R&D Systems Immunology Focus
summer | 2006

Immunology FOCUS

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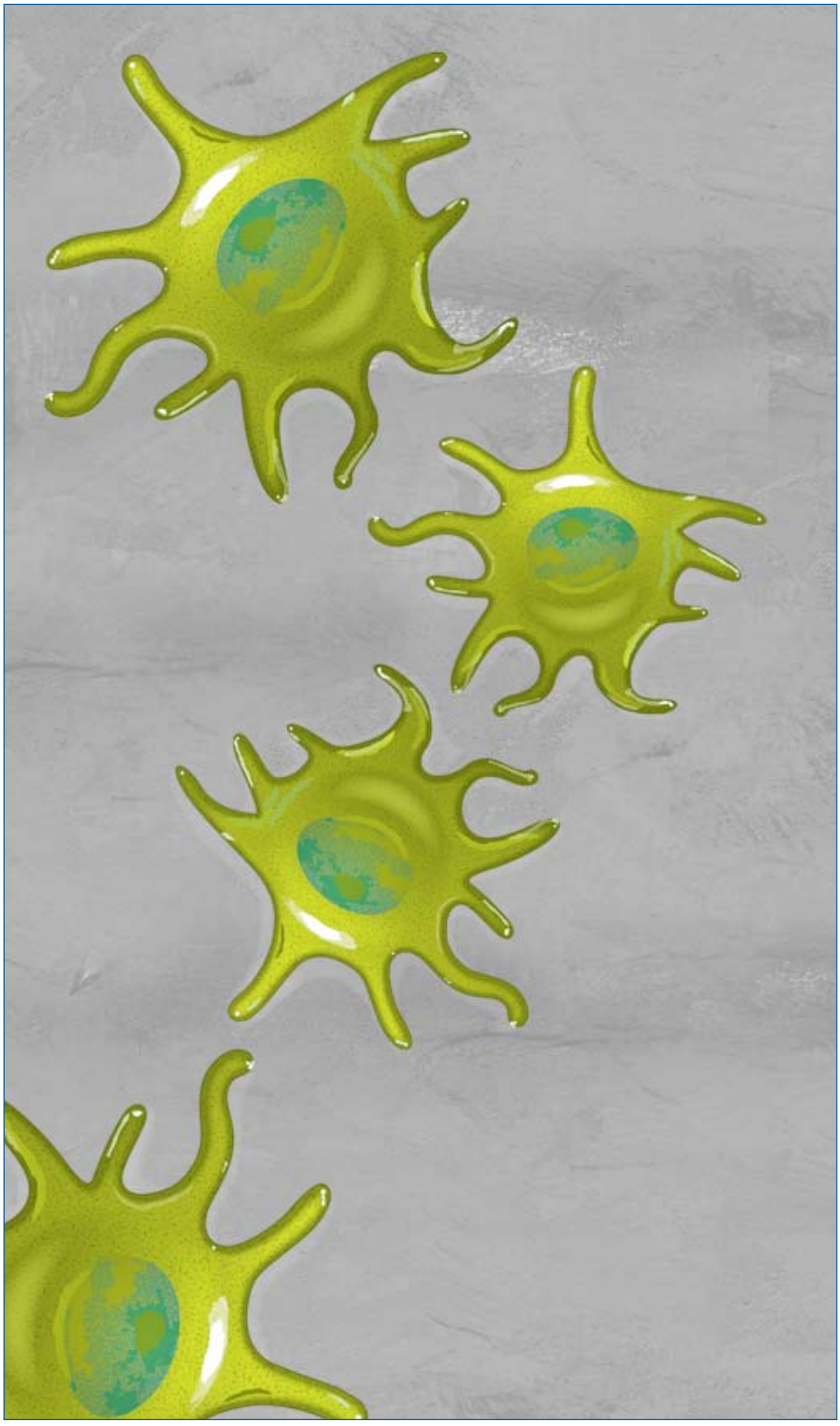


R&D SYSTEMS
Quality | Selection | Performance | Results

Signal Transduction: Kinase & Phosphatase Reagents

Co-inhibitory PD-L2/PD-1 signaling & SHP-2 phosphatase

Regulation of MAP kinase (MAPK) signaling pathways is critical for T cell development, activation, differentiation, and death. MAPKs are activated by the dual phosphorylation of threonine and tyrosine residues resulting in subsequent transcription factor activation. The MAPK signaling pathway in T cells can be triggered by cytokines, growth factors, and ligands for transmembrane receptors. Ligation of the T cell receptor (TCR)/CD3 complex results in rapid activation of PI 3-kinase, which leads to Akt and MAPK activation. Sustaining this signaling often requires a second co-stimulatory signal. T cells express several co-stimulatory molecules including CD28, ICOS, LFA-1, SLAM, 4-1BB, OX40, and CD27. MAPK signaling induced by TCR/CD3 ligation can also be downregulated by co-inhibitory signals. T cells express several co-inhibitory molecules including CTLA-4, PD-1, and BTLA-4. Co-inhibition by PD-1 and its ligand PD-L2 has been attributed to increased SHP-2 phosphatase activity (Figure 1) and the disruption of PI 3-kinase/Akt/MAPK signaling (Figure 2).

