ren-64, REN64)	human: 12 mouse: 15	cytoplasmic protein	activation of NF-κB pathway
IRAK-M (Irak3)	human: 12 mouse: 10	cytoplasmic protein	inhibits IRAK signaling
MyD88	human: 3 mouse: 9 rat: 8	cytoplasmic protein	Myeloid differentiation primary response gene 88, activation of NF-κB pathway
NOD-1 (CARD4)	human: 7 mouse: 6	cytoplasmic protein	intracellular pattern recognition, binds to small peptidoglycans, iE-DAP, interacts with RICK, NF-κB pathway, caspase recruitment domain family
NOD-2 (CARD15, BLAU, IBD1, CD)	human: 16 mouse: 8 or 15	cytoplasmic protein, restricted to monocyte lineage	intracellular pattern recognition, binds to small peptidoglycans, muramyl dipeptide, interacts with RICK, NF-κB pathway, caspase recruitment domain family
TIRAP (Mal, wyatt, Tlr4ap)	human: 11 mouse: 9	cytoplasmic protein	TIRdomain-containing adaptor protein, MyD88-adapter like, activation of NF-κB pathway
TOLLIP (IL1RAcPIP)	human: 11 mouse: 7	cytoplasmic protein	TOLL interacting protein, prevention of IRAK activation

References:
1) Vogel SN, Fitzgerald KA, Fenton MJ. Mol Interv. 2003. 3:466-77. 2) Yamamoto M, Takeda K, Akira S. Mol Immunol. 2004. 40:861-8.
3) Janssens S, Beyaert R. Clin Microbiol Rev. 2003. 16:637-46. 4) Bell JK, Mullen GE, Leifer CA, Mazzoni A, Davies DR, Segal DM. Trends Immunol. 2003. 24:528-33. 5) O'Neill LA. Curr Opin Pharmacol. 2003. 3:396-403. 6) Kopp E, Medzhitov R. Curr Opin Immunol. 2003. 15:396-401.
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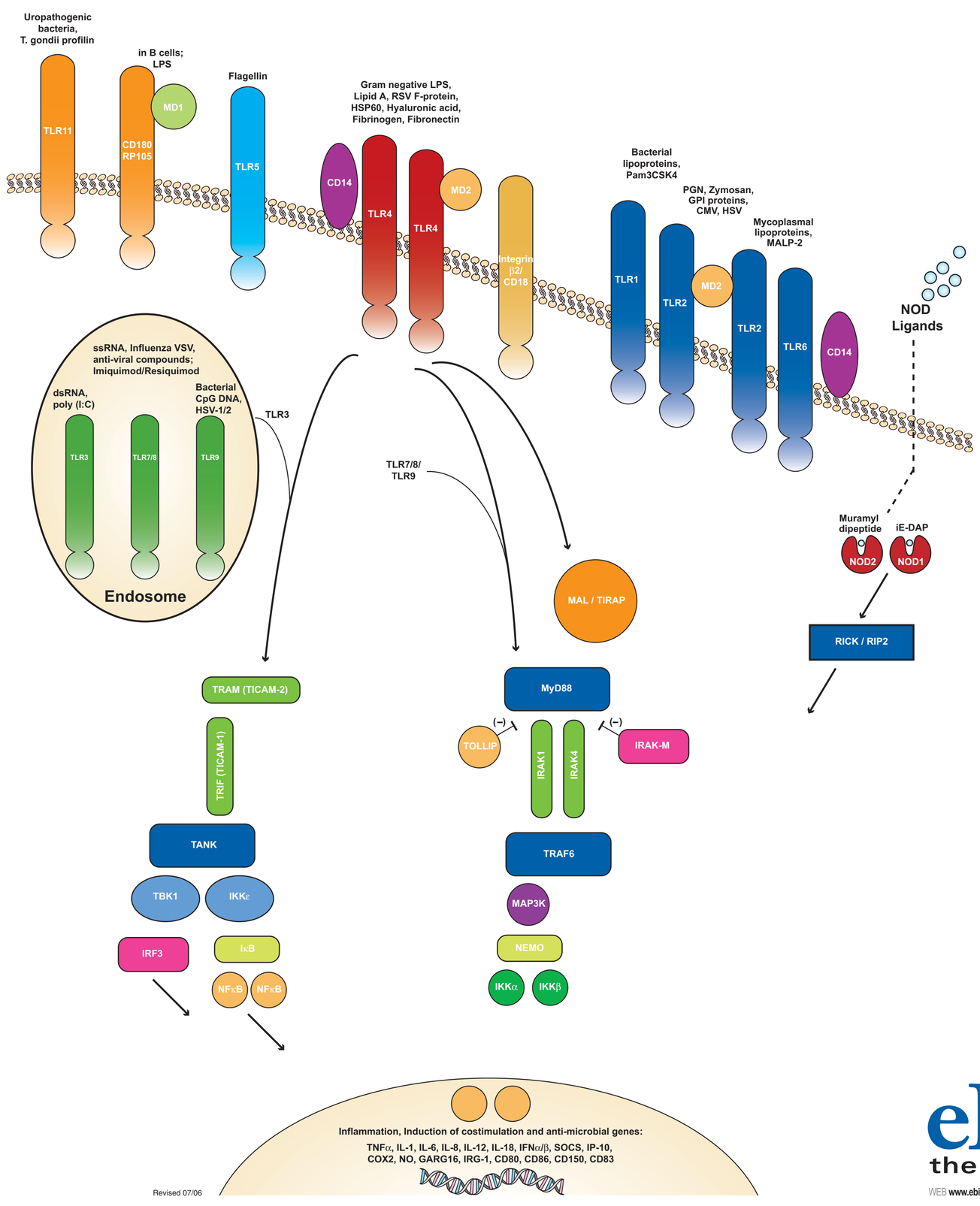

