R&D Systems Immunology Focus
spring | 2007

Immunology FOCUS

Inside

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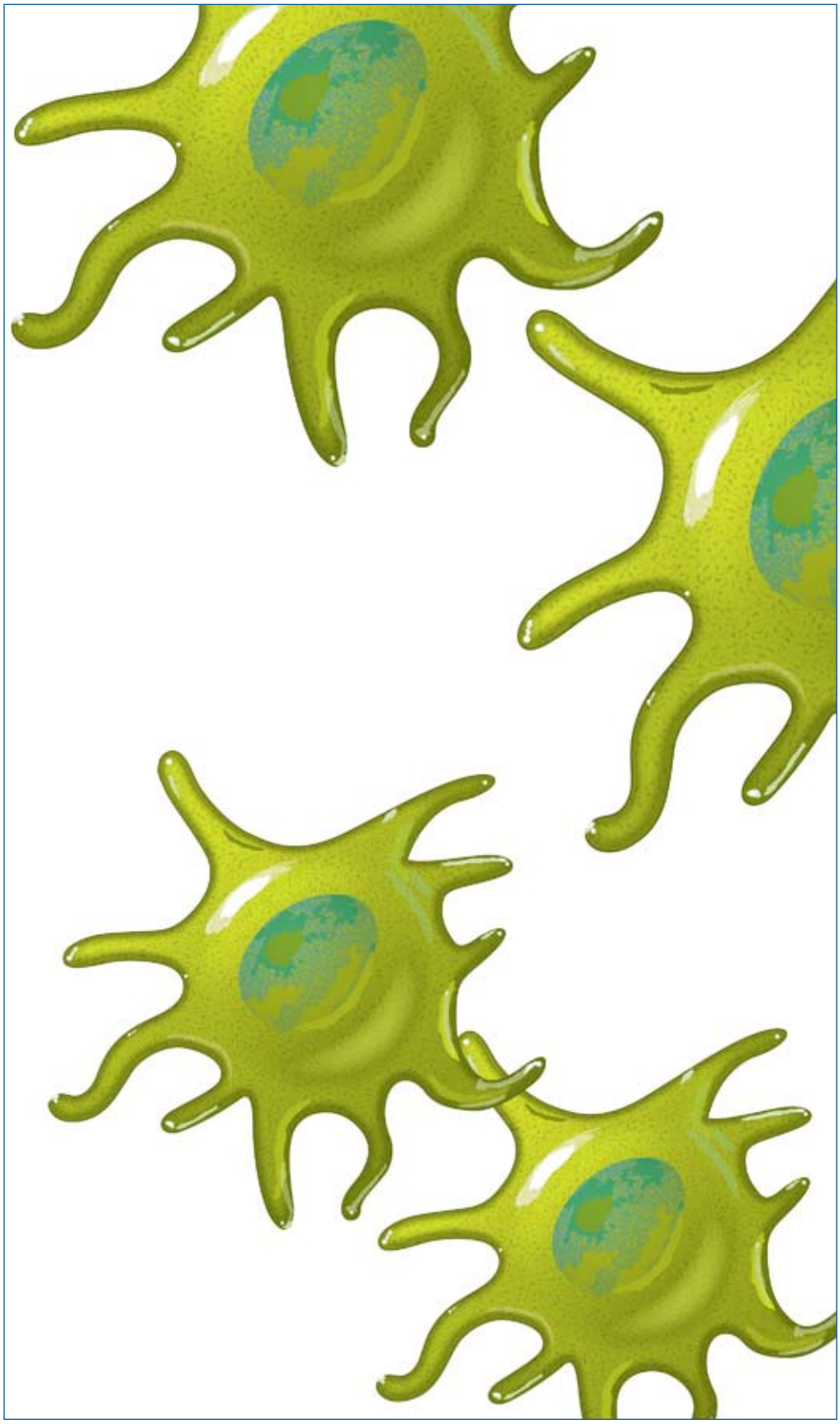
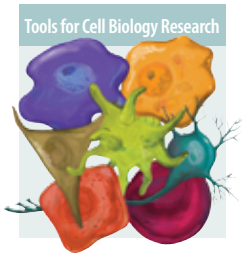




R&D SYSTEMS®

SLAM Family

The SLAM (signaling lymphocyte activation molecule) family proteins belong to the CD2 subset of Ig superfamily cell surface receptors. These proteins are expressed on hematopoietic cells and have extracellular domains containing an Ig V- and a C2-like domain (duplicated in the case of CD229). The cytoplasmic domains of six family members, 2B4 (CD244), SLAM (CD150), CD84, CD229, NTB-A and CRACC, contain multiple copies of cytoplasmic immunoreceptor tyrosine-based switch motifs (ITSM) that recruit the SAP (SLAM-associated protein) family of adaptor proteins.