PD-L2 Co-inhibition Activates SHP-2

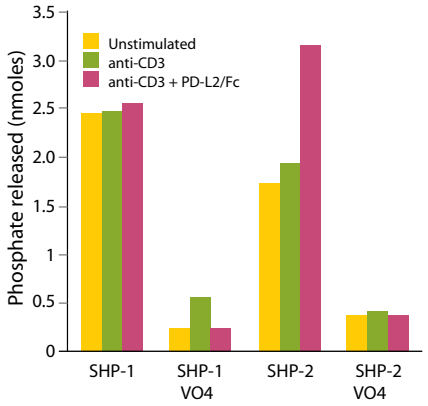


Figure 1. Combined stimulation with anti-CD3 and the co-inhibitory ligand PD-L2, doubles SHP-2 phosphatase activity. Human T cell blasts were unstimulated (BSA; 1 µg/mL), stimulated with an anti-CD3 antibody (500 ng/mL), or stimulated with anti-CD3 and R&D Systems recombinant human PD-L2/Fc (Catalog # 1224-PL). Phosphatase activity was determined for immunoprecipitated SHP-1 and SHP-2 in the presence or absence of the phosphatase inhibitor sodium orthovanadate (VO4). Phosphatase activity was determined using R&D Systems Tyrosine Phosphatase Substrate 1 (Catalog # ES006), followed by phosphate detection using R&D Systems Malachite Green Phosphate Detection Kit (Catalog # DY996).

Figures 1 & 2 used with permission: Saunders, P. A. *et al.* (2005) Eur. J. Immunol. **35**:3561.

KINASE & PHOSPHATASE RESEARCH REAGENTS

Kinases			Phosphatases		
MOLECULE	ANTIBODIES	ELISAs/ASSAYS	MOLECULE	ANTIBODIES	ELISAs/ASSAYS
Akt Family	H M R	H M R	Alkaline Phosphatase*	H M R	
AMPK	H M R		Calcineurin A, B	H M R	
ATM	H M R	H	CD45	H M	H M
CaM Kinase II	Ms		CDC25A, B*	H M R	
CDC2	H M R		DARPP-32	M R	
Chk1, 2	H M R	H M R	DEP-1/CD148*	H M R	H
ERK1, 2	H M R	H M R	LAR	H M R	
ERK3	H		Lyp	H	
ERK5/BMK1	H M		Phosphate Detection Kit		Ms
GSK-3	H M R	H M R	PNUTS	H M R	
IKKγ	H M R		PP1	H M R	
JNK (pan), 1, 2	H M R	H M R	PP2A	H M R	H M R
MARCKS	Ms		PRL-3	H	
MEK1, 2	H M R		PTEN*	H M R	H M R
MKK3, 4, 6*	H M R		PTP β/ζ	H	
MLK4α	H M R		Oxidized PTP Active Site	Ms	
MSK1, 2	H M		PTP-MEG2	H M R	
p38 Family*	H M R	H M R	PTP1B*	H M R	H
p70 S6 Kinase	H M R	H M R	Ser/Thr Phosphatase Substrate I		Ms
PDK-1	H		SHIP	H M R	
PI 3-Kinase p85, p110	H		SHP-1, 2	H M R	H M R
PKR	H		TC-PTP*	H M R	H M R
RSK Family	H M R	H M R	Tyrosine Phosphatase Substrate I		Ms
SGK	H				
Src	H M R				
TOR	H M R	H			
Tyk2	H				

Key: H Human M Mouse R Rat Ms Multi-species *Proteins also available, see our website for details

PD-L2 Co-inhibition Supresses Akt and ERK Phosphorylation

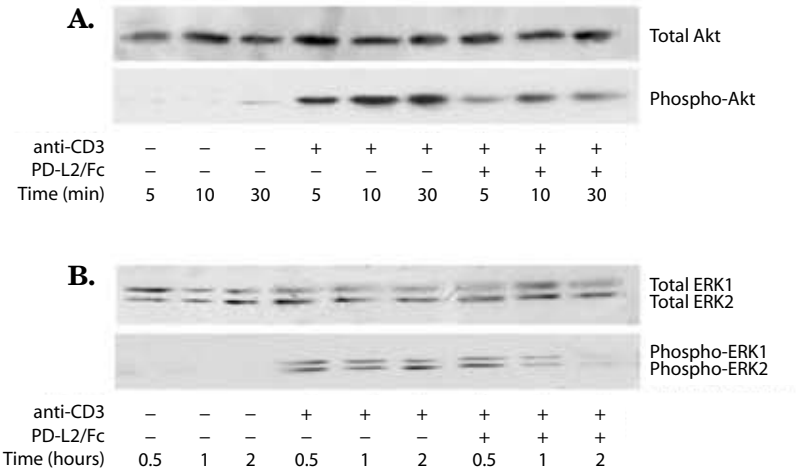


Figure 2. The co-inhibitory ligand PD-L2 suppresses Akt and ERK signaling in stimulated T cells. Human T cell blasts were unstimulated (BSA 1 µg/mL), stimulated with an anti-CD3 antibody (500 ng/mL), or stimulated with anti-CD3 and R&D Systems recombinant human PD-L2/Fc (Catalog # 1224-PL) for the indicated times. Lysates were analyzed by Western blot using **(A)** R&D systems anti-human total or phospho-AKT antibodies (Catalog # AF2055; AF887), or **(B)** anti- human total or phospho-ERK 1/2 antibodies (Catalog # AF1576; AF1018).

Lectin Family

Carbohydrate-binding proteins (lectins) have roles to play in many important processes such as self/non-self recognition, endocytosis, routing and chaperoning of molecules within the cell, and trafficking of cells within the body. C-type lectins, including CL-P1, the monocyte mannose receptor (MMR), mannose binding lectin (MBL), ficolins, and others are active in pathogen recognition. Dendritic cell C-type lectins, such as DC-SIGN, DC-SIGNR, DCAR, DCIR, dectins, and DLEC, are important in dendritic cell trafficking and formation of the immunological synapse. The selectins are also well known for mediating cell trafficking. R&D Systems offers a range of research tools for the study of these lectin subgroups, as well as sialic acid-binding Ig-type lectins (siglecs) and galactose-binding lectins (galectins).