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Applications: **BA** = Functional bioassays **FC** = Fluorescence staining followed by flow cytometric analysis **ICFC** = Intracellular FC **IHC** = Immunohistochemical staining **IHC/F** = IHC of frozen tissue **IHC/P** = IHC of paraffin embedded tissue **NU** = Cytokine- or antibody-mediated neutralization **IP** = Immunoprecipitation **WB** = Immunoblotting

TLRs	Specificity	Clone	Applications	Formats
TLR1	h	GD2.F4	FC, IHC	14-9911, 16-9911, 12-9911
	m	eBioTR23	FC, IP	14-9011, 13-9011, 51-9011
	m	eBioLD5	WB	14-9012
TLR2	h	polyclonal	WB	24-9028 (rabbit serum)
	h	TL2.3	FC, IHC/F	14-9029
	h	TL2.1	FC, BA, IHC/F, IP, WB	14-9922, 16-9922, 13-9922, 11-9922, 12-9922, 17-9922, 51-9922, 53-9922, 56-9922, 57-9922
	m/h	T2.5	FC, BA, IHC, IP	14-9024, 16-9024, 13-9024, 12-9024, 25-9024, 51-9024
	m	6C2	FC, NU, IP	14-9021, 16-9021, 13-9021, 11-9021, 12-9021, 17-9021
	m	mT2.7	FC, IHC/F, IP	14-9022, 13-9022, 12-9022, 11-9022
TLR3	h	TLR3.7	FC, BA, IP	14-9039, 16-9039, 13-9039, 12-9039
	m	polyclonal	WB, FC	24-9031 (rabbit serum)
	m	T3.7C3	WB	14-9032
TLR4	h	polyclonal	WB	24-9048 (rabbit serum), 14-9920
	h	HTA125	FC, BA, IP	14-9917, 16-9917, 13-9917, 12-9917, 15-9917, 17-9917, 25-9917, 51-9917, 53-9917, 56-9917, 57-9917
	m	polyclonal	WB	24-9004 (rabbit serum)
activating	m	UT12	FC, BA, IP, NU	14-9925, 16-9925
activating	m	UT18	FC, BA, IP, NU	14-9926, 16-9926
	m	UT41	FC	14-9041, 13-9041, 12-9041, 51-9041, 53-9041, 57-9041
TLR4/MD-2	m	MTS510	FC, BA, NU, IP	14-9924, 16-9924, 13-9924, 12-9924, 25-9924
	m	eBioMT439	WB	14-9042
TLR5	m/h			Please Inquire
TLR6	h	hPer6	FC, WB	14-9069, 13-9069, 12-9069
	h	eBioTLR6.127	FC, WB, NU	14-9068, 16-9068, 13-9068, 51-9068
	m		WB	Please Inquire
TLR7	m/h	polyclonal	WB	14-9079
	h		FC	Please Inquire
TLR8	m/h	polyclonal	WB	14-9089
TLR9	h	eB72-1665	ICFC, IP, WB	14-9099, 12-9099
	m	polyclonal	FC, WB	14-9092, 13-9092, 12-9092
	m	M9.D6	ICFC, WB	14-9093, 13-9093, 11-9093
TLR10	m/h			Please Inquire
TLR11	m	polyclonal	WB	14-9119
CD11b	h	CBRM1/5	FC, BA, IP	14-0113, 16-0113, 11-0113, 12-0113, 56-0113
	h	ICRF44	FC, BA, IHC	14-0118, 13-0118, 12-0118, 25-0118
	m	M1/70	FC, BA, IHC, IP	14-0112, 16-0112, 13-0112, 11-0112, 12-0112, 15-0112, 17-0112, 25-0112, 10-0112, 19-0112, 36-0112, 51-0112, 53-0112, 57-0112, 27-0112
CD14	h	61D3	FC, BA, IHC	14-0149, 16-0149, 11-0149, 12-0149, 17-0149, 25-0149
	m	Sa2-8	FC, WB, IP	14-0141, 16-0141, 13-0141, 11-0141, 12-0141, 17-0141
CD16	h	eBioC816	FC	14-0168, 16-0168, 13-0168, 12-0168
CD16/32 blocks Fc binding	m	clone 93	FC, BA	14-0161, 16-0161, 13-0161, 11-0161, 12-0161, 17-0161, 25-0161, 10-0161, 19-0161, 36-0161, 56-0161, 57-0161
CD18	h	CLB-LFA-1/1	FC	14-0187, 11-0187, 12-0187
	m	M18/2	FC, BA, WB, IHC, IP	14-0181, 16-0181, 13-0181, 11-0181, 12-0181
CD36	h	mAb	FC	14-0369
	m	No. 72-1	FC	14-0361
CD64	h	10.1	FC, IHC/F	14-0649, 16-0649, 11-0649, 12-0649
CD180/RP105	h	MHR73-11	FC, BA, IHC, IP	14-1809, 16-1809, 13-1809, 12-1809
	m	RP/14	FC, BA, IP	14-1801, 16-1801, 13-1801, 12-1801
F4/80	m	BM8	FC, IHC/F, IHC/P	14-4801, 16-4801, 13-4801, 11-4801, 12-4801, 15-4801, 17-4801, 25-4801, 51-4801, 53-4801, 57-4801, 27-4801
MD-1	m	MD14	FC	14-9921, 13-9921, 12-9921
	m	MD113	FC	14-9931, 16-9931, 13-9931, 12-9931
MD-2	h	9B4	IP	14-9928

