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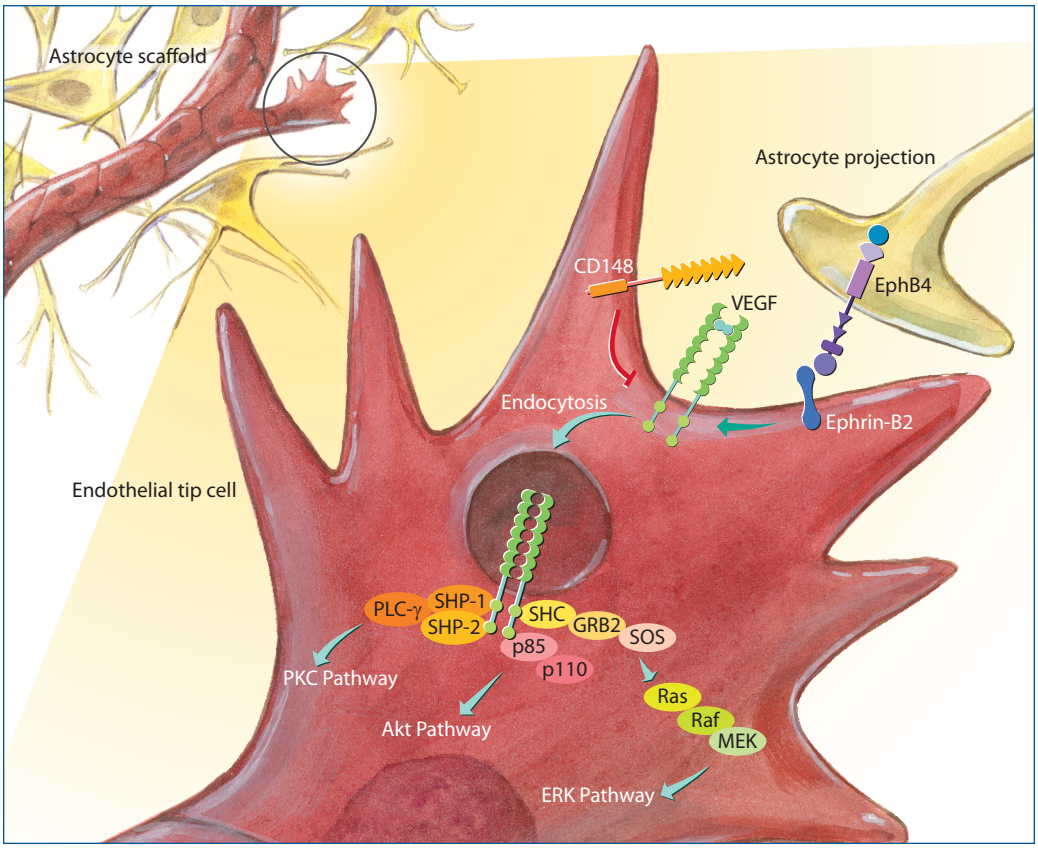

VEGF and Ephrin Signaling Pathways Cooperate During Vessel Guidance. In the developing retina, endothelial sprouts migrate across an astrocyte scaffold.[6] Endothelial tip cell filopodia direct vessel growth by reacting to guidance cues in the surrounding environment. Following stimulation by EphB4, Ephrin-B2 reverse signaling promotes the endocytosis of phosphorylated VEGF R dimers, in a PDZ domain dependent manner.[5,6] Internalized VEGF receptors subsequently exert pro-angiogenic effects via the PKC, Akt, and ERK signaling pathways. VEGF signaling is attenuated by dephosphorylation of VEGF R at the plasma membrane by phosphatases, such as CD148.

Ephrin-B2 Controls VEGF Receptor Endocytosis & Angiogenic Sprouting

Angiogenesis specifically describes the sprouting of new blood vessels from the existing vasculature. This process requires the formation of endothelial tip cells, highly motile cells that invade the surrounding tissue.[1] Endothelial tip cells guide the direction of new blood vessel growth by extending sensory filopodia that react to chemical signals in the surrounding environment. Endothelial cell proliferation and migration are primarily regulated by members of the vascular endothelial growth factor (VEGF) family. VEGF is known to regulate the generation of tip cells through the Notch-1 signaling pathway.[2] However, the underlying mechanisms that direct tip cell migration and vessel guidance remain enigmatic.

Emerging research suggests the cues that govern capillary attraction and repulsion are dependent on the same molecules that orchestrate axon migration. For example, the expression of Ephrin-B2, an established axon guidance molecule, was shown to be essential for early angiogenic remodeling.[3,4] Two recent papers tested the hypothesis that the VEGF and Ephrin signaling pathways cooperate to regulate angiogenic growth.[5,6] Wang and colleagues reported that selective inhibition of the Ephrin-B2 gene in endothelial cells caused a significant decrease in sprouting angiogenesis.[5] In contrast, microinjection of an Ephrin-B2 expression construct into individual cells in an endothelial monolayer induced migration, the dynamic extension of cellular protrusions, and invasive behavior.