Lectin Research Reagents

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ ASSAYS
ASGR1	M	M	
Chondrolectin	H		
CL-P1/COLEC12	H	H M	
CLEC-1	H		
CLEC-2	H		
CLECSF13	M		
CLECSF8	H		
DC-SIGN	H	H	
DC-SIGNR/CD299	H	H	
DCAR	M		
DCIR/CLEC4A	H M		
DEC-205	H		
Dectin-1/CLEC7A, Dectin-2/CLEC6A	H M		
DLEC	H		
Fcε RII	H	H	
Ficolin-2, -3	H		
Galectins	H M	H M	M
Galectin-3 BP	H		

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ ASSAYS
Langerin	H		
Layilin	H M	H M	
LOX-1/SR-E1	H M	H M	
LSECtin/CLEC4G	H		
MAG/Siglec-4a	R	R	
MBL/MBL-1/MBL-2	H M	H M	
MDL-1/CLEC5A	H M		
MGL2	M	M	
MICL/CLEC12A	H M		
MMR	H M	H M	
OCILRP2/CLEC2i	M		
PSGL-1	H	H	
Reg Family	H M R		
E-Selectin	H M R	H M R	H M
L-Selectin	H M R	H M R	H M R
P-Selectin	H M	H M	H M
Siglec Family*	H M R	H M R	H

Key: H Human M Mouse R Rat *See our website @ www.RnDSystems.com for an updated product listing for the siglec family.

Galectin-4 Expression in Human Epithelium

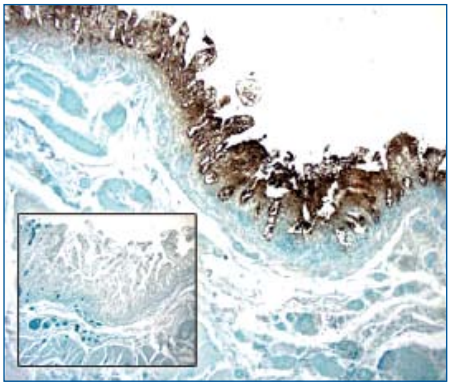


Figure 1. Galectin-4 expression has previously been described in mammalian alimentary tract epithelium.[1] Consistent with this observation, galectin-4 was detected in paraffin-embedded sections of human intestine using R&D Systems polyclonal anti-human galectin-4 antibody (Catalog # AF1227). Tissues were stained using R&D Systems anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue). Little staining is observed in a control section in the absence of the primary antibody (inset).

1. Wooters, M.A. *et al.* (2005) J. Histochem. Cytochem. **53**:197.

PSGL-1-Mediated P-Selectin Adhesion

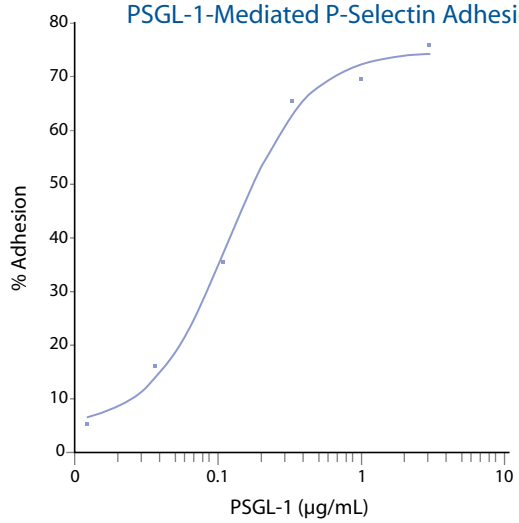


Figure 2. PSGL-1 mediates P-selectin adhesion. CHO cells were transfected with P-selectin and transferred to microplates (5 x 10^5 cells/well) coated with the indicated concentrations of R&D Systems recombinant human PSGL-1 (Catalog # 3345-P5) for 30 minutes. P-selectin-expressing cells adhere to PSGL-1 in a dose-dependent manner.

Flow Cytometric Analysis of MDL-1 Expression

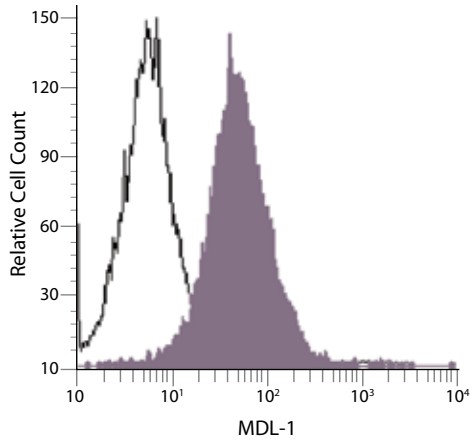


Figure 3. The mouse monocytic cell line RAW264 was stained with R&D Systems phycoerythrin-conjugated anti-mouse MDL-1 antibody (Catalog # FAB1639P; filled histogram) and analyzed by flow cytometry. Cells were also stained with a PE-conjugated isotype control antibody (Catalog # IC006P; open histogram).