Cytokines	Specificity	Clone	Formats
IL-1α	h	CRM8	11-7019
	m	ALF-161	11-7011, 12-7011
IL-1β	h	CRM56	11-7018, 12-7018
	m	polyclonal	51-7113, 53-7113
IL-1β ELISA Ready-SET-Go!	h/m/r		88-7010 (h), 88-7013 (m), 88-7001 (r)
IL-1RA	h	CRM17	11-7015
IL-6	h	MQ2-13A5	11-7069, 12-7069
	m	MP5-20F3	11-7061, 12-7061
IL-6 ELISA Ready-SET-Go!	h/m		88-7066 (h), 88-7064 (m)
IL-12	h	C8.6	12-7129, 51-7129, 53-7129, 57-7129
	m	C17.8	12-7123, 51-7123
IL-12 ELISA Ready-SET-Go!	h	-	88-7126 (p70)
	m	-	88-7121 (p70), 88-7120 (IL-12/IL-23 total p40)
IL-18Rα	h	H44	11-7183, 12-7183
IL-23 p19	m	G23-8	14-7232, 16-7232
	h	eBio473P19	14-7238
	h	eBio25P19	16-7236
IL-23 (p19/p40) Ready-SET-Go!	h/m		88-7237 (p19/p40) (h), 88-7234 (p19/p40) (m)
IL-27	h	mAb	14-1309 (anti-p28), 14-1279, 16-1279 (anti-EBI3)
	m	mAb	14-1301 (anti-p28), 14-1272, 13-1272 (anti-EBI3)
IL-27 Ready-SET-Go!	m		88-7274
MCP-1/CCL2 ELISA RSG!	h/m		88-7399 (h), 88-7391 (m)
TNF-α	h	MAb11	11-7349, 12-7349, 17-7349, 51-7349, 53-7349, 57-7349
	m	MP6-XT22	11-7321, 12-7321, 17-7321, 51-7321, 53-7321, 57-7321
TNF-α ELISA Ready-SET-Go!	h/m		88-7346 (h), 88-7324 (m)

Cell Signaling	Specificity	Clone	Formats
Foxp3	m/r	FJK-16s	13-5773, 11-5773, 12-5773, 17-5773, 51-5773, 57-5775, see web for other formats available
	h/nhp	PCH101	14-4776, 13-4776, 11-4776, 12-4776, 15-4776, 17-4776, 51-4776, 53-4776, 56-4776, 57-4776
	h	236A/E7	14-4777, 13-4777, 12-4777, 17-4777, 53-4777
IKKe (IKKi)	h	polyclonal	14-6329
IKKg (NEMO, FIP3)	h/m/r	polyclonal	14-6335
IRAK	h/m/r	polyclonal	14-6006
IRAK2	h	polyclonal	14-6122 (546 - 564aa), 14-6221 (571 - 590aa)
IRAK4	h	polyclonal	14-6125
IRAK-M	h/m/r	polyclonal	14-6355
MyD88	h/m	polyclonal	14-6222 (internal), 14-6223 (CT)
NAK (TBK1)	h/m	polyclonal	14-6351
NIK (NF-kB inducing kinase)	h	polyclonal	14-6128
NOD2	h	2D9	14-5869
	m		Please Inquire
TIRAP	h/m/r	polyclonal	14-9058

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