A unique feature of Ephrin/Eph signaling is that Ephrin ligands can also act as receptors and Eph receptors can function as ligands. To clarify this bidirectional relationship, an Ephrin ligand binding to an Eph

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INSIDE

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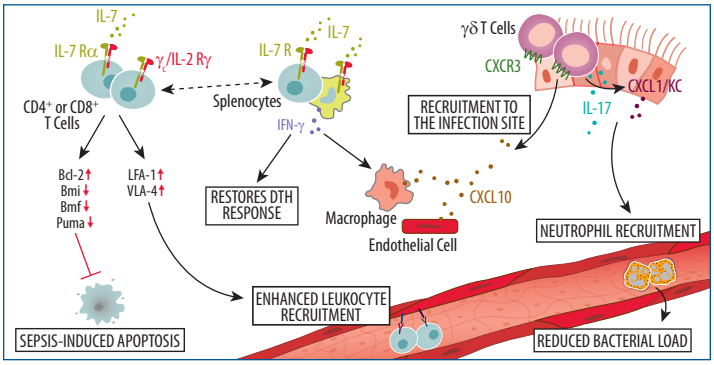
Interleukin-7 Inhibits Lymphocyte Apoptosis & Immunosuppression to Improve Survival in a Mouse Model of Sepsis

Sepsis, also known as systemic inflammatory response syndrome, is a potentially life-threatening condition caused by an uncontrolled inflammatory response to a microbial infection.[1] This response can lead to severe tissue damage, hypotension, and organ dysfunction or failure. The septic response involves both the innate and adaptive immune systems, and occurs in two distinct phases, an initial hyperinflammatory phase, followed by a period of immunoparalysis. While most patients survive the excessive cytokine production that occurs during the hyperinflammatory phase, subsequent immunoparalysis often leads to death due to an inability to eliminate either the primary infection or a secondary infection that develops.[2] In both experimental animal models of sepsis and human sepsis patients, apoptotic depletion of T and B lymphocytes has been suggested to be a key mechanism leading to immunoparalysis.[3-7] Supporting this hypothesis, several groups have shown that anti-apoptotic agents, such as caspase inhibitors and Bcl-2 overexpression, inhibit lymphocyte apoptosis and improve survival in mouse models of sepsis.[8-11]

Anti-apoptotic cytokines that promote lymphocyte proliferation and function, such as IL-7, are also now being investigated as potential therapeutic agents for the treatment of sepsis.[2,12] IL-7 is a type I cytokine belonging to the common cytokine receptor γ-chain (γ_c) family. It plays an important role in the development, survival, proliferation, and/or activation of CD4$^+$ and CD8$^+$ T cells, B cells, and γδ T cells.[13,14] In addition, it inhibits cytokine deprivation-induced T lymphocyte apoptosis by up-regulating the anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1 proteins.[14,15] These activities suggest that IL-7, like other anti-apoptotic agents, may be capable of inhibiting the immunoparalysis phase of sepsis.

A recent study by Unsinger *et al.* sheds more light on how IL-7 affects the septic response.[15] Using a clinically relevant mouse model of sepsis, known as cecal ligation and puncture (CLP) to artificially induce intra-abdominal peritonitis and bacteremia, the authors demonstrated that injection of IL-7 inhibited the sepsis-induced apoptosis of thymocytes, and CD4$^+$ and CD8$^+$ T cell subsets in the spleen and mesenteric lymph nodes. This was partially attributed to an increase in Bcl-2 expression that was detected in CD4$^+$ and CD8$^+$ T cells following IL-7 treatment. In addition, IL-7 prevented the sepsis-induced expression of the pro-apoptotic *Bim*, *Bmf*, and *Puma* genes. Significantly, IL-7 treatment not only inhibited lymphocyte apoptosis, but also improved the overall survival of two different mouse strains following CLP surgery. To identify potential mechanisms by which IL-7 may improve the outcome of sepsis, the effects of IL-7 on the production of pro- and anti-inflammatory cytokines was investigated. While IL-7 did not affect the levels of TNF-α, IL-6, or IL-10 detected in septic mice, it did restore splenocyte production of IFN-γ. Decreased IFN-γ production is a hallmark of sepsis that plays a major role in its lethality, presumably due to decreased resistance to invading microorganisms, reduced macrophage activation, and loss of the delayed-type hypersensitivity (DTH) response.[16] Injection of IL-7 re-established the IFN-γ-dependent DTH response to antigenic challenge that was lacking in septic mice.[15] In addition, it enhanced the expression of the leukocyte adhesion markers, LFA-1 and VLA-4 on CD4$^+$ and CD8$^+$ T cells in these animals, suggesting that it may improve leukocyte recruitment to the infection site. Collectively, these results indicate that IL-7 is capable of preventing several immunological defects that may contribute to septic immunoparalysis, while not affecting the severity of the hyperinflammatory response.

Many of the results reported by Unsinger *et al.* were confirmed by Kasten *et al.*, who also found that IL-7 treatment of septic mice led to a substantial increase in the recruitment of IL-17-secreting γδT cells to the peritoneum following CLP surgery.[17] Increased production of IL-17, coupled with elevated levels of CXCL1/KC, enhanced the early recruitment of neutrophils to the infection site, although it did not affect their activation or function. Enhanced neutrophil recruitment led to a reduction in bacterial load 24 hours following CLP surgery, without causing a significant increase in tissue damage. Together, these findings support the conclusion that IL-7, in addition to promoting T lymphocyte proliferation and function, may also improve survival in septic mice by promoting neutrophil recruitment to the infection site, thereby enhancing the innate immune response.