Regulatory T Cells

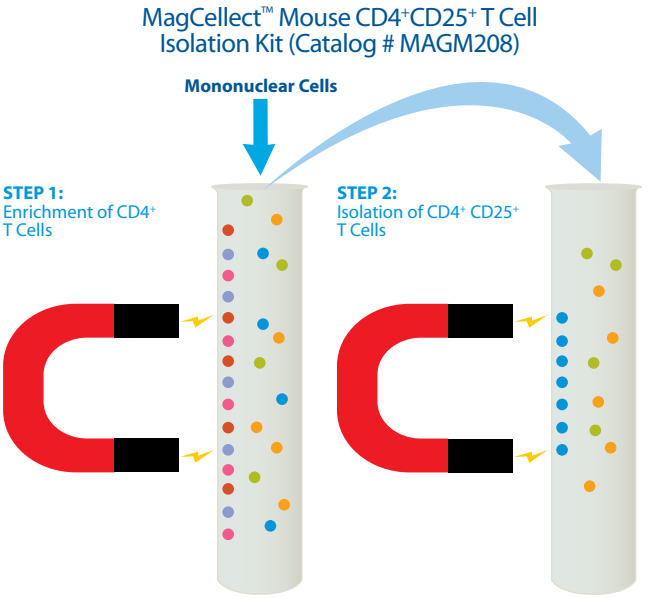
Subsets of regulatory T cells (Tregs), generated from different lineages, play important roles in immune homeostasis, autoimmune disease, anti-tumor responses, and transplantation immunology. Populations of Tregs are found in both $CD4^+$ and $CD8^+$ T cell lineages. Further characterization of Tregs has identified subsets that are $CD4^+CD25^+$, $CD4^+CD25^-$, and $CD8^+CD28^-$. Studies have concluded that the $CD4^+CD25^+$ Treg population constitutes 5 to 10% of peripheral $CD4^+$ T cells in normal individuals.

Multiple attempts have been made to identify markers that can be utilized to distinguish and/or isolate specific Treg subsets. Candidates like integrin αE/CD103 and LAG-3/CD223 are unique to some Treg subsets, while CTLA-4, GITR, or OX40/CD134 are also expressed on activated T cells. Another possible marker, PD-1/CD279 (programmed cell death-1), is expressed intracellularly in Tregs, but is co-expressed with CD25 on the surface of activated $CD4^+$ T cells. The nuclear localization of FoxP3, initally thought to be unique to $CD4^+$ $CD25^+$ cells, precludes its use for cell isolation purposes. R&D Systems offers a wide range of reagents useful for the characterization, study, and/or isolation of Tregs.

Treg Research Reagents

MOLECULES	ANTIBODIES	PROTEINS	ELISAs/ASSAYS	ELISpot	Cell Selection
B7-2/CD86	H M R	H M R			
CD3	H M				H M R
CD4	H M Ca F	H			H M R
CD5	H M				
CD8	H M F				H M R
CD25/IL-2 Rα	H M	H M	H		H M
CD27/TNFRSF7	H M	H M			
CD28	H M	H M			
CD38	H				
CD40 Ligand/TNFSF5	H M	H M	H M		
CD45	H M		H M		
CD69	H M				
CTLA-4	H M	H M			
CXCR4	H M				
Fas/TNFRSF6	H M R	H M R F	H M		
FoxP3	H				
GITR/TNFRSF18	H M	H M	H M		
Granzyme B	M	H M		M	
ICAM-1/CD54	H M R	H M R	H M R		
IFN-γ	H M R Ca CR E F P Pr	H M R B Ca E CR F P Pr	H M R Ca CR F P Pr	H M R Ca CR F P Pr	
IL-2 Rβ	H M	H			
IL-4	H M R Ca CR E F P	H M R B Ca E F CR P Pr	H M R CR F P	H M Ca	
IL-10	H M R Ca CR E F P V	H M R Ca CR E F P V	H M R Ca F P	H M Ca F	
Integrin αE/CD103	M				
LAG-3/CD223	H M	H			
Neuropilin-1	R	R			
OX40/TNFRSF4/CD134	M	H M			
OX40 Ligand/TNFSF4	H M	H M	H		
PD-1	H M	H M			
RANK/TNFRSF11A	H M	H M			
L-Selectin	H M R	H M R	H M R		
P-Selectin	H M	H M	H M		
TLR4	H	H			
TRANCE/TNFSF11	H M	H M	M		

Key: H Human M Mouse R Rat B Bovine Ca Canine CR Cotton Rat E Equine F Feline P Porcine Pr Primate V Viral



STEP 1: Mononuclear cells are incubated with a cocktail of biotinylated antibodies followed by incubation with streptavidin ferrofluid.

Unwanted cells are magnetically separated using the MagCellect magnet (Catalog # MAG997) and discarded, leaving a $CD4^+$-enriched cell population.

STEP 2: $CD4^+$ cells are incubated with a biotinylated CD25 antibody, followed by incubation with streptavidin ferrofluid.

Magnetically tagged $CD4^+CD25^+$ T cells (cyan cells) are isolated using the MagCellect magnet.

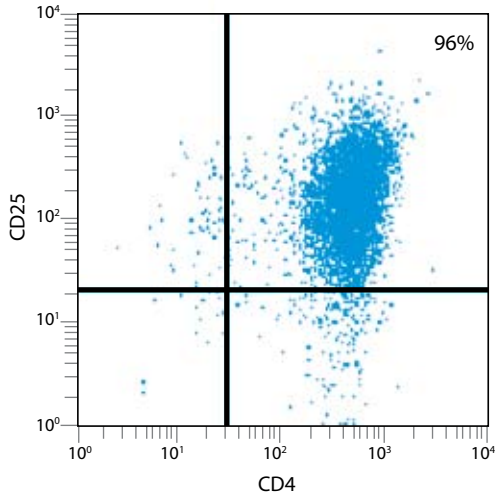


Figure 1. Isolation of mouse $CD4^+CD25^+$ T cells from activated splenocytes using R&D Systems MagCellect Mouse $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (Catalog # MAGM208). Dot plots represent dual staining of all viable cells recovered using the MagCellect Kit and analyzed by flow cytometry. Cells were stained with R&D Systems CD4-Fluorescein antibody (Catalog # FAB554F) and CD25-Phycoerythrin (PE) antibody (Catalog # FAB2438P). Approximately 96% of the cells recovered are $CD4^+CD25^+$ cells.

Natural Killer Cells

Natural killer (NK) cells are lymphocytes of the innate immune system that function as both cytolytic effectors and regulators of immune responses. NK cells express a large number of receptors that deliver either activating or inhibitory signals, and the relative balance of these signals controls NK cell activity.