Most SLAM family receptors have homophilic interactions, although 2B4 binds CD48. Ligation of SLAM receptors induces the phosphorylation of the cytoplasmic ITSMs, which serve as the docking sites for the SH2 domain of SAP-related adaptors SAP, EAT-2 and ERT (in rodents only). In turn, SAP binds the SH3 domain of Fyn and recruits it to the SLAM/SAP complex. Receptor-associated Fyn can subsequently phosphorylate tyrosine residues on the receptor to mediate downstream cell signaling.

SLAM family proteins participate in the development, differentiation, activation, and regulation of effector functions of B cells, T cells, NK cells and NKT cells. The involvement of SAP and SLAM family proteins in immune regulation is illustrated by their altered expression in autoimmunity and the association of SAP mutations with X-linked lymphoproliferative disease.

SLAM Family & Associated Molecules

MOLECULE	ANTIBODIES	PROTEINS
2B4/SLAMF4/CD244	H M	H M
BLAME/SLAMF8	H	
CD2	H M	
CD2F-10/SLAMF9	M	
CD48/SLAMF2	H M	H M
CD58/LFA-3	H	H
CD84/SLAMF5	H	H
CD229/SLAMF3/Ly-9	H M	
CRACC/SLAMF7/CD319	H	
Fyn	H M R	
NTB-A/SLAMF6	H M	
SLAM/CD150/SLAMF-1	H	

Key: H Human M Mouse R Rat

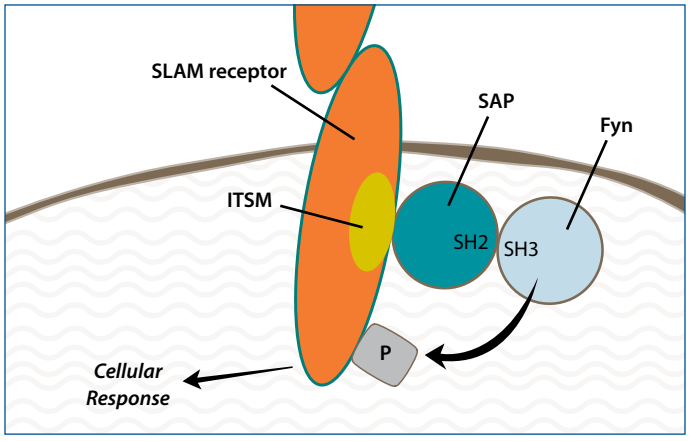


Figure 1. Fyn-mediated phosphorylation of SLAM receptor via SAP coupling.

Detection of CD229 by Flow Cytometry

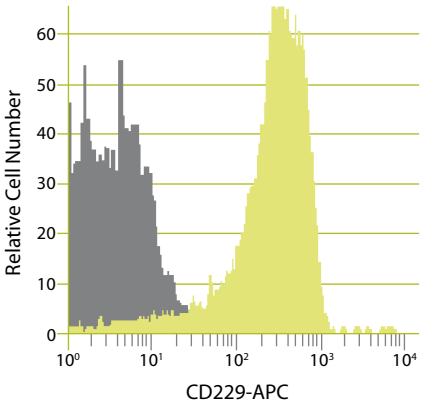


Figure 2. Human peripheral blood lymphocytes were stained with allophycocyanin-conjugated anti-human CD229 antibody (Catalog # FAB1898A; green histogram). Control cells were stained with isotype control antibody (Catalog # IC003A; gray histogram).

Double-Staining of CD3 and CRACC

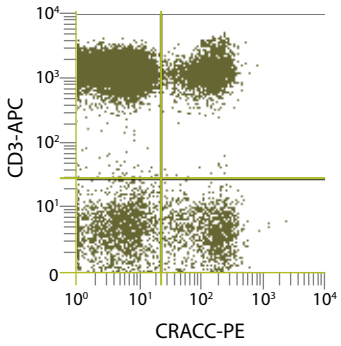


Figure 3. Whole blood lymphocytes double stained with phycoerythrin-conjugated anti-human CRACC (Catalog # FAB1906P) and allophycocyanin-conjugated anti-human CD3 (Catalog # FAB100A).

CD58/LFA-3 in Human Tonsil

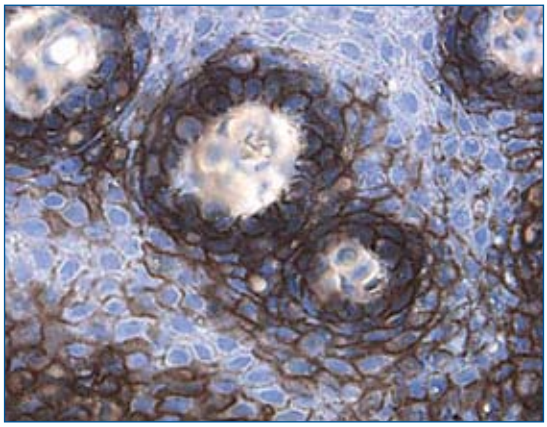


Figure 4. Detection of CD58 in paraffin-embedded human tonsil tissue sections using goat anti-human CD58 affinity-purified polyclonal antibody (Catalog # AF1689). Tissues were stained using anti-goat HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue).