IL-7-induced Effects Improve the Immune Response in a Mouse Model of Sepsis. IL-7 treatment improves the immune response to sepsis by inhibiting the apoptosis of CD4$^+$ and CD8$^+$ T cells and promoting their expression of LFA-1 and VLA-4. Additionally, IL-7 treatment restores splenocyte production of IFN-γ to re-establish the delayed-type hypersensitivity (DTH) response and promote the recruitment of γδ T cells to the infection site. IL-17 secretion by γδ T cells, along with mesothelial cell production of CXCL1 at the infection site, promotes neutrophil recruitment and reduces the bacterial load.

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A Role for Galectin-3 in Angiogenesis

Certain physiological processes, such as embryonic development, reproductive function, and wound healing, require the formation of new blood vessels. The cellular mechanism by which new blood vessels are formed from the existing vasculature is termed angiogenesis. Angiogenesis is a multi-step process that involves the concerted action of several cytokines and growth factors that exert chemotactic, mitogenic, and modulatory effects on endothelial cells.[1]

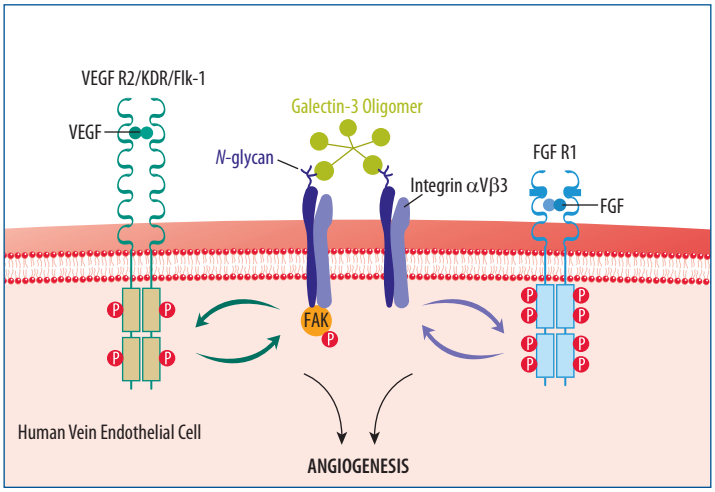
It has been demonstrated that carbohydrate-binding proteins and their respective glycoconjugate ligands also play an essential role in angiogenesis.[2,3] Specifically, recent research has shown that Galectin-3 promotes angiogenesis.[4,5] Galectin-3 is one member of the Galectin family of lectins that specifically bind to *N*-acetyl-lactosamine-containing glycoproteins.[6] All galectins contain at least one carbohydrate recognition domain (CRD) in their C-termini. However, Galectin-3 is the sole member of the family that contains one CRD linked to a proline, glycine and tyrosine-rich repeat N-terminal domain. The N-terminal half of Galectin-3 mediates oligomerization upon ligand binding.[6] Galectin-3 is mainly found in the cytosol, but can be secreted to function extracellularly, where it binds to cell surface glycoconjugates containing β-galactosides.[6]

A role for Galectin-3 in angiogenesis was first revealed after research demonstrated that it influences chemotaxis and differentiation of human umbilical vein endothelial cells (HUVECs). *In vitro*, Galectin-3 is both chemotactic and induces capillary tubule formation in HUVECs.[4] Galectin-3 also promotes angiogenesis *in vivo*.[4,5] These processes are dependent upon both carbohydrate recognition and oligomerization by Galectin-3.[4,5]

A recent paper by Markowska *et al.* elucidated a potential mechanism by which Galectin-3 mediates angiogenesis.[5] These authors discovered that Galectin-3 participates in angiogenesis induced by vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Decreasing Galectin-3 expression in HUVECs by small interfering RNA reduced VEGF- and bFGF- mediated migration and capillary tubule formation.[5] Additionally, both growth factors failed to induce neovascularization in Galectin-3-deficient mice.[5] Mass spectrometry analysis of HUVEC lysate proteins isolated with a Galectin-3 affinity column, identified Integrin αVβ3 as a binding partner with putative involvement in Galectin-3-mediated effects on angiogenesis.[5] In general, integrin clustering triggers the activation of intracellular signaling molecules, such as focal adhesion kinase (FAK).[7] Specifically, Integrin αVβ3 has been shown to be involved in growth factor-mediated angiogenesis.[7,8,9,10] Markowska and colleagues demonstrated that treating HUVECs with Galectin-3 promoted Integrin αVβ3 clustering and FAK activation.[5] Moreover, pretreatment with antibodies against Integrin αVβ3 inhibited Galectin-3-induced HUVEC migration and capillary tubule formation.[5]

It was hypothesized that Galectin-3 interacts with complex *N*-glycans present on Integrin αVβ3, activating signaling pathways that influence VEGF- and bFGF-mediated angiogenesis. The authors generated HUVECs expressing Integrin αVβ3 with reduced expression of the high affinity Galectin-3 ligands, β1,6-*N*-acetylglucosamine (GlcNAc)-branched *N*-glycans. This was accomplished by transfecting HUVECs with lentivirus carrying small hairpin RNA (shRNA) constructs directed against human *N*-acetylglucosaminyl-transferase V (GnTV), the enzyme that synthesizes the β1,6GlcNAc-branched arm in *N*-glycans.[5,11,12] Disrupting GnTV expression in HUVECs attenuated binding of Galectin-3 to cell surface glycoproteins.[5] These GnTV shRNA-transfected cells did not migrate or form tubule structures when exposed to either Galectin-3, VEGF, or bFGF.[5] In addition, Galectin-3 and both growth factors failed to promote angiogenesis *in vivo* in *GnTV*$^{-/-}$ mice.[5]

In addition to the key physiological processes that require angiogenesis, many disease states also trigger abnormal capillary growth.[4] For example, new blood vessel formation is central for the growth, invasion, and metastasis of cancer cells.[1] Galectin-3 serum levels have been found to be higher in cancer patients compared to healthy controls.[13] Elucidation of a cellular mechanism by which Galectin-3 modulates angiogenesis may provide new avenues for pharmacologically regulating pathological neovascularization.