NK cells are activated upon detection of abnormalities in target cells such as the loss of MHC class I expression or up-regulation of stress-induced ligands in response to infection or neoplastic transformation. Indeed, many viruses have evolved strategies to evade detection by NK cells or to modulate their activity.

A variety of receptors trigger the NK cytolytic activity directed toward certain tumor targets, virally infected cells, and even normal immune system constituents such as immature dendritic cells. NK cells are also important regulators of the adaptive immune system via their ability to secrete a number of cytokines in response to immune activation.

NK Cell Research Reagents

MOLECULE	ANTIBODIES	PROTEINS	ELISA/ASSAYS
2B4/SLAMF4	H M		
CD155/PVR	H	H	
CD69	H M		
CD8	H M F	H	
CD94	H		
CRACC/SLAMF7	H		
DNAM-1	H	H	
Fcγ RIII/CD16	H M	H M	
H60	M	M	
ILT2/CD85j	H	H	
Integrin α2/CD49B	H M		
KIR/CD158	H		
KIR2DL1	H		
KIR2DL3	H		
KIR2DL4/CD158d	H	H	
KIR2DS4	H		
KIR3DL1	H		
LFA-3/CD58	H	H	
LMIR1/CD300A	H		
LMIR2/CD300c	H		
MICA	H	H	H
MICB	H	H	H
MULT-1	M	M	
NCAM-1/CD56	H	H	
Nectin-2/CD112	H	H	
NKG2A	H		
NKG2C	H		
NKG2D	H M	H M	
NKp30	H	H	
NKp44	H	H	
NKp46/NCR1	H M	H M	
NKp80/KLRF1	H		
NTB-A/SLAMF6	H		
Rae-1	M		
Rae-1α		M	
Rae-1β		M	
Rae-1γ	M	M	
Rae-1δ		M	
Rae-1ε	M	M	
SLAM/CD150	H		
SLAMF3/CD229	H M		
ULBP-1	H	H	
ULBP-2	H	H	
ULBP-3	H	H	

Key: H Human M Mouse F Feline

NKp46 & NCAM-1-Expressing PBLs

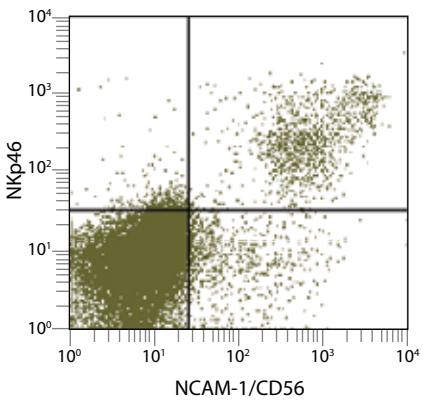


Figure 1. Human peripheral blood lymphocytes (PBL) were examined by flow cytometry to determine the relative number of cells expressing cell surface NKp46/CD335 and/or NCAM-1/CD56. Cells were double stained with R&D Systems anti-human NKp46 allophycocyanin (AP)-conjugated antibody (Catalog # FAB1850A) and an anti-human NCAM-1/CD56 Phycoerythrin (PE)-conjugated antibody.

MICB & ULBP-3 Binding to NKG2D

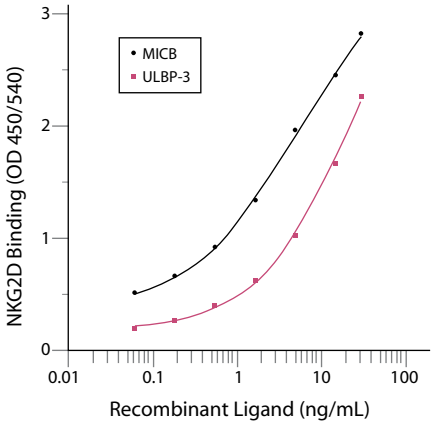


Figure 2. NKG2D binding to recombinant ligands MICB and ULBP-3. R&D Systems recombinant human NKG2D/Fc (Catalog Number 1299-NK) was immobilized at 2 µg/mL in a microtiter plate. After blocking with 1% BSA, R&D Systems recombinant MICB/Fc (Catalog # 1599-MB) or ULBP-3/Fc (Catalog # 1517-UL), were added at the indicated concentrations. Ligand binding was detected with R&D Systems biotinylated polyclonal antibodies specific for MICB (Catalog # BAF1599) or ULBP-3 (Catalog # BAF1517).

ULBP-2 Expression in Colon Cancer Tissue

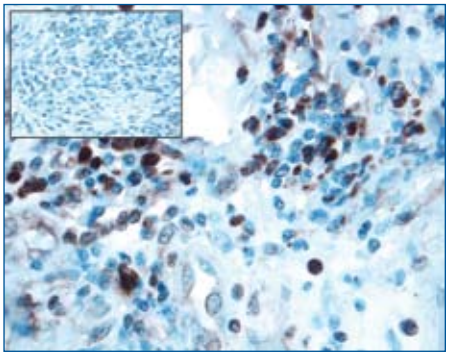


Figure 3. Tumor cell-expressed ULBP-2 functions as a ligand for the NK cell-activating receptor NKG2D, and has putative involvement in tumor surveillance. ULBP-2 was detected in paraffin-embedded human colon cancer tissue sections using R&D Systems anti-human ULBP-2 polyclonal antibody (Catalog # AF1298). Tissues were first subjected to an antigen-retrieval procedure using R&D Systems Antigen Retrieval Reagent-Basic (Catalog # CTS013). Sections were stained using R&D Systems anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue). The inset shows control staining in the absence of primary antibody.