Dendritic Cell Subsets in Immune Regulation

Myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) represent the two major DC subsets that differ in phenotype and function, but are complementary in immune regulation. In humans, the classic CD11c$^+$ mDC is well characterized as the prime IL-12 producer that preferentially induces the Th1 response. In contrast, the IL-3 Rα/CD123$^+$ pDC subset (also known as natural IFN-producing cells (IPC)) is renowned for its potent type I IFN production and believed to bias towards the Th2 response. Further studies revealed that DC are more sophisticated and complex than initially described. In addition to their cytokine production, DC's intrinsic phenotype, activation stage, and activation modes all play roles in dictating a specific immune response. Recent findings have underscored the dynamic interactions of DC with other immune cells such as NK cells, Treg cells, T cells, and B cells. This dynamic and mutual interaction is critical in maintaining the balance between immunity and tolerance. R&D Systems offers a wide range of research reagents for the isolation, characterization, and culturing of DC, and the study of DC-regulated immune responses.

Products for Dendritic Cell Isolation, Characterization, & Culturing

MOLECULE	ANTIBODIES	PROTEINS
Lineage Markers		
CD2	H M	
CD3	H M	
CD4	H M Ca F	H
CD8	H M F	
CD14	H M	H M
Fcγ RIII/CD16	H M	H M
Glycophorin A	H	
Gr-1/Ly-6G	M	
NCAM-1/CD56	H	H
Siglec-2/CD22	H M	H M
Maturation-Related Markers		
CD83	H M	H M
MMR/CD206	H M	H M
Other Subset Markers		
DLEC/CLEC4C/BDCA2	H	
IL-3 Rα/CD123	H M	H M
ILT2/CD85j	H	H
ILT3/CD85k	H	
ILT4/CD85d	H	H
ILT5/CD85a	H	
Integrin αM/CD11b	H M	
Integrin αX/CD11c	H	
Langerin/CLEC4K	H	
Neuropilin-1/BDCA-4	R	H R
Thrombomodulin/CD141/BDCA-3	M	H

Key: Ca Canine F Feline H Human M Mouse R Rat

Products for Dendritic Cell Isolation, Characterization, & Culturing

MOLECULE	ANTIBODIES	PROTEINS
Functional Markers		
B7-1/CD80	H M R	H M R
B7-2/CD86	H M R	H M R
B7-H3	H M	H M
CCR6	H M	
CCR7	H M	
CD40/TNFRSF5	H M	H M
CD58/LFA-3	H	H
DC-SIGN/CLEC4L	H	H
DEC-205/CLEC13B	H	
Dectin-1/CLEC7A	H M	
ICAM-1/CD54	H M R	H M R
PD-L2/B7-DC	H M	H M
TLR1	H M	
TLR2	H M	M
TLR3	H M	H M
TLR4	H M	H
TLR6	M	M
TLR9	H	

CD83 Detection by Flow Cytometry

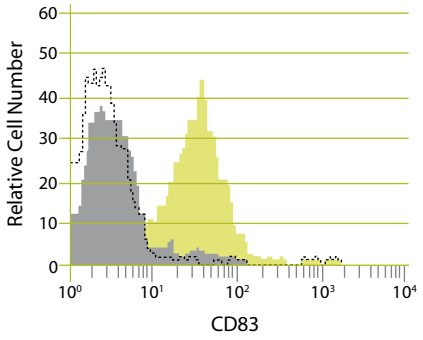
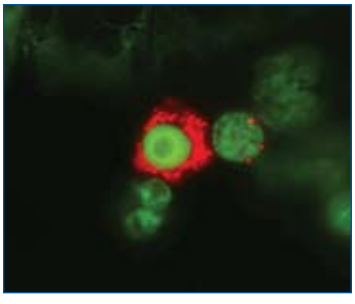


Figure 1. CD14$^+$ monocytes were isolated from human peripheral blood using MagCellect Human CD14$^+$ Cell Isolation Kit (Catalog # MAGH105). Immature monocyte-derived dendritic cells (MoDC) were generated by culturing for 7 days in Human StemXVivo™ Serum-Free Dendritic Cell Base Media (Catalog # CCM003) with GM-CSF (Catalog # 215-GM) and IL-4 (Catalog # 204-IL; gray histogram). LPS was added on day 7 to induce maturation. On day 9, mature MoDC were stained with PE-conjugated anti-human CD83 monoclonal antibody (Catalog # FAB1774P; green histogram). Isotype-matched controls are shown as dotted histogram.



IL-3 Rα in Human Peripheral Blood Lymphocytes

Figure 2. Detection of IL-3 Rα-producing cells in human PBLs with mouse anti-human IL-3 Rα (Catalog # MAB301). Cells were stained with anti-goat Cy™3 (red) and FluoroNissl™ Green counterstain.