Galectin-3 Activation of Integrin αVβ3 in Growth Factor-mediated Angiogenesis. Galectin-3, a β-galactoside binding protein, has been previously implicated as participating in angiogenesis; however, the mechanism Galectin-3 employs was still unknown. Markowska and colleagues demonstrated that Galectin-3 binds to complex *N*-glycans on Integrin αVβ3, triggering integrin clustering and FAK activation.[5] Activated Integrin αVβ3 subsequently influences VEGF- and bFGF-induced angiogenesis. This study complements a wealth of research that has implicated angiogenic activity to be dependent upon the coordinated actions of integrins and growth factor receptors. Integrin αVβ3, specifically the β3 subunit, complexes with VEGF R2 and FGF R1 upon ligand binding.[9,10,14] The interaction between Integrin αVβ3 and VEGF R2 is critical for VEGF-induced VEGF R2 phosphorylation and activation of VEGF R2 signaling pathways.[7,9,10] Integrin αVβ3 is also required for the sustained ERK1/2 activation observed during bFGF-induced angiogenesis.[15] In a similar manner, growth factors have also been shown to modulate integrin functioning. VEGF and FGF receptor activation increases the affinity of Integrin αVβ3 for its ligands.[8,9]

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Milk Fat Globule-EGF Factor 8 Protein & Tissue Repair

Milk Fat Globule-EGF Factor 8 Protein (MFG-E8), also known as Lactadherin, is a pleiotropic secreted glycoprotein that promotes mammary gland morphogenesis, angiogenesis, and tumor progression, inhibits blood coagulation, and can be cleaved to release an internal amyloidogenic fragment known as Medin. MFG-E8 also plays an important role in tissue homeostasis and the prevention of inflammation.[1] It contains discrete domains that enable it to serve as a bridge between apoptotic bodies and phagocytes. The discoidin domains bind to phosphatidylserine (PS), which is exposed on the surface of apoptotic cell bodies but not on viable cells.[2] An RGD motif (Arg-Gly-Asp) then mediates binding of MFG-E8 to Integrin αVβ3 or αVβ5 on phagocytic cells, triggering the uptake and destruction of the apoptotic material.[2,3] Clearance of apoptotic bodies prevents their transformation into necrotic masses and thereby prevents the inflammatory response that occurs surrounding tissue necrosis. Apoptotic cells are generated during normal tissue homeostasis and do not trigger inflammation.

MFG-E8 contributes to debris clearance and tissue repair in a variety of situations. During the germinal center reaction in the spleen and lymph nodes, it enables the clearance of apoptotic B cells by tingible body macrophages.[4,5] MFG-E8 null mice are unable to perform this function and are at heightened risk for developing autoimmunity.[4,5] Additionally, MFG-E8 limits disease progression by promoting the engulfment of apoptotic bodies in atherosclerotic plaques and prion-infected brain by macrophages and microglia, respectively.[6,7] It reduces inflammation and disease progression in colitis by preventing Osteopontin from binding and activating Integrin αVβ3.[8] MFG-E8 binding to PS on the surface of injured intestinal epithelial cells promotes their migration and the regeneration of epithelial integrity.[9]

An additional mechanism for MFG-E8 mediated tissue protection has recently been described, one that does not rely upon clearance of apoptotic bodies or binding to the above mentioned molecules. Based on the known functions of MFG-E8, Atabi *et al.* investigated whether it could be involved in the development of tissue fibrosis that can result from increased apoptosis and inflammation.[10] These authors employed a mouse model of bleomycin-induced pulmonary fibrosis, which is characterized by a buildup of interstitial Collagen. MFG-E8 was upregulated following bleomycin treatment, and mice deficient in MFG-E8 expression showed exaggerated fibrotic responses. Despite these observations, MFG-E8 did not appear to mediate the clearance of apoptotic cells. It did, however, affect the accumulation of Collagen. MFG-E8 participated in the removal of excess Collagen from fibrotic lungs, while its presence did not affect the rates of Collagen synthesis or degradation. The interaction of MFG-E8 with Collagen was mediated by the first discoidin domain, which is consistent with the Collagen-binding function of discoidin domains found in other proteins. MFG-E8/Collagen complexes were subsequently cleared by macrophages, although this did not involve the RGD motif of MFG-E8 or any of several integrins tested (including αVβ3 and αVβ5).

Therefore, MFG-E8 functions as a bridge to clear tissue debris through at least two distinct mechanisms. It can bind directly to PS on apoptotic cell bodies or to fibrotic Collagen. However, while the clearance of MFG-E8/apoptotic body complexes by macrophages is mediated by defined integrins, it remains to be determined how macrophages remove complexes of MFG-E8 and Collagen.