Innate Immunity and Dendritic Cells

Dendritic cells (DC) are immune system sentinels, as well as regulators of cytokine production and inflammatory responses to pathogens. Initiation of the innate immune response begins with the recognition of a pathogen, which is mediated via the germline-encoded pattern recognition receptors (PRRs) expressed on DCs, innate lymphocytes, and other cell types. Non-clonal PRRs have the ability to recognize pathogen-associated molecular patterns (PAMPs). Activation of PRRs triggers downstream signaling, resulting in specific anti-viral, anti-fungal, or anti-bacterial responses. R&D Systems has developed a wide range of research reagents useful for the study of DCs as well as lymphocytes of the innate immune system.

Dendritic Cell Research Reagents

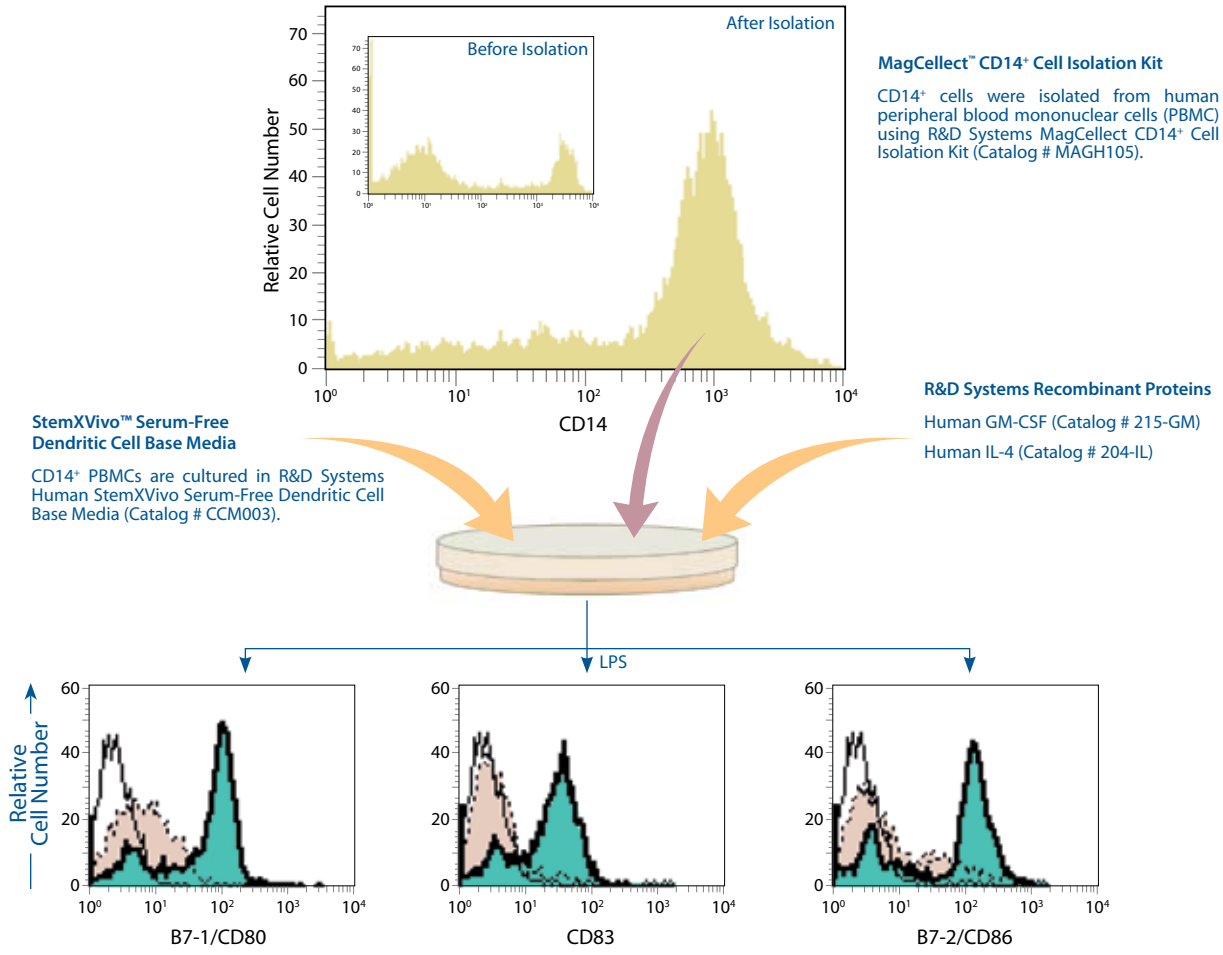
MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ASSAYS
B7-1/CD80	H M R	H M R	H
B7-2/CD86	H M R	H M R	
B7-H1, H2, H3	H M	H M	
CCR6	H M		
CCR7	H		
CD14	H M	H M	H
CD40/TNFRSF5	H M	H M	M
CD58/LFA-3	H	H	
CD83	H M	M	
CLEC-1, -2	H		
DC-SIGN	H	H	

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ASSAYS
DC-SIGNR/CD299	H	H	
DCAR	M		
DCIR/CLEC4A	H M		
DEC-205	H		
Dectin-1, -2	H M		
DLEC	H		
ICAM-1/CD54	H M R	H M R	H M R
ICAM-2/CD102	H M	H M	
ILT2/CD85j	H	H	
ILT3/CD85k	H		
ILT4/CD85d	H	H	

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ASSAYS
ILT5/CD85a	H		
Langerin	H		
MMR	H M	H M	
PD-L2	H M	H M	
TLR1	H M		
TLR2	H M	M	
TLR3	H M	H M	
TLR4	H	H	
TLR6	M	M	

Key: H Human M Mouse R Rat

Isolation of CD14+ Monocytes and Induction of Mature Monocyte-derived Dendritic Cells



LPS upregulates markers of mature dendritic cells. Immature CD14+ monocyte-derived cells (tan) and LPS-treated mature dendritic cells (aqua) were stained with the indicated FITC-conjugated R&D Systems antibodies (B7-1/CD80, Catalog # FAB140F; CD83, Catalog # FAB1774F; B7-2/CD86, Catalog # FAB141F) and assessed by flow cytometry. Isotype-matched control staining is also shown (open histograms).