StemXVivo is a trademark of R&D Systems, Inc. Cy is a trademark of GE Healthcare Bio-Sciences. FluoroNissl is a trademark of Molecular Probes.

New Roles for Semaphorins

Semaphorins were originally known for their role in controlling neuronal guidance through their interaction with multimeric receptor complexes comprised of subunits from the Neuropilin and Plexin families. Several Semaphorins, soluble and membrane-bound, are now known to regulate the immune system.

The secreted Semaphorins, Sema3A and Sema3C inhibit activation and migration of T cells or monocytes. The transmembrane proteins, Sema4A, Sema4D, and Sema6A, influence the differentiation or activation of T cells, B cells, dendritic cells, and Langerhans cells. Sema7A, a GPI-linked member, stimulates monocytes yet inhibits T cell functions. In addition, two viral Semaphorins have been shown to modulate immune responses.

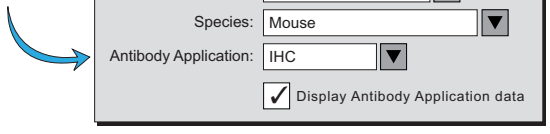
Immune Semaphorin signal transduction involves interaction with receptors associated with, as well as distinct from other semaphorin-regulated processes. For example, Sema3A appears to signal through a Neuropilin-1/Plexin A1 receptor. However, TIM-2, a member of the T cell immunoglobulin and mucin (TIM) gene family, is the only well characterized immune receptor for Sema4A, although a Plexin family member has also been implicated. Sema4D can signal either through CD72 or Plexin B1. Integrin β1 subunits and Plexin C1 are involved in Sema7A signaling.

Semaphorin-related Products

MOLECULE	ANTIBODIES	PROTEINS
CD72	M	
Integrin β1/CD29	H M	
Neuropilin-1	R	H R
Neuropilin-2	H R	H R
Plexin B1	H	
Plexin C1	H	
Semaphorin 3A	H	H
Semaphorin 3C	M	
Semaphorin 3E	H	H M
Semaphorin 3F		M
Semaphorin 6A	H M	H
Semaphorin 6B	H M	H
Semaphorin 6C	H M	
Semaphorin 6D	H	
Semaphorin 7A	H M	
TIM-2	M	

Key: H Human M Mouse R Rat

Locate your antibody quickly using our website antibody application filter



Semaphorin 6A-mediated Growth Cone Collapse

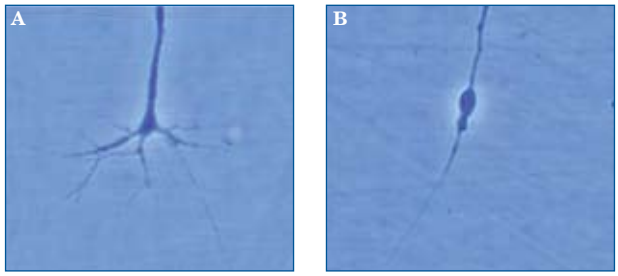


Figure 1. (A) A fully extended dorsal root ganglia (DRG) growth cone growing on a laminin-coated tissue plate in the presence of β-NGF (Catalog # 256-GF). **(B)** A collapsed DRG growth cone following treatment with Semaphorin-6A (Catalog # 1146-S6).

Plexin C1 Detection in Monocytes by Flow Cytometry

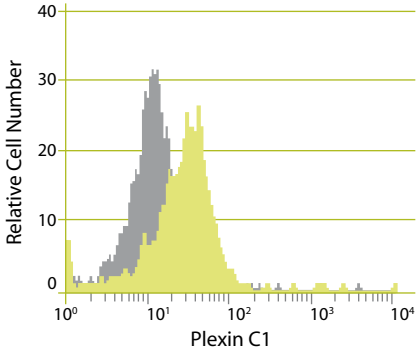


Figure 2. Blood-derived monocytes were stained with goat anti-human Plexin-C1 (Catalog # AF3887; green histogram) or control antibody (Catalog # AB-108-C; gray histogram), followed by PE-conjugated anti-goat antibody (Catalog # F0107).

Splenocyte Expression of CD72

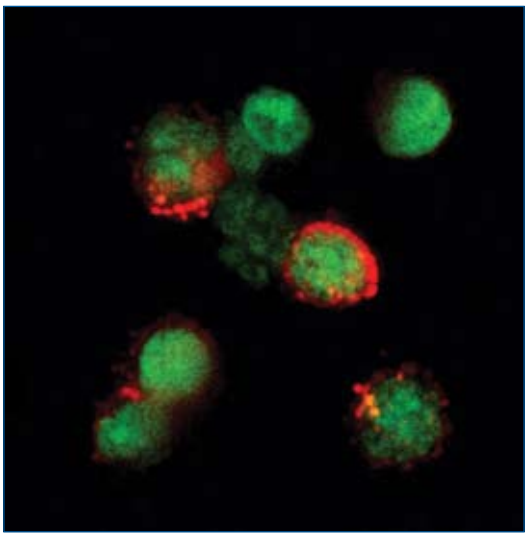


Figure 3. Detection of CD72 in mouse splenocytes using goat anti-mouse CD72 antibody (Catalog # AF1279) followed by a Rhodamine Red™ anti-goat antibody. Cells were counterstained with FluoroNissl Green.