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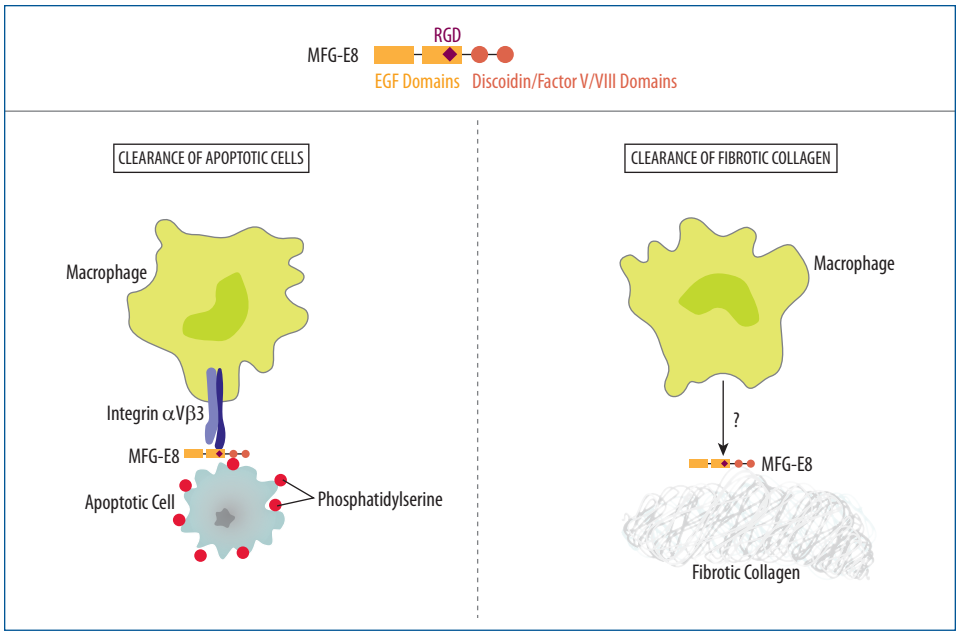

MFG-E8 Functions as a Bridging Molecule to Mediate Phagocytic Clearance of both Apoptotic Cells and Excess Collagen. MFG-E8 mediates the clearance of cellular debris by binding to phosphatidylserine on apoptotic cell bodies and Integrins αVβ3 or αVβ5 on phagocytes. A similar bridging function helps to alleviate fibrosis by binding of MFG-E8 to excess Collagen and unknown molecule(s) on macrophages.

RECENT CITATIONS:
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   Goat Anti-Mouse IL-15 Rα Antigen Affinity-purified Polyclonal Antibody (Catalog # AF551)

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2. Hoe, E. *et al.* (2010) Functionally significant differences in expression of disease-associated IL-7 receptor alpha haplotypes in CD4 T cells and dendritic cells. J. Immunol. **184**:2512.

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   Application: Bioassay

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   Human IL-7 Biotinylated Fluorokine® Kit (Catalog # NF700)

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   Recombinant Mouse IL-6 (Catalog # 406-ML)
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   Application: Bioassay

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   Application: Flow cytometry and ELISA development

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   Application: *In vivo*

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   Sample: Mouse
   Application: *In vivo*

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   Sample: Mouse dendritic cells
   Application: Flow Cytometry

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   Application: Immunohistochemistry

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   Sample: Human CD4 T cells
   Application: Western blot, immunoprecipitation, and flow cytometry

   Goat Anti-Human Common γ Chain Antigen Affinity-purified Polyclonal Antibody (Catalog # AF284)

   Sample: Human $CD4^+$ T cells
   Application: Western blot and immunoprecipitation

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Toronto, Ontario, Canada . . . . . June 15-18, 2011

10th World Congress on Inflammation
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TECHNICAL NOTE: A Novel Glycosyltransferase Activity Assay

Glycosyltransferases are enzymes that catalyze the transfer of a monosaccharide moiety from a glycosyl donor to an acceptor substrate. The majority are classified as Leloir enzymes that utilize nucleotide sugars as donors and produce nucleotide phosphates as part of the reaction. Assaying glycosyltransferase activity can be challenging. The most common method has been to monitor the transfer of radiolabeled sugars from donor to acceptor molecules. Various non-radioactive assays have also been developed; however, most of these have been customized for specific glycosyltransferases.

R&D Systems has developed a versitile, non-radioactive assay for measuring glycosyltransferase activity (Figure 1).[1] The assay involves the use of a specific phosphatase that liberates phosphate from nucleotides generated during the glycosyltransferase reaction. The phosphate levels are then assessed using malachite green phosphate detection reagents. Because the concentration of released phosphate is directly proportional to the number of sugar molecules transferred, kinetic parameters of glycosyltransferases can be measured. In addition, this colorimetric assay is conducted in a 96-well plate, making it amenable to high-throughput studies.

To demonstrate the utility of this technique, we assessed the activities of the enzymes *Clostridium difficile* toxin B (TcdB; Figure 2A), human O-Glucosyltransferase I (KTELC1; Figure 2B), and human ST6GAL1 (Figure 2C). TcdB and KTELC1 are glucosyltransferases with UDP-Glucose hydrolase activity that produce UDP as a byproduct of the reaction. The phosphatase CD39L3 hydrolyzes nucleotide β-phosphates and was used to liberate free phosphate from UDP. In contrast, ST6GAL1 is a sialyltransferase that catalyzes the transfer of sialic acid in α2,6 linkage to Galβ1-4GlcNAc structures on *N*-glycans. For this reaction, CMP-NeuAc was used as a donor substrate and *N*-acetyllactosamine (LN) was used as the acceptor. The 5′ nucleotidase CD73 was used to liberate free phosphate from CMP produced in the reaction. For all three glycosyltransferase reactions, accurate measurements of the specific activity (pmol/min/μg) versus donor substrate concentration were obtained by measuring free phosphate levels using malachite green detection reagents (Figure 2).