Co-Stimulation/-Inhibition: The B7 Family & Associated Molecules

The spectrum of B7 family co-regulatory molecules has expanded since the original members, B7-1 (CD80) and B7-2 (CD86), were described. It now includes seven members with roles inside and outside of lymphoid tissues. PD-L1 (B7-H1) and PD-L2 (B7-DC) play important roles in regulating T cell activation and tolerance by delivering mainly inhibitory signals through T cell programmed death-1 (PD-1). B7-H2 (B7h, ICOSL) is a ligand for inducible co-stimulator (ICOS), an effector of T cell responses and T cell-dependent B cell responses. ICOS engagement stimulates the production of cytokines including IL-10, indicating B7-H2 might influence $CD4^+CD25^+$ T regulatory cells, tolerance, and autoimmunity. Two more B7 homologs, B7-H3 and B7-H4 (B7x, B7-S1), bind to and influence activated T cells, although the receptors they engage are not yet known. B7-H3 shows both stimulatory and inhibitory actions, while B7-H4 is an unequivocal negative regulator of T cell responses. B7-1 and B7-2 act through CD28 as co-stimulators and CTLA-4 as a co-inhibitor.

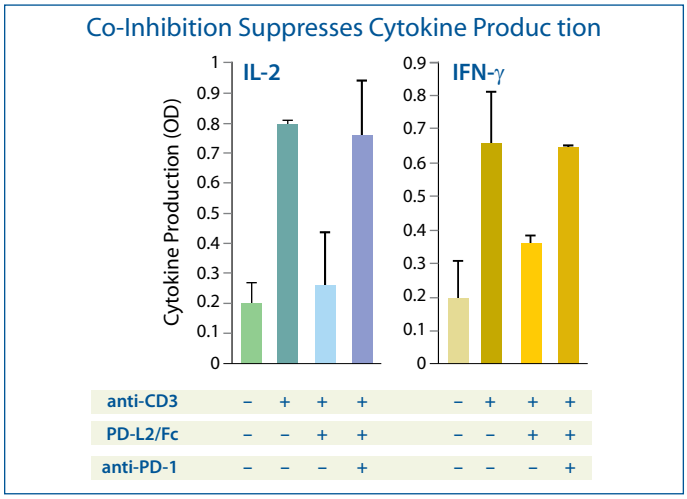


Figure 1. The co-inhibitory ligand PD-L2 suppresses cytokine production in stimulated T cells. PHA human T cell blasts were stimulated with an anti-CD3 antibody (500 ng/mL), or stimulated with anti-CD3 supplemented with R&D Systems recombinant PD-L2/Fc chimera (Catalog # 1224-PL; 30 mg/mL), or treated with anti-CD3, recombinant PD-L2/Fc, and R&D Systems human anti-PD-1 monoclonal antibody (Catalog # MAB1086). After a 2 day incubation, supernatants were collected and assessed using R&D Systems IL-2 (Catalog # D2050) or IFN-γ (Catalog # DIF50) Quantikine® ELISA kits. Cytokine stimulation is suppressed by PD-L2, which is in turn inhibited by a blocking antibody to its receptor, PD-1.

Figure used with permission: Saunders, P. A. *et al.* (2005) Eur. J. Immunol. **35**:3561.

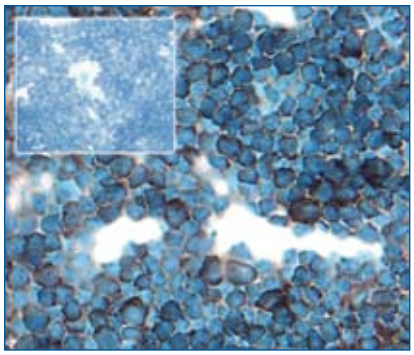


Figure 2. Detection of PD-L2 in cryostat tissue sections of mouse thymus using of R&D Systems anti-mouse PD-L2 affinity-purified antibody (Catalog # AF1022). Tissues were stained using R&D Systems anti-goat HRP-DAB Cell and Tissue Staining Kit (Catalog CTS008; brown) and counterstained with hematoxylin (blue). No staining is seen in control sections in the absence of primary antibody (inset). This antibody is also validated for Western blot and receptor blockade.

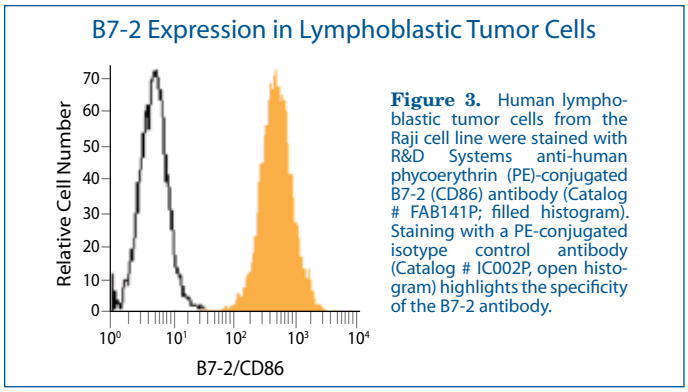


Figure 3. Human lymphoblastic tumor cells from the Raji cell line were stained with R&D Systems anti-human phycoerythrin (PE)-conjugated B7-2 (CD86) antibody (Catalog # FAB141P; filled histogram). Staining with a PE-conjugated isotype control antibody (Catalog # IC002P, open histogram) highlights the specificity of the B7-2 antibody.