Rhodamine Red is a trademark of Molecular Probes.

Interferons & Other Class II Cytokines

Type I interferons (IFN-α/β) have now been known for 50 years. Originally thought to simply "interfere" with viral replication, they are now known to play a broad role in microbial resistance. Type I IFN signals through a receptor complex containing two distinct Class II cytokine receptor subunits. These receptors are characterized by the presence of tandem fibronectin type III domains and contain no WSXWS motifs. To date, twelve Class II cytokine receptors that form heteromeric receptor complexes for approximately 30 ligands (Class II cytokines) have been identified. In addition to the type I IFNs, class II cytokines include, the type II interferon (IFN-γ), the antiviral proteins IL-28 (IFN-λ2), IL-28B (IFN-λ3), IL-29 (IFN-λ1), and the IL-10 family cytokines. Ligand binding causes the activation of cytoplasmic signal transduction pathway molecules including STAT1, STAT2, STAT3, STAT5, and Tyk2. IFNs exert a wide range of activities, including antiviral, immunomodulatory, pro-apoptotic, and anti-angiogenic functions, while IL-10 family molecules appear to be principally immunomodulatory, affecting both the pro- and anti-inflammatory arms of the immune response.

Interferon-related Products

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ASSAYS	PRIMER PAIRS
BLIMP1	H			
Coagulation Factor III/Tissue Factor	H M	H M		
IFN-α	H M CR P	H M R CR F	H M	
IFN-α/β R1	H M	M		
IFN-α/β R2	H M			
IFN-β	H M R	H M R	H M	
IFN-γ	H M R B Ca CR E F P Pr	H M R B Ca CR E F P Pr	H M R Ca CR F P Pr	H M R
IFN-γ R1	H M	H M	H M	
IFN-γ R2	H M			
IFN-ω	H	H	H	
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 R
IL-10 Rα	H M	H M		
IL-10 Rβ	H	H		
IL-19	H M	H M		H
IL-20	H M	H M	H M	H M
IL-20 Rα	H M	H		
IL-20 Rβ	H	H		
IL-22	H M	H M R	H M R	
IL-22 R	H			
IL-22BP	H M	H M		
IL-24	H M	H		M R
IL-26/AK155	H	H		H
IL-28A/IFN-λ2	H	H	H	
IL-28B/IFN-λ3	M	M	M	
IL-29/IFN-λ1	H	H	H	
Jak1	M			
Jak2	M R			
Limitin	M	M		
STAT1	H M		H M	
STAT2	H M		H	
STAT3	H M R		H M	
STAT5a/b	H M			
STAT5a	H M			
STAT5b	H M			
Tyk2	H			

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

IL-10 in Mouse PBLs

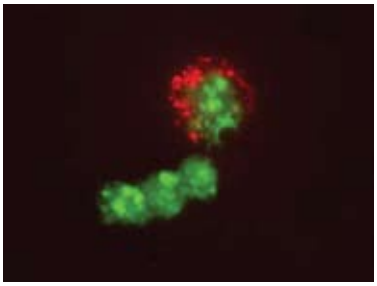


Figure 1. Indirect immunofluorescent staining of mouse PBLs to detect IL-10 producing cells. IL-10 was detected with goat anti-rat/mouse IL-10 (Catalog # AF519). Cells were stained with anti-goat Rhodamine Red X and counterstained with FluoroNissl Green.

Detection of Active Mouse STAT1 p91

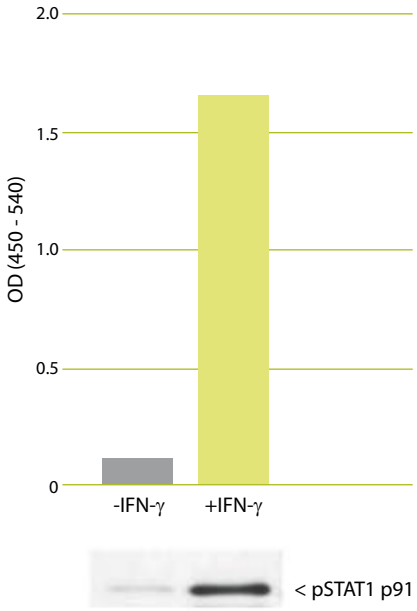


Figure 2. Mouse myeloid leukemia (M1) cells were treated with mouse IFN-γ (Catalog # 485-MI). Active STAT1 p91 nuclear extracts were prepared and assayed with the DuoSet® IC activity assay (Catalog # DYC-1490). The results correlate well with Western blot data obtained using the same nuclear extracts.