R&D Systems has used this technique to examine the activities of a wide range of glycosyltransferases. For more details, see our recent publication[1] or visit our website at **www.RnDSystems.com/go/Glycosyltransferase** to request a copy of our scientific poster, Universal Phosphatase-Coupled Glycosyltransferase Assay.

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1. Wu, Z.L. *et al.* (2010) Glycobiology [Epub ahead of print].

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NOW AVAILABLE
Glycosyltransferase Activity Kit
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This kit contains the specific phosphatase CD39L3, buffers, and phosphate detection reagents to measure the activity of glycosyltransferases that use diphosphonucleotide sugars as donor substrates.

Phosphatase-coupled Glycosyltransferase Reactions

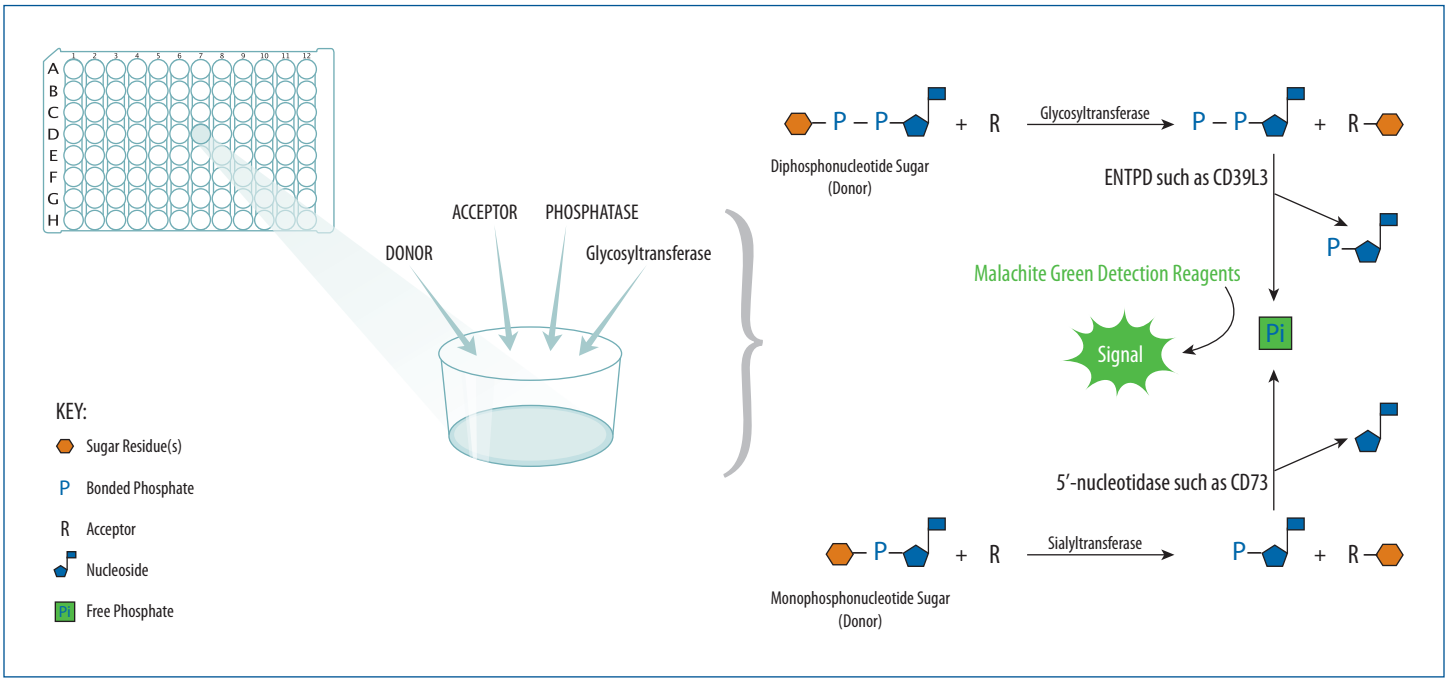

Figure 1. The Phosphatase-coupled Glycosyltransferase Assay. This non-radioactive assay couples glycosyltransferase reactions with specific phosphatases to generate free phosphate groups, which are then measured. The specific phosphatase is chosen based on the phosphonucleotide produced during the reaction. Liberated free phosphate is then measured using malachite green detection reagents, and the levels are used as a measure of enzyme activity.