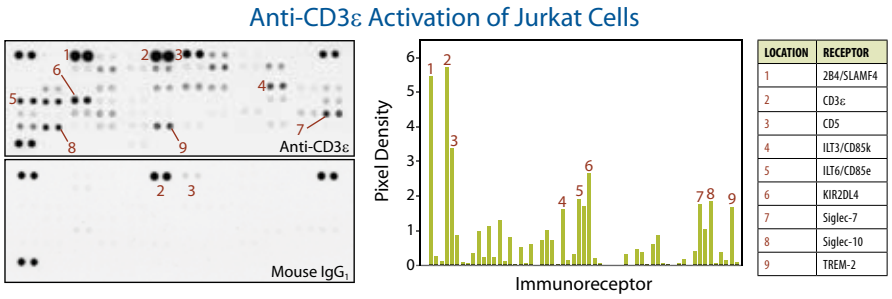
Co-Stimulation/Co-Inhibition Research Reagents

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ASSAYS	PRIMER PAIRS
2B4/SLAMF4	H M			
4-1BB/TNFRSF9	H M	H M	H M	
4-1BB Ligand/TNFSF9	H M	H M		
B7-1/CD80	H M R	H M R	H	
B7-2/CD86	H M R	H M R		
B7-H1	H M	H M		
B7-H2	H M	H M		
B7-H3	H M	H M		
B7-H4	M	M		
BLAME/SLAMF8	H			
BTLA	H M			
CD27/TNFRSF7	H M	H M		H
CD27 Ligand/TNFSF7	M	M	M	M
CD28	H M	H M		
CD30/TNFRSF8	H M	H M	M	H M R
CD30 Ligand/TNFSF8	H M	H M	M	H
CD48/SLAMF2	H			
CD84/SLAMF5	H	H		
CD229/SLAMF3	H M			
CD2F-10/SLAMF9	M			
CRACC/SLAMF7	H			
CTLA-4	H M	H M		
HVEM/TNFRSF14	H M	H		
ICOS	H M	H M		
LIGHT/TNFSF14	H M	H M	H	H
NTB-A/SLAMF6	H			
OX40/TNFRSF4	M	H M		
OX40 Ligand/TNFSF4	H M	H M	H	
PD-1	H M	H M		
PD-L2	H M	H M		
SLAM	H			

Key: H Human M Mouse R Rat

Proteome Profiler™ Phospho-Immunoreceptor Array: ITAM/ITIM-Associated Receptors

The Human Phospho-Immunoreceptor Array (Catalog # ARY004) is a rapid, sensitive, and economical tool used to simultaneously detect the relative levels of tyrosine phosphorylation of 59 ITAM/ITIM-associated immunoreceptors, without the need for immunoprecipitation/ Western blots. The array kit includes buffers, nitrocellulose membranes spotted in duplicate with carefully selected capture antibodies, and detection antibodies. Relative phosphorylation levels can then be assessed by chemiluminescence. The conditions used in the array protocol maintain the non-covalent association of activation receptors with phosphorylated, ITAM-containing transmembrane signaling adapters. This kit can also be adapted to provide a total immunoreceptor profile.



LOCATION	RECEPTOR
1	2B4/SLAMF4
2	CD3ε
3	CD5
4	ILT3/CD85k
5	ILT6/CD85e
6	KIR2DL4
7	Siglec-7
8	Siglec-10
9	TREM-2

Figure 1. The Human Phospho-Immunoreceptor Array (Catalog # ARY004) detects multiple tyrosine-phosphorylated ITAM/ITIM-associated immunoreceptors in lysates prepared from cells activated by antibody-mediated crosslinking. Jurkat T cells were incubated with a mouse monoclonal anti-CD3ε antibody (Catalog # MAB100; left/upper panel) or a mouse IgG1 isotype control (Catalog # MAB002; left/lower panel) for 10 minutes, followed by incubation with a goat anti-mouse IgG antibody (Catalog # AF007). Changes in phosphorylation were quantified using image analysis software (histogram; middle panel). Receptors undergoing significant phosphorylation and their location on the array are highlighted in the table (right panel). All membranes are spotted in duplicate and positive control spots are located in the upper left, lower left, and upper right corners of the array.

Human Phospho-Immunoreceptor Array Panel (Catalog # ARY004):

- 2B4/SLAMF4
- BLAME/SLAMF8
- BTLA
- CD3ε
- CD5
- CD6
- CD28
- CD84/SLAMF5
- CD229/SLAMF3
- CEACAM-1
- CLEC-1
- CLEC-2
- CRACC/SLAMF7
- CTLA-4/CD152
- DCIR/CLEC4A
- Dectin-1/CLEC7A
- DNAM-1
- Fcε RII/CD23
- Fcγ RIIA
- Fcγ RIIIA/B
- FcRH1/IRTA5
- FcRH2/IRTA4
- FcRH4/IRTA1
- FcRH5/IRTA2
- ILT2/CD85j
- ILT3/CD85k
- ILT4/CD85d
- ILT5/CD85a
- ILT6/CD85e
- Integrin β3/CD61
- KIR2DL4
- LAIR-1
- LAIR-2
- LMIR1/CD300A
- LMIR2/CD300C
- LMIR3/CD300LF
- LMIR6/CD300LE
- MDL-1/CLEC5A
- NKp30/NCR3
- NKp44/NCR2
- NKp46/NCR1
- NKp80/KLRF1
- NTB-A/SLAMF6
- PD-1
- PECAM/CD31
- SHIP-1
- SHP-1
- SHP-2
- Siglec-2/CD22
- Siglec-3/CD33
- Siglec-5
- Siglec-7
- Siglec-9
- Siglec-10
- SIRP-β1
- SLAM/CD150/SLAMF1
- TREM-1
- TREM-2
- TREML1/TLT-1

Quality | Selection | Performance | Results

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