DuoSet is a registered trademark of R&D Systems, Inc.

A New Pole in Helper T Cells: Th17 Polarization

The recently described Th17 lineage is another subset of $CD4^+$ effector T cells that is distinct from, and antagonized by, the Th1 and Th2 lineages. In the mutual presence of interleukin (IL)-6 and transforming growth factor (TGF)-β, activated Th_0 cells express the transcription factor RORγt, leading to the predominant secretion of IL-17(A, F) and IL-22. In addition, IL-23 is essential for expansion and maintenance of this lineage. Mounting evidence indicates that Th17 cells, similar to Th1 and Th2 cells, are essential players in the response to specific pathogens, and when not properly regulated, can lead to autoimmune pathologies. Studies find elevated numbers of Th17 cells in wild-type mice relative to IL-23-deficient mice and suggest an important role for IL-23 and/or IL-17 in several extracellular bacterial infection models. Studies with IL-23 knockout mice have also implicated Th17 cells in autoimmune models: experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA). IL-22 is an IL-10 cytokine family member that has recently been identified as an inflammatory mediator of psoriasis by signaling through STAT3 in endothelial cells. STAT3, activated by IL-6 and IL-23, is also critical to Th17 differentiation and expansion.

Th Subset-related Molecules

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ASSAYS	PRIMER PAIRS
IFN-γ	H M R B Ca CR E F P Pr	H M R B Ca CR E F P Pr	H M R Ca CR F P Pr	H M R
IFN-γ R1	H M	H M	H M	
IFN-γ R2	H M			
IL-4	H M R B Ca CR E F P	H M R B Ca CR E F P Pr	H M R CR F P	H M
IL-4 R	H M	H M		
IL-6	H M R Ca CR E F P	H M R Ca CR E F P	H M R Ca P	H M R
IL-6 R	H M	H M	H	
IL-12	H M R P	H M R Ca F P Pr	H M	H M R
IL-12 R β1	H M	H M		
IL-12 R β2	H	H		
IL-12/IL-23 p40	H M R Ca F P	H M Ca F	H M P	H M R
IL-17	H M	H M	H M	H M
IL-17F	H M	H M		
IL-17 R	H M	H M		
IL-22	H M	H M R	H M R	
IL-22 BP	H M	H M		
IL-22 R	H	H		
IL-23	H M	H M R		
IL-23 R	H M	H M		
IL-27	H M	H M	M	H M
STAT3	H M R		H M	
TGF-β1	H	H M P	H M R Ca P	H M
TGF-β RI/ALK-5	H M	M		
TGF-β RII	H M	H M		
TGF-β RIIb	H	H		
TIM-3	H M			
TRAF-6	H			

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

Cell Surface Staining of IL-23 R by Flow Cytometry

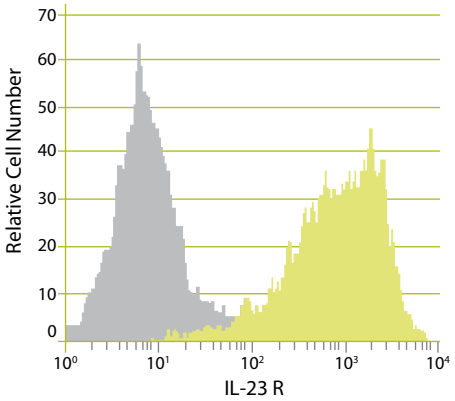


Figure 1. IL-23 R was detected in human erythroleukemia cells (K562) with anti-human IL-23 R (Catalog # AF1400; green histogram) or isotype control antibody (Catalog # AB-108-C; gray histogram). Cells were stained with PE-conjugated anti-goat antibody (Catalog # F0107).

IL-6 in Activated Mouse Cells

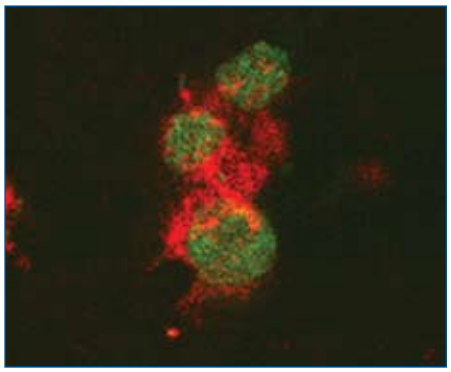


Figure 2. Detection of IL-6-producing cells in activated mouse T lymphocytes using anti-rat IL-6 (Catalog # AF506). Cells were stained with Cy2-conjugated anti-goat secondary antibody and counterstained with FluoroNissl Green.