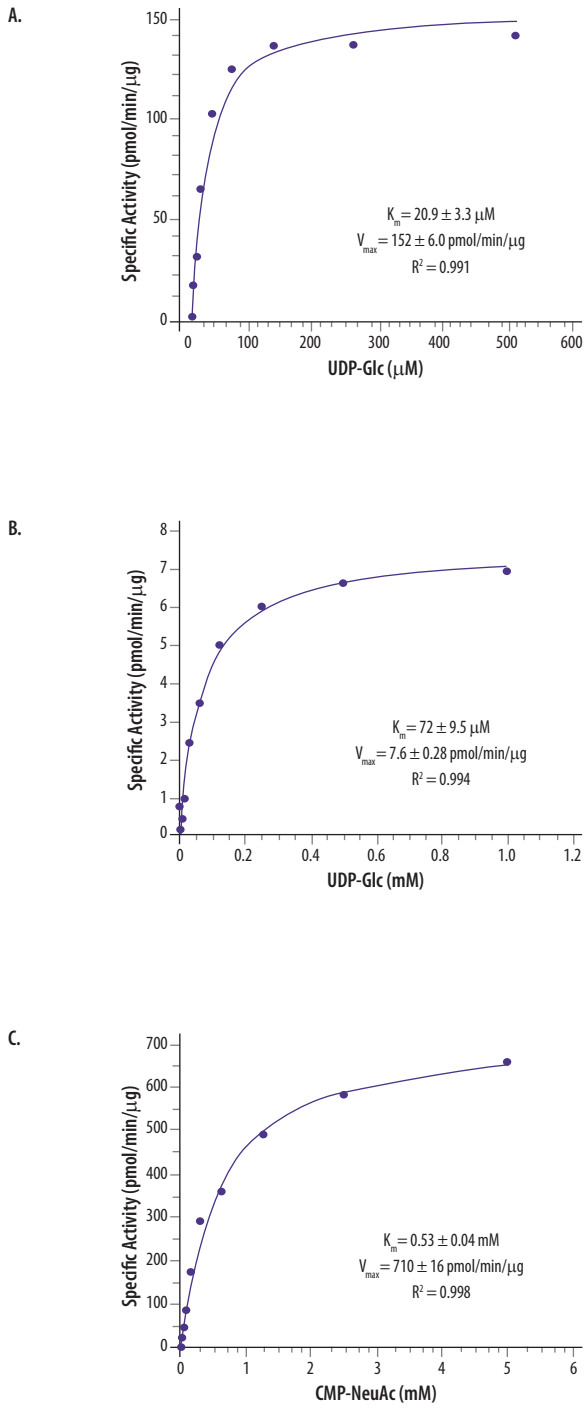

Figure 2. Measuring Enzyme Activity Using the Phosphatase-coupled Glycosyltransferase Assay. (**A**) The activities of Recombinant TcdB (Catalog # 6246-GT) and (**B**) Recombinant Human KTELC1 (Catalog # 6437-GT) were measured using the donor substrate UDP-Glucose (acceptor H_2O). Both glycosyltransferase reactions were carried out in the presence of Recombinant Human CD39L3 phosphatase (Catalog # 4400-EN). Free phosphate was detected using the Malachite Green Phosphate Detection Kit (Catalog # DY996). (**C**) The activity of Recombinant Human ST6GAL1 (Catalog # 5924-GT) was measured using the donor substrate CMP-NeuAC (acceptor LN). The glycosyltransferase reaction was carried out in the presence of Recombinant Human CD73 phosphatase (Catalog # 4488-EN). Free phosphate was detected using the Malachite Green Phosphate Detection Kit (Catalog # DY996).

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receptor induces *forward* signaling that is dependent on a catalytically active intracellular Eph kinase domain. In addition, Ephrin ligands can also *reverse* signal into their host cell.[7] For transmembrane Ephrin-B ligands, reverse signaling is dependent on tyrosine phosphorylation sites and a PDZ binding motif in the cytoplasmic domain.[8]

To investigate the importance of reverse signaling during angiogenesis, Sawamiphak and colleagues created Ephrin-B2ΔV mice, in which the cytoplasmic PDZ binding motif of Ephrin-B2 was impaired by targeted deletion of a single valine residue.[6] The authors reported decreased angiogenic vessel sprouting and reduced filopodia density in the retinas of Ephrin-B2ΔV mice compared to wild-type control littermates. These data support the hypothesis that Ephrin-B2 reverse signaling controls vessel sprouting by promoting cell tip filopodia extension during angiogenesis.

The effects of VEGF are dependent on the internalization of VEGF receptors, which subsequently promotes signaling in the endosomal compartment.[2] Both studies highlighted here examined the role of Ephrin-B2 in VEGF-induced angiogenesis. Sawamiphak *et al.* showed that the Ephrin-B2 PDZ domain was required for VEGF-A-induced internalization and activation of VEGF Receptor 2 (VEGF R2) in endothelial cells.[6] In parallel, Wang *et al.* showed that knockout of the Ephrin-B2 gene severely impaired VEGF-C-induced VEGF R3 endocytosis and pathway activation, suggesting Ephrin-B2 and VEGF are functionally linked.[5] Interestingly, in the absence of VEGF, recombinant Ephrin-B2 or EphB4 triggered internalization of VEGF R3 but did not activate markers of VEGF signaling. This supports the theory that Ephrin and VEGF pathways cooperate to promote angiogenesis.[5]