ELISpot Detection of IL-17-producing Splenocytes

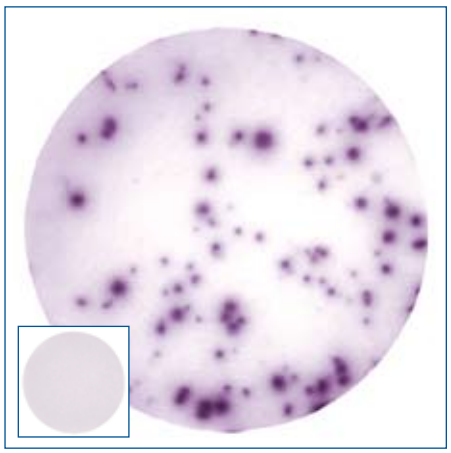


Figure 3. Mouse splenocytes (10^5 cells per well) were cultured in the absence (inset) or presence of PMA and calcium ionophore. The frequency of IL-17 producing cells was measured with the mouse IL-17 ELISpot kit (Catalog # EL421).

Chemokine Receptors: Orchestrating the Immunological Synapse

Chemokines and their G protein-coupled transmembrane receptors play critical roles in the success of T cell activation and response. Maturing dendritic cells (DC) migrate to the T cell zones of the lymph node (LN) through upregulation of chemokine receptor CCR7 and LN production of CCR7 ligands, CCL19/MIP-3β and CCL21/6Ckine. CCR7 expression also directs naïve T cells to the LN and encourages interaction with CCL21/6Ckine-producing antigen-presenting dendritic cells (APC). CCR7 ligation on T cells activates LFA-1 adhesion and facilitates formation of an LFA-1/ICAM-mediated tether from the T cell to the APC, a characteristic of immunological synapse (IS) organization. Subsequently, T cell chemokine receptors CCR5 and CXCR4 are recruited to the IS by APC-secretion of their ligands, CCL5/RANTES and CXCL12/SDF-1, respectively, thus stabilizing the IS. CCL5/RANTES and CXCL12/SDF-1 may also have dual roles as co-stimulators that enhance T cell activation. In preparation for exiting the T zone, primed T cells downregulate CCR7 and upregulate target tissue-specific chemokine receptors. For example, CXCR5 directs primed T cells to the LN follicle in order to assist B cells. In addition, chemokine receptors may participate in polarized immune responses since CCR5 and CXCR3 are associated with helper T (Th) type 1 cells and CCR3, CCR4, and CCR8 are preferentially expressed on Th2 cells.

Chemokine Receptors

MOLECULE	ANTIBODIES	PRIMER PAIRS
CCR1	H	H M R
CCR10	H M	
CCR2	H	H M
CCR3	H M	H M R
CCR4	H	H M R
CCR5	H	H M R
CCR6	H M	H M R
CCR7	H M	H M
CCR8	H	H M
CCR9	H M	
Chem R23	H	
CX_3CR1		H M R
CXCR1/IL-8 RA	H	H M R
CXCR2/IL-8 RB	H M	H M R
CXCR3	H M	H M R
CXCR4	H M	H M
CXCR5	H	H M R
CXCR6	H M	
D6	H	
HCR/CRAM-A/B	H	
HM74A/GPR109A	H	
XCR1		H M

Key: Ca Canine CR Cotton Rat F Feline H Human M Mouse R Rat RM Rhesus/Macaque

Chemokines

MOLECULE	ANTIBODIES	PROTEINS	ELISAs/ASSAYS
CCL2/MCP-1	H M Ca CR	H M R Ca	H M Ca
CCL3/MIP-1α	H M CR	H M CR	H M
CCL3L1/MIP-1α Isoform LD78β		H	
CCL4/MIP-1β	H M CR	H M CR	H M
CCL4L1/LAG-1		H	
CCL5/RANTES	H M CR	H M CR F	H M
CCL18/PARC	H	H	H
CCL19/MIP-3β	H M	H M	H M
CCL21/6Ckine	H M	H M	H M
CCL22/MDC	H M	H M	H M
CXCL9/MIG	H M	H M	H M
CXCL10/IP-10 /CRG-2	H M CR	H M CR	H M
CXCL12/SDF-1	H M	H M F RM	H M

CCR7 Detection by Flow Cytometry

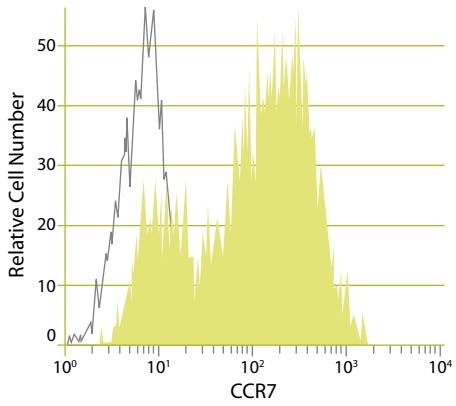


Figure 1. Monocyte-derived dendritic cells were stained with anti-human CCR7 phycoerythrin-conjugated monoclonal antibody (Catalog # FAB197P; green histogram), or an appropriate mouse IgG2A isotype control (Catalog # IC003P; open histogram).

CCL21/6Ckine Immunohistochemistry

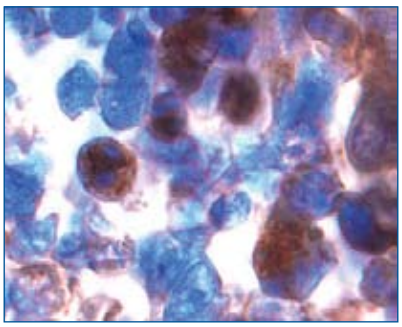


Figure 2. Detection of CCL21/6Ckine in immersion-fixed human tonsil tissue sections using goat anti-human CCL21/6Ckine affinity-purified polyclonal antibody (Catalog # AF366). Tissues were stained using anti-goat HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue).

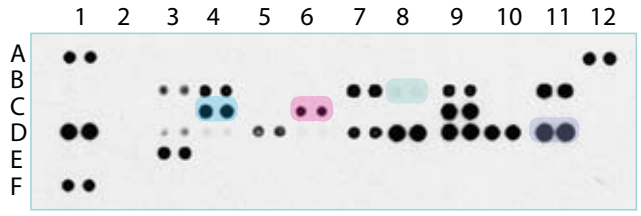
Proteome Profiler Mouse Cytokine Array, Panel A

R&D Systems Mouse Cytokine Array, Panel A (Catalog # ARY006) is a rapid, sensitive, and economical tool used to simultaneously detect the relative levels of 40 different cytokines, chemokines, and more in a single sample. No specialized equipment is necessary. Carefully selected capture antibodies have been spotted in duplicate on nitrocellulose membranes. Cell culture supernatant, cell lysate, serum, or plasma samples are diluted and mixed with a cocktail of biotinylated detection antibodies. The sample/antibody mixture is then incubated with the array. Any analyte/detection antibody complex present is bound by its cognate immobilized capture antibody on the membrane. Streptavidin-Horseradish Peroxidase and chemiluminescent detection reagents are added and a signal is produced in proportion to the amount of analyte bound.

KIT CONTENTS (Catalog # ARY006):

- 4 membranes with capture antibodies spotted in duplicate
- Detection antibody cocktail
- Streptavidin-HRP
- Transparency overlay
- Detailed protocol
- Buffers

A.



B.

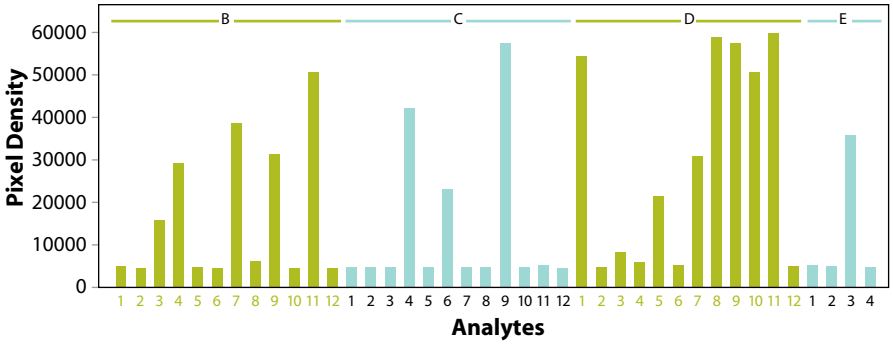


Figure 1. Proteome Profiler Mouse Cytokine Array, Panel A (Catalog # ARY006) was used to profile 40 cytokines, chemokines, and more in a single sample of culture supernatant following the treatment of mouse splenocytes with LPS. After chemiluminescent detection (**A**), the array data were quantified to generate a protein profile (histogram; **B**). The table (**C**) shows the analytes detected and their location on the membrane.

C.

COORDINATE	ANALYTE
B-1	BCA-1/CXCL13
B-2	C5a
B-3	G-CSF
B-4	GM-CSF
B-5	I-309/CCL1
B-6	Eotaxin/CCL11
B-7	sICAM-1
B-8	IFN-γ
B-9	IL-1α
B-10	IL-1β
B-11	IL-1ra
B-12	IL-2
C-1	IL-3
C-2	IL-4
C-3	IL-5
C-4	IL-6
C-5	IL-7
C-6	IL-10
C-7	IL-13
C-8	IL-12 p70

COORDINATE	ANALYTE
C-9	IL-16
C-10	IL-17
C-11	IL-23
C-12	IL-27
D-1	IP-10/CXCL10
D-2	I-TAC/CXCL11
D-3	KC
D-4	M-CSF
D-5	MCP-5/CCL12
D-6	JE/MCP-1/CCL2
D-7	MIG/CXCL9
D-8	MIP-1α/CCL3
D-9	MIP-1β/CCL4
D-10	MIP-2
D-11	RANTES/CCL5
D-12	SDF-1/CXCL12
E-1	TARC
E-2	TIMP-1
E-3	TNF-α
E-4	TREM-1

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