Further studies by Sawamiphak and colleagues tested the hypothesis that Ephrin-B2 regulates VEGF signaling during pathological angiogenesis. In an orthotopic glioma model, tumor size in Ephrin-B2ΔV mice was severely reduced compared to tumors in wild-type control littermates.[6] This effect was associated with a substantial decrease in tumor vascularization, indicating that a common mechanism of angiogenesis occurs in physiological and pathological settings. Because blockade of VEGF/VEGF R signaling is routinely used as a treatment for cancer patients, inhibition of Ephrin-B2 may represent an alternative or combinatorial anti-angiogenic therapy.[9] This strategy is supported by a recent study that used a specific EphB4 kinase inhibitor to suppress VEGF-driven angiogenesis *in vivo*.[10] In addition, inhibition of Ephrin-B2/EphB4 signaling reduced tumor size in a transgenic mouse model of pancreatic cancer (RIP1-Tag2), an effect that was enhanced by combinatorial blockade of Notch signaling.[11]

References

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R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
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Fax: (612) 656-4400
info@RnDSystems.com

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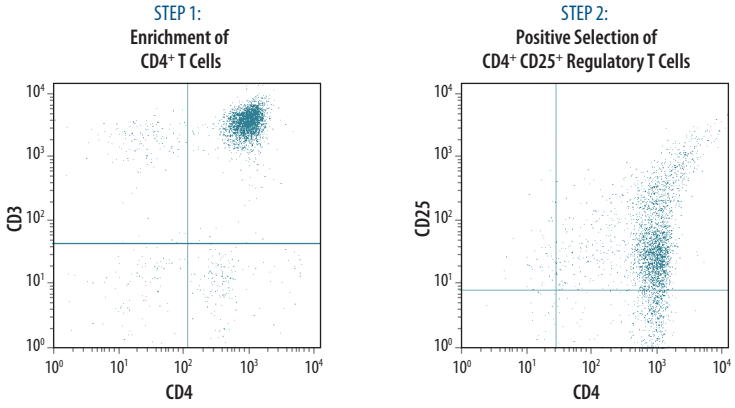
NEW TOOLS: Products for Regulatory T Cell Research

R&D Systems has recently introduced several new products for regulatory T (Treg) cell research, including a MagCellect™ Rat $CD4^+CD25^+$ Regulatory T Cell Selection Kit (Catalog # MAGR304)*, and Human, Mouse, and Rat Regulatory T Cell Multi-Color Flow Cytometry Kits (Catalog # FMC013, FMC014, FMC015, respectively). The Treg cell selection kit is designed to isolate rat $CD4^+CD25^+$ Treg cells from a splenocyte suspension using a two-step procedure that combines both negative and positive selection techniques. $CD4^+$ cells are initially enriched by negative selection, whereby unwanted cells are tagged and magnetically removed. $CD25^+$ cells are subsequently isolated from the enriched $CD4^+$ population by positive selection. Using this kit, the purity of the isolated $CD4^+CD25^+$ population is typically between 75-85%. For identification of Treg cells by flow cytometry, we also now offer Treg Multi-Color Flow Cytometry Kits for several different species. These kits include fluorochrome-conjugated antibodies against CD4, CD25, and FoxP3 that can be used together for single-step staining of human, mouse, or rat Treg cells. In addition, several new proteins and fluorochrome-conjugated antibodies (Table 1) add to a growing list of reagents that are available for functional studies on Treg cells. For a complete listing of Treg-related products, please visit our website at **www.RnDSystems.com/go/Treg**.

* Human and Mouse $CD4^+CD25^+$ Regulatory T Cell Selection Kits (Catalog # MAGH104 & MAGM208, respectively) are also available.



Isolation of Rat $CD4^+CD25^+$ Regulatory T Cells using the MagCellect Kit. $CD4^+$ T cells (Step 1) and $CD4^+CD25^+$ regulatory T cells (Step 2) were isolated from rat splenocytes using the two-step negative/positive selection protocol from the MagCellect Rat $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (Catalog # MAGR304). Following the first and the second isolation steps, the cells were stained with the indicated antibodies and detected by flow cytometry. $CD25^+$ T cells are typically a heterogeneous population that expresses different levels of CD25. Both $CD25^{dim}$ and $CD25^{high}$ cells are isolated with this kit.

Table 1. New Proteins & Antibodies for Treg Cell Research

PRODUCTS	CATALOG #
Recombinant Human LRRC32/GARP	6055-LR
Recombinant Mouse LRRC32/GARP	6229-LR
APC-conjugated Goat Anti-Human/Mouse/Rat FoxP3 Antigen Affinity-purified Polyclonal Antibody	IC3240A
APC- and PerCP-conjugated Goat Anti-Human LAG-3 Antigen Affinity-purified Polyclonal Antibodies	FAB2319A & FAB2319C
PE-conjugated Goat Anti-Mouse LAG-3 Antigen Affinity-purified Polyclonal Antibody	FAB3328P
Alexa Fluor® 488-conjugated Mouse Anti-Human/Mouse LAP (TGF-β1) Monoclonal Antibody	FAB2463G
APC-, PE-, and PerCP-conjugated Mouse Anti-Human/Mouse IL-12/IL-35 p35 Monoclonal Antibodies**	IC2191A, IC2191P, & IC2191C

** These antibodies, along with R&D Systems Mouse Anti-Human/Mouse IL-12/IL-35 p35 Monoclonal Antibody (Catalog # MAB1570), were cited in the most highly ranked article in immunology from the Faculty of 1000 Post-Publication Peer Review published by The Scientist.[1,2]

1. Zielinska, E. (2011) The Scientist [Epub Feb. 8].
2. Collison, L.W. *et al.* (2010) Nat. Immunol. **11**:1093.

This symbol denotes references that cite the use of R&D Systems products.